ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2015 March 21; 21(11): 3157-3448

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

A peer-reviewed, online, open-access journal of gastroenterology and hepatology

Editorial Board

2014-2017

The *World Journal of Gastroenterology* Editorial Board consists of 1378 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 68 countries, including Algeria (2), Argentina (7), Australia (31), Austria (9), Belgium (11), Brazil (20), Brunei Darussalam (1), Bulgaria (2), Cambodia (1), Canada (26), Chile (4), China (163), Croatia (2), Cuba (1), Czech (6), Denmark (2), Egypt (9), Estonia (2), Finland (6), France (20), Germany (58), Greece (31), Guatemala (1), Hungary (15), Iceland (1), India (33), Indonesia (2), Iran (10), Ireland (9), Israel (18), Italy (195), Japan (151), Jordan (1), Kuwait (1), Lebanon (7), Lithuania (1), Malaysia (1), Mexico (11), Morocco (1), Netherlands (5), New Zealand (4), Nigeria (3), Norway (6), Pakistan (6), Poland (12), Portugal (8), Puerto Rico (1), Qatar (1), Romania (10), Russia (3), Saudi Arabia (2), Singapore (7), Slovenia (2), South Africa (1), South Korea (69), Spain (51), Sri Lanka (1), Sudan (1), Sweden (12), Switzerland (5), Thailand (7), Trinidad and Tobago (1), Tunisia (2), Turkey (55), United Kingdom (49), United States (180), Venezuela (1), and Vietnam (1).

Alfred King Lam, *Gold Coast*
Ian C Lawrance, *PerthFremantle*
Barbara Anne Leggett, *Brisbane*
Daniel A Lemberg, *Sydney*
Rupert W Leong, *Sydney*
Finlay A Macrae, *Victoria*
Vance Matthews, *Melbourne*
David L Morris, *Sydney*
Reme Mountifield, *Bedford Park*
Hans J Netter, *Melbourne*
Nam Q Nguyen, *Adelaide*
Liang Qiao, *Westmead*
Rajvinder Singh, *Adelaide*
Ross Cyril Smith, *StLeonards*
Kevin J Spring, *Sydney*
Debbie Trinder, *Fremantle*
Daniel R van Langenberg, *Box Hill*
David Ian Watson, *Adelaide*
Desmond Yip, *Garran*
Li Zhang, *Sydney*

Austria

Felix Aigner, *Innsbruck*
Gabriela A Berlakovich, *Vienna*
Herwig R Cerwenka, *Graz*
Peter Ferenci, *Wien*
Alfred Gangl, *Vienna*
Kurt Lenz, *Linz*
Markus Peck-Radosavljevic, *Vienna*
Markus Raderer, *Vienna*
Stefan Riss, *Vienna*

Belgium

Michael George Adler, *Brussels*
Benedicte Y De Winter, *Antwerp*
Mark De Ridder, *Jette*
Olivier Detry, *Liege*
Denis Dufrane Dufrane, *Brussels*
Sven M Francque, *Edegem*
Nikos Kotzampassakis, *Liège*
Geert KMM Robaeys, *Genk*
Xavier Sagaert, *Leuven*
Peter Starkel, *Brussels*
Eddie Wisse, *Keerbergen*

Brazil

SMP Balzan, *Santa Cruz do Sul*
JLF Caboclo, *Sao jose do rio preto*
Fábio Guilherme Campos, *Sao Paulo*
Claudia RL Cardoso, *Rio de Janeiro*
Roberto J Carvalho-Filho, *Sao Paulo*
Carla Daltro, *Salvador*
José Sebastiao dos Santos, *Ribeirao Preto*
Eduardo LR Mello, *Rio de Janeiro*
Sthela Maria Murad-Regadas, *Fortaleza*
Claudia PMS Oliveira, *Sao Paulo*
Júlio C Pereira-Lima, *Porto Alegre*
Marcos V Perini, *Sao Paulo*
Vietla Satyanarayana Rao, *Fortaleza*
Raquel Rocha, *Salvador*
AC Simoes e Silva, *Belo Horizonte*
Mauricio F Silva, *Porto Alefre*
Aytan Miranda Sipahi, *Sao Paulo*
Rosa Leonôra Salerno Soares, *Niterói*
Cristiane Valle Tovo, *Porto Alegre*
Eduardo Garcia Vilela, *Belo Horizonte*

Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*

Bulgaria

Tanya Kirilova Kadiyska, *Sofia*
Mihaela Petrova, *Sofia*

Cambodia

Francois Rouet, *Phnom Penh*

Canada

Brian Bressler, *Vancouver*
Frank J Burczynski, *Winnipeg*
Wangxue Chen, *Ottawa*
Francesco Crea, *Vancouver*
Mirko Diksic, *Montreal*
Jane A Foster, *Hamilton*
Hugh J Freeman, *Vancouver*
Shahrokh M Ghobadloo, *Ottawa*
Yuewen Gong, *Winnipeg*
Philip H Gordon, *Quebec*
Rakesh Kumar, *Edmonton*
Wolfgang A Kunze, *Hamilton*
Patrick Labonte, *Laval*
Zhikang Peng, *Winnipeg*
Jayadev Raju, *Ottawa*
Maitreyi Raman, *Calgary*
Giada Sebastiani, *Montreal*
Maida J Sewitch, *Montreal*
Eldon A Shaffer, *Alberta*
Christopher W Teshima, *Edmonton*
Jean Sévigny, *Québec*
Pingchang Yang, *Hamilton*
Pingchang Yang, *Hamilton*
Eric M Yoshida, *Vancouver*
Bin Zheng, *Edmonton*

Chile

Marcelo A Beltran, *La Serena*
Flavio Nervi, *Santiago*
Adolfo Parra-Blanco, *Santiago*
Alejandro Soza, *Santiago*

China

Zhao-Xiang Bian, *Hong Kong*
San-Jun Cai, *Shanghai*
Guang-Wen Cao, *Shanghai*
Long Chen, *Nanjing*
Ru-Fu Chen, *Guangzhou*
George G Chen, *Hong Kong*
Li-Bo Chen, *Wuhan*
Jia-Xu Chen, *Beijing*
Hong-Song Chen, *Beijing*
Lin Chen, *Beijing*
Yang-Chao Chen, *Hong Kong*
Zhen Chen, *Shanghai*
Ying-Sheng Cheng, *Shanghai*
Kent-Man Chu, *Hong Kong*
Zhi-Jun Dai, *Xi'an*
Jing-Yu Deng, *Tianjin*
Yi-Qi Du, *Shanghai*
Zhi Du, *Tianjin*
Hani El-Nezami, *Hong Kong*
Bao-Ying Fei, *Hangzhou*
Chang-Ming Gao, *Nanjing*
Jian-Ping Gong, *Chongqing*
Zuo-Jiong Gong, *Wuhan*
Jing-Shan Gong, *Shenzhen*
Guo-Li Gu, *Beijing*
Yong-Song Guan, *Chengdu*
Mao-Lin Guo, *Luoyang*
Jun-Ming Guo, *Ningbo*
Yan-Mei Guo, *Shanghai*
Xiao-Zhong Guo, *Shenyang*
Guo-Hong Han, *Xi'an*
Ming-Liang He, *Hong Kong*
Peng Hou, *Xi'an*
Zhao-Hui Huang, *Wuxi*
Feng Ji, *Hangzhou*
Simon Law, *Hong Kong*
Yu-Yuan Li, *Guangzhou*
Meng-Sen Li, *Haikou*
Shu-De Li, *Shanghai*
Zong-Fang Li, *Xi'an*
Qing-Quan Li, *Shanghai*
Kang Li, *Lasa*
Han Liang, *Tianjin*
Xing'e Liu, *Hangzhou*
Zheng-Wen Liu, *Xi'an*
Xiao-Fang Liu, *Yantai*
Bin Liu, *Tianjin*
Quan-Da Liu, *Beijing*
Hai-Feng Liu, *Beijing*
Fei Liu, *Shanghai*
Ai-Guo Lu, *Shanghai*
He-Sheng Luo, *Wuhan*
Xiao-Peng Ma, *Shanghai*
Yong Meng, *Shantou*
Ke-Jun Nan, *Xi'an*
Siew Chien Ng, *Hong Kong*
Simon SM Ng, *Hong Kong*
Zhao-Shan Niu, *Qingdao*
Bo-Rong Pan, *Xi'an*
Di Qu, *Shanghai*
Rui-Hua Shi, *Nanjing*
Bao-Min Shi, *Shanghai*
Xiao-Dong Sun, *Hangzhou*
Si-Yu Sun, *Shenyang*
Guang-Hong Tan, *Haikou*
Wen-Fu Tang, *Chengdu*
Anthony YB Teoh, *Hong Kong*
Wei-Dong Tong, *Chongqing*
Eric Tse, *Hong Kong*
Hong Tu, *Shanghai*
Rong Tu, *Haikou*
Jian-She Wang, *Shanghai*
Kai Wang, *Jinan*
Xiao-Ping Wang, *Xianyang*
Dao-Rong Wang, *Yangzhou*
De-Sheng Wang, *Xi'an*
Chun-You Wang, *Wuhan*
Ge Wang, *Chongqing*

Xi-Shan Wang, *Harbin*
Wei-hong Wang, *Beijing*
Zhen-Ning Wang, *Shenyang*
Wai Man Raymond Wong, *Hong Kong*
Chun-Ming Wong, *Hong Kong*
Jian Wu, *Shanghai*
Sheng-Li Wu, *Xi'an*
Wu-Jun Wu, *Xi'an*
Qing Xia, *Chengdu*
Yan Xin, *Shenyang*
Dong-Ping Xu, *Beijing*
Jian-Min Xu, *Shanghai*
Wei Xu, *Changchun*
Ming Yan, *Jinan*
Xin-Min Yan, *Kunming*
Yi-Qun Yan, *Shanghai*
Feng Yang, *Shanghai*
Yong-Ping Yang, *Beijing*
He-Rui Yao, *Guangzhou*
Thomas Yau, *Hong Kong*
Winnie Yeo, *Hong Kong*
Jing You, *Kunming*
Jian-Qing Yu, *Wuhan*
Ying-Yan Yu, *Shanghai*
Wei-Zheng Zeng, *Chengdu*
Zong-Ming Zhang, *Beijing*
Dian-Liang Zhang, *Qingdao*
Ya-Ping Zhang, *Shijiazhuang*
You-Cheng Zhang, *Lanzhou*
Jian-Zhong Zhang, *Beijing*
Ji-Yuan Zhang, *Beijing*
Hai-Tao Zhao, *Beijing*
Jian Zhao, *Shanghai*
Jian-Hong Zhong, *Nanning*
Ying-Qiang Zhong, *Guangzhou*
Ping-Hong Zhou, *Shanghai*
Yan-Ming Zhou, *Xiamen*
Tong Zhou, *Nanchong*
Li-Ming Zhou, *Chengdu*
Guo-Xiong Zhou, *Nantong*
Feng-Shang Zhu, *Shanghai*
Jiang-Fan Zhu, *Shanghai*
Zhao-Hui Zhu, *Beijing*

Croatia

Tajana Filipec Kanizaj, *Zagreb*
Mario Tadic, *Zagreb*

Cuba

Damian Casadesus, *Havana*

Czech

Jan Bures, *Hradec Kralove*
Marcela Kopacova, *Hradec Kralove*
Otto Kucera, *Hradec Kralove*
Marek Minarik, *Prague*
Pavel Soucek, *Prague*
Miroslav Zavoral, *Prague*

Denmark

Vibeke Andersen, *Odense*
E Michael Danielsen, *Copenhagen*

Egypt

Mohamed MM Abdel-Latif, *Assiut*
Hussein Atta, *Cairo*
Ashraf Elbahrawy, *Cairo*
Mortada Hassan El-Shabrawi, *Cairo*
Mona El Said El-Raziky, *Cairo*
Elrashdy M Redwan, *New Borg Alrab*
Zeinab Nabil Ahmed Said, *Cairo*
Ragaa HM Salama, *Assiut*
Maha Maher Shehata, *Mansoura*

Estonia

Margus Lember, *Tartu*
Tamara Vorobjova, *Tartu*

Finland

Marko Kalliomäki, *Turku*
Thomas Kietzmann, *Oulu*
Kaija-Leena Kolho, *Helsinki*
Eija Korkeila, *Turku*
Heikki Makisalo, *Helsinki*
Tanja Pessi, *Tampere*

France

Armando Abergel Clermont, *Ferrand*
Elie K Chouillard, *Polssy*
Pierre Cordelier, *Toulouse*
Pascal P Crenn, *Garches*
Catherine Daniel, *Lille*
Fanny Daniel, *Paris*
Cedric Dray, *Toulouse*
Benoit Foligne, *Lille*
Jean-Noel Freund, *Strasbourg*
Hervé Guillou, *Toulouse*
Nathalie Janel, *Paris*
Majid Khatib, *Bordeaux*
Jacques Marescaux, *Strasbourg*
Jean-Claude Marie, *Paris*
Driffa Moussata, *Pierre Benite*
Hang Nguyen, *Clermont-Ferrand*
Hugo Perazzo, *Paris*
Alain L Servin, *Chatenay-Malabry*
Chang Xian Zhang, *Lyon*

Germany

Stavros A Antoniou, *Monchengladbach*
Erwin Biecker, *Siegburg*
Hubert E Blum, *Freiburg*
Thomas Bock, *Berlin*
Katja Breitkopf-Heinlein, *Mannheim*
Elke Cario, *Essen*
Güralp Onur Ceyhan, *Munich*
Angel Cid-Arregui, *Heidelberg*
Michael Clemens Roggendorf, *München*
Christoph F Dietrich, *Bad Mergentheim*
Valentin Fuhrmann, *Hamburg*
Nikolaus Gassler, *Aachen*
Andreas Geier, *Wuerzburg*
Markus Gerhard, *Munich*
Anton Gillessen, *Muenster*
Thorsten Oliver Goetze, *Offenbach*
Daniel Nils Gotthardt, *Heidelberg*
Robert Grützmann, *Dresden*
Thilo Hackert, *Heidelberg*
Joerg Haier, *Muenster*
Claus Hellerbrand, *Regensburg*
Harald Peter Hoensch, *Darmstadt*
Jens Hoeppner, *Freiburg*
Richard Hummel, *Muenster*
Jakob Robert Izbicki, *Hamburg*
Gernot Maximilian Kaiser, *Essen*
Matthias Kapischke, *Hamburg*
Michael Keese, *Frankfurt*
Andrej Khandoga, *Munich*
Jorg Kleeff, *Munich*
Alfred Koenigsrainer, *Tuebingen*
Peter Christopher Konturek, *Saalfeld*
Michael Linnebacher, *Rostock*
Stefan Maier, *Kaufbeuren*
Oliver Mann, *Hamburg*
Marc E Martignoni, *Munic*
Thomas Minor, *Bonn*
Oliver Moeschler, *Osnabrueck*
Jonas Mudter, *Eutin*
Sebastian Mueller, *Heidelberg*
Matthias Ocker, *Berlin*
Andreas Ommer, *Essen*
Albrecht Piiper, *Frankfurt*
Esther Raskopf, *Bonn*
Christoph Reichel, *Bad Brückenau*
Elke Roeb, *Giessen*
Udo Rolle, *Frankfurt*
Karl-Herbert Schafer, *Zweibrücken*
Peter Schemmer, *Heidelberg*
Andreas G Schreyer, *Regensburg*
Manuel A Silva, *Penzberg*
Georgios C Sotiropoulos, *Essen*
Ulrike S Stein, *Berlin*
Dirk Uhlmann, *Leipzig*
Michael Weiss, *Halle*
Hong-Lei Weng, *Mannheim*
Karsten Wursthorn, *Hamburg*

Greece

Alexandra Alexopoulou, *Athens*
Nikolaos Antonakopoulos, *Athens*
Stelios F Assimakopoulos, *Patras*
Grigoris Chatzimavroudis, *Thessaloniki*
Evangelos Cholongitas, *Thessaloniki*
Gregory Christodoulidis, *Larisa*
George N Dalekos, *Larissa*
Urania Georgopoulou, *Athens*
Eleni Gigi, *Thessaloniki*
Stavros Gourgiotis, *Athens*
Leontios J Hadjileontiadis, *Thessaloniki*
Thomas Hyphantis, *Ioannina*
Ioannis Kanellos, *Thessaloniki*
Stylianos Karatapanis, *Rhodes*
Michael Koutsilieris, *Athens*
Spiros D Ladas, *Athens*
Theodoros K Liakakos, *Athens*
Emanuel K Manesis, *Athens*
Spilios Manolakopoulos, *Athens*
Gerassimos John Mantzaris, *Athens*
Athanasios D Marinis, *Piraeus*

Nikolaos Ioannis Nikiteas, *Athens*
Konstantinos X Papamichael, *Athens*
George Sgourakis, *Athens*
Konstantinos C Thomopoulos, *Patras*
Konstantinos Triantafyllou, *Athens*
Christos Triantos, *Patras*
Georgios Zacharakis, *Athens*
Petros Zezos, *Alexandroupolis*
Demosthenes E Ziogas, *Ioannina*

Guatemala

Carlos Maria Parellada, *Guatemala*

Hungary

Mihaly Boros, *Szeged*
Tamás Decsi, *Pécs*
Gyula Farkas, *Szeged*
Andrea Furka, *Debrecen*
Y vette Mandi, *Szeged*
Peter L Lakatos, *Budapest*
Pal Miheller, *Budapest*
Tamás Molnar, *Szeged*
Attila Olah, *Gyor*
Maria Papp, *Debrecen*
Zoltan Rakonczay, *Szeged*
Ferenc Sipos, *Budapest*
Miklós Tanyi, *Debrecen*
Tibor Wittmann, *Szeged*

Iceland

Tryggvi Bjorn Stefánsson, *Reykjavík*

India

Brij B Agarwal, *New Delhi*
Deepak N Amarapurkar, *Mumbai*
Shams ul Bari, *Srinagar*
Sriparna Basu, *Varanasi*
Runu Chakravarty, *Kolkata*
Devendra C Desai, *Mumbai*
Nutan D Desai, *Mumbai*
Suneela Sunil Dhaneshwar, *Pune*
Radha K Dhiman, *Chandigarh*
Pankaj Garg, *Mohali*
Uday C Ghoshal, *Lucknow*
Kalpesh Jani, *Vadodara*
Premashis Kar, *New Delhi*
Jyotdeep Kaur, *Chandigarh*
Rakesh Kochhar, *Chandigarh*
Pradyumna K Mishra, *Mumbai*
Asish K Mukhopadhyay, *Kolkata*
Imtiyaz Murtaza, *Srinagar*
P Nagarajan, *New Delhi*
Samiran Nundy, *Delhi*
Gopal Pande, *Hyderabad*
Benjamin Perakath, *Vellore*
Arun Prasad, *New Delhi*
D Nageshwar Reddy, *Hyderabad*
Lekha Saha, *Chandigarh*
Sundeep Singh Saluja, *New Delhi*
Mahesh Prakash Sharma, *New Delhi*
Sadiq Saleem Sikora, *Bangalore*
Sarman Singh, *New Delhi*
Rajeev Sinha, *Jhansi*
Rupjyoti Talukdar, *Hyderabad*
Rakesh Kumar Tandon, *New Delhi*
Narayanan Thirumoorthy, *Coimbatore*

Indonesia

David Handojo Muljono, *Jakarta*
Andi Utama, *Jakarta*

Iran

Arezoo Aghakhani, *Tehran*
Seyed Mohsen Dehghani, *Shiraz*
Ahad Eshraghian, *Shiraz*
Hossein Khedmat, *Tehran*
Sadegh Massarrat, *Tehran*
Marjan Mohammadi, *Tehran*
Roja Rahimi, *Tehran*
Farzaneh Sabahi, *Tehran*
Majid Sadeghizadeh, *Tehran*
Farideh Siavoshi, *Tehran*

Ireland

Gary Alan Bass, *Dublin*
David J Brayden, *Dublin*
Ronan A Cahill, *Dublin*
Glen A Doherty, *Dublin*
Liam J Fanning, *Cork*
Barry Philip McMahon, *Dublin*
RossMcManus, *Dublin*
Dervla O'Malley, *Cork*
Sinead M Smith, *Dublin*

Israel

Dan Carter, *Ramat Gan*
Jorge-Shmuel Delgado, *Metar*
Eli Magen, *Ashdod*
Nitsan Maharshak, *Tel Aviv*
Shaul Mordechai, *Beer Sheva*
Menachem Moshkowitz, *Tel Aviv*
William Bahij Nseir, *Nazareth*
Shimon Reif, *Jerusalem*
Ram Reifen, *Rehovot*
Ariella Bar-Gil Shitrit, *Jerusalem*
Noam Shussman, *Jerusalem*
Igor Sukhotnik, *Haifa*
Nir Wasserberg, *Petach Tiqwa*
Jacob Yahav, *Rehovot*
Doron Levi Zamir, *Gedera*
Shira Zelber-Sagi, *Haifa*
Romy Zemel, *Petach-Tikva*

Italy

Ludovico Abenavoli, *Catanzaro*
Luigi Elio Adinolfi, *Naples*
Carlo Virginio Agostoni, *Milan*
Anna Alisi, *Rome*
Piero Luigi Almasio, *Palermo*
Donato Francesco Altomare, *Bari*
Amedeo Amedei, *Florence*
Pietro Andreone, *Bologna*
Imerio Angriman, *Padova*
Vito Annese, *Florence*
Paolo Aurello, *Rome*
Salavtore Auricchio, *Naples*
Gian Luca Baiocchi, *Brescia*
Gianpaolo Balzano, *Milan*
Antonio Basoli, *Rome*
Gabrio Bassotti, *San Sisto*
Mauro Bernardi, *Bologna*
Alberto Biondi, *Rome*
Ennio Biscaldi, *Genova*
Massimo Bolognesi, *Padua*
Luigi Bonavina, *Milano*
Aldo Bove, *Chieti*
Raffaele Bruno, *Pavia*
Luigi Brusciano, *Napoli*
Giuseppe Cabibbo, *Palermo*
Carlo Calabrese, *Bologna*
Daniele Calistri, *Meldola*
Vincenza Calvaruso, *Palermo*
Lorenzo Camellini, *Reggio Emilia*
Marco Candela, *Bologna*
Raffaele Capasso, *Naples*
Lucia Carulli, *Modena*
Renato David Caviglia, *Rome*
Luigina Cellini, *Chieti*
Giuseppe Chiarioni, *Verona*
Claudio Chiesa, *Rome*
Michele Cicala, *Roma*
Rachele Ciccocioppo, *Pavia*
Sandro Contini, *Parma*
Gaetano Corso, *Foggia*
Renato Costi, *Parma*
Alessandro Cucchetti, *Bologna*
Rosario Cuomo, *Napoli*
Giuseppe Currò, *Messina*
Paola De Nardi, *Milano*
Giovanni D De Palma, *Naples*
Raffaele De Palma, *Napoli*
Giuseppina De Petro, *Brescia*
Valli De Re, *Aviano*
Paolo De Simone, *Pisa*
Giuliana Decorti, *Trieste*
Emanuele Miraglia del Giudice, *Napoli*
Isidoro Di Carlo, *Catania*
Matteo Nicola Dario Di Minno, *Naples*
Massimo Donadelli, *Verona*
Mirko D'Onofrio, *Verona*
Maria Pina Dore, *Sassari*
Luca Elli, *Milano*
Massimiliano Fabozzi, *Aosta*
Massimo Falconi, *Ancona*
Ezio Falletto, *Turin*
Silvia Fargion, *Milan*
Matteo Fassan, *Verona*
Gianfranco Delle Fave, *Roma*
Alessandro Federico, *Naples*
Francesco Feo, *Sassari*
Davide Festi, *Bologna*
Natale Figura, *Siena*
Vincenzo Formica, *Rome*
Mirella Fraquelli, *Milan*
Marzio Frazzoni, *Modena*
Walter Fries, *Messina*
Gennaro Galizia, *Naples*
Andrea Galli, *Florence*
Matteo Garcovich, *Rome*
Eugenio Gaudio, *Rome*
Paola Ghiorzo, *Genoa*
Edoardo G Giannini, *Genova*

Luca Gianotti, *Monza*
Maria Cecilia Giron, *Padova*
Alberto Grassi, *Rimini*
Gabriele Grassi, *Trieste*
Francesco Greco, *Bergamo*
Luigi Greco, *Naples*
Antonio Grieco, *Rome*
Fabio Grizzi, *Rozzano*
Laurino Grossi, *Pescara*
Simone Guglielmetti, *Milan*
Tiberiu Hershcovici, *Jerusalem*
Calogero Iacono, *Verona*
Enzo Ierardi, *Bari*
Amedeo Indriolo, *Bergamo*
Raffaele Iorio, *Naples*
Paola Iovino, *Salerno*
Angelo A Izzo, *Naples*
Loreta Kondili, *Rome*
Filippo La Torre, *Rome*
Giuseppe La Torre, *Rome*
Giovanni Latella, *L'Aquila*
Salvatore Leonardi, *Catania*
Massimo Libra, *Catania*
Anna Licata, *Palermo*
C armela Loguercio, *Naples*
Amedeo Lonardo, *Modena*
Carmelo Luigiano, *Catania*
Francesco Luzza, *Catanzaro*
Giovanni Maconi, *Milano*
Antonio Macrì, *Messina*
Mariano Malaguarnera, *Catania*
Francesco Manguso, *Napoli*
Tommaso Maria Manzia, *Rome*
Daniele Marrelli, *Siena*
Gabriele Masselli, *Rome*
Sara Massironi, *Milan*
Giuseppe Mazzarella, *Avellino*
Michele Milella, *Rome*
Giovanni Milito, *Rome*
Antonella d'Arminio Monforte, *Milan*
Fabrizio Montecucco, *Genoa*
Giovanni Monteleone, *Rome*
Mario Morino, *Torino*
Vincenzo La Mura, *Milan*
Gerardo Nardone, *Naples*
Riccardo Nascimbeni, *Brescia*
Gabriella Nesi, *Florence*
Giuseppe Nigri, *Rome*
Erica Novo, *Turin*
Veronica Ojetti, *Rome*
Michele Orditura, *Naples*
Fabio Pace, *Seriate*
Lucia Pacifico, *Rome*
Omero Alessandro Paoluzi, *Rome*
Valerio Pazienza, *San Giovanni Rotondo*
Rinaldo Pellicano, *Turin*
Adriano M Pellicelli, *Rome*
Nadia Peparini, *Ciampino*
Mario Pescatori, *Rome*
Antonio Picardi, *Rome*
Alberto Pilotto, *Padova*
Alberto Piperno, *Monza*
Anna Chiara Piscaglia, *Rome*
Maurizio Pompili, *Rome*
Francesca Romana Ponziani, *Rome*
Cosimo Prantera, *Rome*
Girolamo Ranieri, *Bari*
Carlo Ratto, *Tome*
Barbara Renga, *Perugia*
Alessandro Repici, *Rozzano*
Maria Elena Riccioni, *Rome*
Lucia Ricci-Vitiani, *Rome*
Luciana Rigoli, *Messina*
Mario Rizzetto, *Torino*
Ballarin Roberto, *Modena*
Roberto G Romanelli, *Florence*
Claudio Romano, *Messina*
Luca Roncucci, *Modena*
Cesare Ruffolo, *Treviso*
L ucia Sacchetti, *Napoli*
Rodolfo Sacco, *Pisa*
Lapo Sali, *Florence*
Romina Salpini, *Rome*
Giulio Aniello, *Santoro Treviso*
Armando Santoro, *Rozzano*
Edoardo Savarino, *Padua*
Marco Senzolo, *Padua*
Annalucia Serafino, *Rome*
Giuseppe S Sica, *Rome*
Pierpaolo Sileri, *Rome*
Cosimo Sperti, *Padua*
Vincenzo Stanghellini, *Bologna*
Cristina Stasi, *Florence*
Gabriele Stocco, *Trieste*
Roberto Tarquini, *Florence*
Mario Testini, *Bari*
Guido Torzilli, *Milan*
Guido Alberto Massimo, *Tiberio Brescia*
Giuseppe Toffoli, *Aviano*
Alberto Tommasini, *Trieste*
Francesco Tonelli, *Florence*
Cesare Tosetti Porretta, *Terme*
Lucio Trevisani, *Cona*
Guglielmo M Trovato, *Catania*
Mariapia Vairetti, *Pavia*
Luca Vittorio Valenti, *Milano*
Mariateresa T Ventura, *Bari*
Giuseppe Verlato, *Verona*
Alessandro Vitale, *Padova*
Marco Vivarelli, *Ancona*
Giovanni Li Volti, *Catania*
Giuseppe Zanotti, *Padua*
Vincenzo Zara, *Lecce*
Gianguglielmo Zehender, *Milan*
Anna Linda Zignego, *Florence*
Rocco Antonio Zoccali, *Messina*
Angelo Zullo, *Rome*

Japan

Yasushi Adachi, *Sapporo*
Takafumi Ando, *Nagoya*
Masahiro Arai, *Tokyo*
Makoto Arai, *Chiba*
Takaaki Arigami, *Kagoshima*
Itaru Endo,*Yokohama*
Munechika Enjoji, *Fukuoka*
Shunji Fujimori, *Tokyo*
Yasuhiro Fujino, *Akashi*
Toshiyoshi Fujiwara, *Okayama*
Yosuke Fukunaga, *Tokyo*
Toshio Fukusato, *Tokyo*
Takahisa Furuta, *Hamamatsu*
Osamu Handa, *Kyoto*
Naoki Hashimoto, *Osaka*
Yoichi Hiasa, *Toon*
Masatsugu Hiraki, *Saga*
Satoshi Hirano, *Sapporo*
Keiji Hirata, *Fukuoka*
Toru Hiyama, *Higashihiroshima*
Akira Hokama, *Nishihara*
Shu Hoteya, *Tokyo*
Masao Ichinose, *Wakayama*
Tatsuya Ide, *Kurume*
Masahiro Iizuka, *Akita*
Toshiro Iizuka, *Tokyo*
Kenichi Ikejima, *Tokyo*
Tetsuya Ikemoto, *Tokushima*
Hiroyuki Imaeda, *Saitama*
Atsushi Imagawa, *Kan-onji*
Hiroo Imazu, *Tokyo*
Shuji Isaji, *Tsu*
Toru Ishikawa, *Niigata*
Toshiyuki Ishiwata, *Tokyo*
Soichi Itaba, *Kitakyushu*
Yoshiaki Iwasaki, *Okayama*
Tatehiro Kagawa, *Isehara*
Satoru Kakizaki, *Maebashi*
Naomi Kakushima, *Shizuoka*
Terumi Kamisawa, *Tokyo*
Akihide Kamiya, *Isehara*
Osamu Kanauchi, *Tokyo*
Tatsuo Kanda, *Chiba*
Shin Kariya, *Okayama*
Shigeyuki Kawa, *Matsumoto*
Takumi Kawaguchi, *Kurume*
Takashi Kawai, *Tokyo*
Soo Ryang Kim, *Kobe*
Shinsuke Kiriyama, *Gunma*
Tsuneo Kitamura, *Urayasu*
Masayuki Kitano, *Osakasayama*
Hirotoshi Kobayashi, *Tokyo*
Hironori Koga, *Kurume*
Takashi Kojima, *Sapporo*
Satoshi Kokura, *Kyoto*
Shuhei Komatsu, *Kyoto*
Tadashi Kondo, *Tokyo*
Yasuteru Kondo, *Sendai*
Yasuhiro Kuramitsu, *Yamaguchi*
Yukinori Kurokawa, *Osaka*
Shin Maeda, *Yokohama*
Koutarou Maeda, *Toyoake*
Hitoshi Maruyama, *Chiba*
Atsushi Masamune, *Sendai*
Hiroyuki Matsubayashi, *Suntogun*
Akihisa Matsuda, *Inzai*
Hirofumi Matsui, *Tsukuba*
Akira Matsumori, *Kyoto*
Yoichi Matsuo, *Nagoya*
Y Matsuzaki, *Ami*
Toshihiro Mitaka, *Sapporo*
Kouichi Miura, *Akita*
Shinichi Miyagawa, *Matumoto*
Eiji Miyoshi, *Suita*
Toru Mizuguchi, *Sapporo*
Nobumasa Mizuno, *Nagoya*
Zenichi Morise, *Nagoya*
Tomohiko Moriyama, *Fukuoka*
Kunihiko Murase, *Tusima*
Michihiro Mutoh, *Tsukiji*
Akihito Nagahara, *Tokyo*
Hikaru Nagahara, *Tokyo*
Hidenari Nagai, *Tokyo*
Koichi Nagata, *Shimotsuke-shi*

Masaki Nagaya, *Kawasaki*
Hisato Nakajima, *Nishi-Shinbashi*
Toshifusa Nakajima, *Tokyo*
Hiroshi Nakano, *Kawasaki*
Hiroshi Nakase, *Kyoto*
Toshiyuki Nakayama, *Nagasaki*
Takahiro Nakazawa, *Nagoya*
Shoji Natsugoe, *Kagoshima City*
Tsutomu Nishida, *Suita*
Shuji Nomoto, *Naogya*
Sachiyo Nomura, *Tokyo*
Takeshi Ogura, *Takatsukishi*
Nobuhiro Ohkohchi, *Tsukuba*
Toshifumi Ohkusa, *Kashiwa*
Hirohide Ohnishi, *Akita*
Teruo Okano, *Tokyo*
Satoshi Osawa, *Hamamatsu*
Motoyuki Otsuka, *Tokyo*
Michitaka Ozaki, *Sapporo*
Satoru Saito, *Yokohama*
Chouhei Sakakura, *Kyoto*
Naoaki Sakata, *Sendai*
Ken Sato, *Maebashi*
Toshiro Sato, *Tokyo*
Tomoyuki Shibata, *Toyoake*
H Shimada, *Tokyo*
Tomohiko Shimatani, *Kure*
Yukihiro Shimizu, *Nanto*
Tadashi Shimoyama, *Hirosaki*
Masayuki Sho, *Nara*
Ikuo Shoji, *Kobe*
Atsushi Sofuni, *Tokyo*
Takeshi Suda, *Niigata*
M Sugimoto, *Hamamatsu*
Ken Sugimoto, *Hamamatsu*
Haruhiko Sugimura, *Hamamatsu*
Shoichiro Sumi, *Kyoto*
Hidekazu Suzuki, *Tokyo*
Masahiro Tajika, *Nagoya*
Hitoshi Takagi, *Takasaki*
Toru Takahashi, *Niigata*
Yoshihisa Takahashi, *Tokyo*
Shinsuke Takeno, *Fukuoka*
Akihiro Tamori, *Osaka*
Kyosuke Tanaka, *Tsu*
Shinji Tanaka, *Hiroshima*
Atsushi Tanaka, *Tokyo*
Yasuhito Tanaka, *Nagoya*
Shinji Tanaka, *Tokyo*
Minoru Tomizawa, *Yotsukaido City*
Kyoko Tsukiyama-Kohara, *Kagoshima*
Takuya Watanabe, *Niigata*
Kazuhiro Watanabe, *Sendai*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Yokkaichi*
Hiroshi Yamamoto, *Otsu*
Kosho Yamanouchi, *Nagasaki*
Ichiro Yasuda, *Gifu*
Yutaka Yata, *Maebashi-city*
Shin-ichi Yokota, *Sapporo*
Norimasa Yoshida, *Kyoto*
Hiroshi Yoshida, *Tama-City*
Hitoshi Yoshiji, *Kashihara*
Kazuhiko Yoshimatsu, *Tokyo*
Kentaro Yoshioka, *Toyoake*
Nobuhiro Zaima, *Nara*

Jordan
Khaled Ali Jadallah, *Irbid*

Kuwait
Islam Khan, *Kuwait*

Lebanon
Bassam N Abboud, *Beirut*
Kassem A Barada, *Beirut*
Marwan Ghosn, *Beirut*
Iyad A Issa, *Beirut*
Fadi H Mourad, *Beirut*
AIa Sharara, *Beirut*
Rita Slim, *Beirut*

Lithuania
Antanas Mickevicius, *Kaunas*

Malaysia
Huck Joo Tan, *Petaling Jaya*

Mexico
Richard A Awad, *Mexico City*
Carlos R Camara-Lemarroy, *Monterrey*
Norberto C Chavez-Tapia, *Mexico City*
Wolfgang Gaertner, *Mexico City*
Diego Garcia-Compean, *Monterrey*
Arturo Panduro, *Guadalajara*
OT Teramoto-Matsubara, *Mexico City*
Felix Tellez-Avila, *Mexico City*
Omar Vergara-Fernandez, *Mexico City*
Saúl Villa-Trevino, *Cuidad de México*

Morocco
Samir Ahboucha, *Khouribga*

Netherlands
Robert J de Knegt, *Rotterdam*
Tom Johannes Gerardus Gevers, *Nijmegen*
Menno Hoekstra, *Leiden*
BW Marcel Spanier, *Arnhem*
Karel van Erpecum, *Utrecht*

New Zealand
Leo K Cheng, *Auckland*
Andrew Stewart Day, *Christchurch*
Jonathan Barnes Koea, *Auckland*
Max Petrov, *Auckland*

Nigeria
Olufunmilayo Adenike Lesi, *Lagos*
Jesse Abiodun Otegbayo, *Ibadan*
Stella Ifeanyi Smith, *Lagos*

Norway
Trond Berg, *Oslo*
Trond Arnulf Buanes, *Krokkleiva*
Thomas de Lange, *Rud*
Magdy El-Salhy, *Stord*
Rasmus Goll, *Tromso*
Dag Arne Lihaug Hoff, *Aalesund*

Pakistan
Zaigham Abbas, *Karachi*
Usman A Ashfaq, *Faisalabad*
Muhammad Adnan Bawany, *Hyderabad*
Muhammad Idrees, *Lahore*
Saeed Sadiq Hamid, *Karachi*
Yasir Waheed, *Islamabad*

Poland
Thomas Brzozowski, *Cracow*
Magdalena Chmiela, *Lodz*
Krzysztof Jonderko, *Sosnowiec*
Anna Kasicka-Jonderko, *Sosnowiec*
Michal Kukla, *Katowice*
Tomasz Hubert Mach, *Krakow*
Agata Mulak, *Wroclaw*
Danuta Owczarek, *Kraków*
Piotr Socha, *Warsaw*
Piotr Stalke, *Gdansk*
Julian Teodor Swierczynski, *Gdansk*
Anna M Zawilak-Pawlik, *Wroclaw*

Portugal
Marie Isabelle Cremers, *Setubal*
Ceu Figueiredo, *Porto*
Ana Isabel Lopes, *LIsbon*
M Paula Macedo, *Lisboa*
Ricardo Marcos, *Porto*
Rui T Marinho, *Lisboa*
Guida Portela-Gomes, *Estoril*
Filipa F Vale, *Lisbon*

Puerto Rico
Caroline B Appleyard, *Ponce*

Qatar
Abdulbari Bener, *Doha*

Romania
Mihai Ciocirlan, *Bucharest*
Dan LucianDumitrascu, *Cluj-Napoca*
Carmen Fierbinteanu-Braticevici, *Bucharest*
Romeo G Mihaila, *Sibiu*
Lucian Negreanu, *Bucharest*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*
Ioan Sporea, *Timisoara*
Letiția Adela Maria Streba, *Craiova*
Anca Trifan, *Iasi*

Russia

Victor Pasechnikov, *Stavropol*
Vasiliy Ivanovich Reshetnyak, *Moscow*
Vitaly Skoropad, *Obninsk*

Saudi Arabia

Abdul-Wahed N Meshikhes, *Dammam*
M Ezzedien Rabie, *Khamis Mushait*

Singapore

Brian KP Goh, *Singapore*
Richie Soong, *Singapore*
Ker-Kan Tan, *Singapore*
Kok-Yang Tan, *Singapore*
Yee-Joo Tan, *Singapore*
Mark Wong, *Singapore*
Hong Ping Xia, *Singapore*

Slovenia

Matjaz Homan, *Ljubljana*
Martina Perse, *Ljubljana*

South Korea

Sang Hoon Ahn, *Seoul*
Seung Hyuk Baik, *Seoul*
Soon Koo Baik, *Wonju*
Soo-Cheon Chae, *Iksan*
Byung-Ho Choe, *Daegu*
Suck Chei Choi, *Iksan*
Hoon Jai Chun, *Seoul*
Yeun-Jun Chung, *Seoul*
Young-Hwa Chung, *Seoul*
Ki-Baik Hahm, *Seongnam*
Sang Young Han, *Busan*
Seok Joo Han, *Seoul*
Seung-Heon Hong, *Iksan*
Jin-Hyeok Hwang, *Seoungnam*
Jeong Won Jang, *Seoul*
Jin-Young Jang, *Seoul*
Dae-Won Jun, *Seoul*
Young Do Jung, *Kwangju*
Gyeong Hoon Kang, *Seoul*
Sung-Bum Kang, *Seoul*
Koo Jeong Kang, *Daegu*
Ki Mun Kang, *Jinju*
Chang Moo Kang, *Seodaemun-gu*
Gwang Ha Kim, *Busan*
Sang Soo Kim, *Goyang-si*
Jin Cheon Kim, *Seoul*
Tae Il Kim, *Seoul*
Jin Hong Kim, *Suwon*
Kyung Mo Kim, *Seoul*
Kyongmin Kim, *Suwon*
Hyung-Ho Kim, *Seongnam*
Seoung Hoon Kim, *Goyang*
Sang Il Kim, *Seoul*
Hyun-Soo Kim, *Wonju*
Jung Mogg Kim, *Seoul*
Dong Yi Kim, *Gwangju*
Kyun-Hwan Kim, *Seoul*
Jong-Han Kim, *Ansan*
Sang Wun Kim, *Seoul*
Ja-Lok Ku, *Seoul*
Kyu Taek Lee, *Seoul*
Hae-Wan Lee, *Chuncheon*
Inchul Lee, *Seoul*
Jung Eun Lee, *Seoul*
Sang Chul Lee, *Daejeon*
Song Woo Lee, *Ansan-si*
Hyuk-Joon Lee, *Seoul*
Seong-Wook Lee, *Yongin*
Kil Yeon Lee, *Seoul*
Jong-Inn Lee, *Seoul*
Kyung A Lee, *Seoul*
Jong-Baeck Lim, *Seoul*
Eun-Yi Moon, *Seoul*
SH Noh, *Seoul*
Seung Woon Paik, *Seoul*
Won Sang Park, *Seoul*
Sung-Joo Park, *Iksan*
Kyung Sik Park, *Daegu*
Se Hoon Park, *Seoul*
Yoonkyung Park, *Gwangju*
Seung-Wan Ryu, *Daegu*
Il Han Song, *Cheonan*
Myeong Jun Song, *Daejeon*
Yun Kyoung Yim, *Daejeon*
Dae-Yeul Yu *Daejeon*

Spain

Mariam Aguas, *Valencia*
Raul J Andrade, *Málaga*
Antonio Arroyo, *Elche*
Josep M Bordas, *Barcelona*
Lisardo Boscá, *Madrid*
Ricardo Robles Campos, *Murcia*
Jordi Camps, *Reus*
Carlos Cervera *Barcelona*
Alfonso Clemente, *Granada*
Pilar Codoner-Franch, *Valencia*
Fernando J Corrales, *Pamplona*
Fermin Sánchez de Medina, *Granada*
Alberto Herreros de Tejada, *Majadahonda*
Enrique de-Madaria, *Alicante*
JE Dominguez-Munoz, *Santiago de Compostela*
Vicente Felipo, *Valencia*
CM Fernandez-Rodriguez, *Madrid*
Carmen Frontela-Saseta, *Murcia*
Julio Galvez, *Granada*
Maria Teresa García, *Vigo*
MI Garcia-Fernandez, *Málaga*
Emilio Gonzalez-Reimers, *La Laguna*
Marcel Jimenez, *Bellaterra*
Angel Lanas, *Zaragoza*
Juan Ramón Larrubia, *Guadalajara*
Antonio Lopez-Sanroman, *Madrid*
Vicente Lorenzo-Zuniga, *Badalona*
Alfredo J Lucendo, *Tomelloso*
Vicenta Soledad Martinez-Zorzano, *Vigo*
José Manuel Martin-Villa, *Madrid*
Julio Mayol, *Madrid*
Manuel Morales-Ruiz, *Barcelona*
Alfredo Moreno-Egea, *Murcia*
Albert Pares, *Barcelona*
Maria Pellise, *Barcelona*
José Perea, *Madrid*
Miguel Angel Plaza, *Zaragoza*
María J Pozo, *Cáceres*
Enrique Quintero, *La Laguna*
Jose M Ramia, *Madrid*
Francisco Rodriguez-Frias, *Barcelona*
Silvia Ruiz-Gaspa, *Barcelona*
Xavier Serra-Aracil, *Barcelona*
Vincent Soriano, *Madrid*
Javier Suarez, *Pamplona*
Carlos Taxonera, *Madrid*
M Isabel Torres, *Jaén*
Manuel Vazquez-Carrera, *Barcelona*
Benito Velayos, *Valladolid*
Silvia Vidal, *Barcelona*

Sri Lanka

Arjuna Priyadarsin De Silva, *Colombo*

Sudan

Ishag Adam, *Khartoum*

Sweden

Roland G Andersson, *Lund*
Bergthor Björnsson, *Linkoping*
Johan Christopher Bohr, *Örebro*
Mauro D'Amato, *Stockholm*
Thomas Franzen, *Norrkoping*
Evangelos Kalaitzakis, *Lund*
Riadh Sadik, *Gothenburg*
Per Anders Sandstrom, *Linkoping*
Ervin Toth, *Malmö*
Konstantinos Tsimogiannis, *Vasteras*
Apostolos V Tsolakis, *Uppsala*

Switzerland

Gieri Cathomas, *Liestal*
Jean Louis Frossard, *Geneve*
Christian Toso, *Geneva*
Stephan Robert Vavricka, *Zurich*
Dominique Velin, *Lausanne*

Thailand

Thawatchai Akaraviputh, *Bangkok*
P Yoysungnoen Chintana, *Pathumthani*
Veerapol Kukongviriyapan, *Muang*
Vijittra Leardkamolkarn, *Bangkok*
Varut Lohsiriwat, *Bangkok*
Somchai Pinlaor, *Khaon Kaen*
D Wattanasirichaigoon, *Bangkok*

Trinidad and Tobago

B Shivananda Nayak, *Mount Hope*

Tunisia

Ibtissem Ghedira, *Sousse*
Lilia Zouiten-Mekki, *Tunis*

Turkey

Inci Alican, *Istanbul*

Mustafa Altindis, *Sakarya*
Mutay Aslan, *Antalya*
Oktar Asoglu, *Istanbul*
Yasemin Hatice Balaban, *Istanbul*
Metin Basaranoglu, *Ankara*
Yusuf Bayraktar, *Ankara*
Süleyman Bayram, *Adiyaman*
Ahmet Bilici, *Istanbul*
Ahmet Sedat Boyacioglu, *Ankara*
Züleyha Akkan Cetinkaya, *Kocaeli*
Cavit Col, *Bolu*
Yasar Colak, *Istanbul*
Cagatay Erden Daphan, *Kirikkale*
Mehmet Demir, *Hatay*
Ahmet Merih Dobrucali, *Istanbul*
Gülsüm Ozlem Elpek, *Antalya*
Ayse Basak Engin, *Ankara*
Eren Ersoy, *Ankara*
Osman Ersoy, *Ankara*
Yusuf Ziya Erzin, *Istanbul*
Mukaddes Esrefoglu, *Istanbul*
Levent Filik, *Ankara*
Ozgur Harmanci, *Ankara*
Koray Hekimoglu, *Ankara*
Abdurrahman Kadayifci, *Gaziantep*
Cem Kalayci, *Istanbul*
Selin Kapan, *Istanbul*
Huseyin Kayadibi, *Adana*
Sabahattin Kaymakoglu, *Istanbul*
Metin Kement, *Istanbul*
Mevlut Kurt, *Bolu*
Resat Ozaras, *Istanbul*
Elvan Ozbek, *Adapazari*
Cengiz Ozcan, *Mersin*
Hasan Ozen, *Ankara*
Halil Ozguc, *Bursa*
Mehmet Ozturk, *Izmir*
Orhan V Ozkan, *Sakarya*
Semra Paydas, *Adana*
Ozlem Durmaz Suoglu, *Istanbul*
Ilker Tasci, *Ankara*
Müge Tecder-ünal, *Ankara*
Mesut Tez, *Ankara*
Serdar Topaloglu, *Trabzon*
Murat Toruner, *Ankara*
Gokhan Tumgor, *Adana*
Oguz Uskudar, *Adana*
Mehmet Yalniz, *Elazig*
Mehmet Yaman, *Elazig*
Veli Yazisiz, *Antalya*
Yusuf Yilmaz, *Istanbul*
Ozlem Yilmaz, *Izmir*
Oya Yucel, *Istanbul*
Ilhami Yuksel, *Ankara*

United Kingdom

Nadeem Ahmad Afzal, *Southampton*
Navneet K Ahluwalia, *Stockport*
Yeng S Ang, *Lancashire*
Ramesh P Arasaradnam, *Coventry*
Ian Leonard Phillip Beales, *Norwich*
John Beynon, *Swansea*
Barbara Braden, *Oxford*
Simon Bramhall, *Birmingham*
Geoffrey Burnstock, *London*
Ian Chau, *Sutton*
Thean Soon Chew, *London*
Helen G Coleman, *Belfast*
Anil Dhawan, *London*
Sunil Dolwani, *Cardiff*
Piers Gatenby, *London*
Anil T George, *London*
Pasquale Giordano, *London*
Paul Henderson, *Edinburgh*
Georgina Louise Hold, *Aberdeen*
Stefan Hubscher, *Birmingham*
Robin D Hughes, *London*
Nusrat Husain, *Manchester*
Matt W Johnson, *Luton*
Konrad Koss, *Macclesfield*
Anastasios Koulaouzidis, *Edinburgh*
Simon Lal, *Salford*
John S Leeds, *Aberdeen*
JK K Limdi, *Manchester*
Hongxiang Liu, *Cambridge*
Michael Joseph McGarvey, *London*
Michael Anthony Mendall, *London*
Alexander H Mirnezami, *Southampton*
J Bernadette Moore, *Guildford*
Claudio Nicoletti, *Norwich*
Savvas Papagrigoriadis, *London*
Sylvia LF Pender, *Southampton*
David Mark Pritchard, *Liverpool*
James A Ross, *Edinburgh*
Kamran Rostami, *Worcester*
Xiong Z Ruan, *London*
Dina Tiniakos, *Newcastle upon Tyne*
Frank I Tovey, *London*
Dhiraj Tripathi, *Birmingham*
Vamsi R Velchuru, *Great Yarmouth*
Nicholas T Ventham, *Edinburgh*
Diego Vergani, *London*
Jack Westwood Winter, *Glasgow*
Terence Wong, *London*
Ling Yang, *Oxford*

United States

Daniel E Abbott, *Cincinnati*
Ghassan K Abou-Alfa, *New York*
Julian Abrams, *New York*
David William Adelson, *Los Angeles*
Jonathan Steven Alexander, *Shreveport*
Tauseef Ali, *Oklahoma City*
Mohamed R Ali, *Sacramento*
Rajagopal N Aravalli, *Minneapolis*
Hassan Ashktorab, *Washington*
Shashi Bala, *Worcester*
Charles F Barish, *Raleigh*
P Patrick Basu, *New York*
Robert L Bell, *Berkeley Heights*
David Bentrem, *Chicago*
Henry J Binder, *New Haven*
Joshua Bleier, *Philadelphia*
Wojciech Blonski, *Johnson City*
Kenneth Boorom, *Corvallis*
Brian Boulay, *Chicago*
Carla W Brady, *Durham*
Kyle E Brown, *Iowa City*
Adeel A Butt, *Pittsburgh*
Weibiao Cao, *Providence*
Andrea Castillo, *Cheney*
Fernando J Castro, *Weston*
Adam S Cheifetz, *Boston*
Xiaoxin Luke Chen, *Durham*
Ramsey Cheung, *Palo Alto*
Parimal Chowdhury, *Little Rock*
Edward John Ciaccio, *New York*
Dahn L Clemens, *Omaha*
Yingzi Cong, *Galveston*
Laura Iris Cosen-Binker, *Boston*
Joseph John Cullen, *Lowa*
Mark J Czaja, *Bronx*
Mariana D Dabeva, *Bronx*
Christopher James Damman, *Seattle*
Isabelle G De Plaen, *Chicago*
Punita Dhawan, *Nashville*
Hui Dong, *La Jolla*
Wael El-Rifai, *Nashville*
Sukru H Emre, *New Haven*
Paul Feuerstadt, *Hamden*
Josef E Fischer, *Boston*
Laurie N Fishman, *Boston*
Joseph Che Forbi, *Atlanta*
Temitope Foster, *Atlanta*
Amy E Foxx-Orenstein, *Scottsdale*
Daniel E Freedberg, *New York*
Shai Friedland, *Palo Alto*
Virgilio George, *Indianapolis*
Ajay Goel, *Dallas*
Oliver Grundmann, *Gainesville*
Stefano Guandalini, *Chicago*
Chakshu Gupta, *St. Joseph*
Grigoriy E Gurvits, *New York*
Xiaonan Han, *Cincinnati*
Mohamed Hassan, *Jackson*
Martin Hauer-Jensen, *Little Rock*
Koichi Hayano, *Boston*
Yingli Hee, *Atlanta*
Samuel B Ho, *San Diego*
Jason Ken Hou, *Houston*
Lifang Hou, *Chicago*
K-Qin Hu, *Orange*
Jamal A Ibdah, *Columbia*
Robert Thomas Jensen, *Bethesda*
Huanguang "Charlie" Jia, *Gainesville*
Rome Jutabha, *Los Angeles*
Andreas M Kaiser, *Los Angeles*
Avinash Kambadakone, *Boston*
David Edward Kaplan, *Philadelphia*
Randeep Kashyap, *Rochester*
Rashmi Kaul, *Tulsa*
Ali Keshavarzian, *Chicago*
Amir Maqbul Khan, *Marshall*
Nabeel Hasan Khan, *New Orleans*
Sahil Khanna, *Rochester*
Kusum K Kharbanda, *Omaha*
Hyun Sik Kim, *Pittsburgh*
Joseph Kim, *Duarte*
Jae S Kim, *Gainesville*
Miran Kim, *Providence*
Timothy R Koch, *Washington*
Burton I Korelitz, *New York*
Betsy Kren, *Minneapolis*
Shiu-Ming Kuo, *Buffalo*
Michelle Lai, *Boston*
Andreas Larentzakis, *Boston*
Edward Wolfgang Lee, *Los Angeles*
Daniel A Leffler, *Boston*
Michael Leitman, *New York*

World Journal of Gastroenterology

Contents

Weekly Volume 21 Number 11 March 21, 2015

World Journal of Gastroenterology
Volume 21 Number 11 March 21, 2015

Contents

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

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

PUBLICATION DATE
March 21, 2015

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3157

World J Gastroenterol 2015 March 21; 21(11): 3157-3165
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Update on the management of pancreatic cancer: Surgery is not enough

Daniel Ansari, Adam Gustafsson, Roland Andersson

Daniel Ansari, Adam Gustafsson, Roland Andersson, Department of Surgery, Clinical Sciences Lund, Lund University and Skåne University Hospital, SE-221 85 Lund, Sweden
Author contributions: Ansari D and Gustafsson A performed the literature search; Andersson R designed the study and revised the manuscript; all authors were involved in manuscript writing and read and approved the final manuscript.
Conflict-of-interest: The authors declare no conflict of interest.

Correspondence to: Roland Andersson, MD, PhD, Department of Surgery, Clinical Sciences Lund, Lund University and Skåne University Hospital, Getingevägen 4, SE-221 85 Lund, Sweden. roland.andersson@med.lu.se
Telephone: +46-46-172359
Received: November 12, 2014
Peer-review started: November 15, 2014
First decision: December 11, 2014
Revised: December 19, 2014
Accepted: February 5, 2015
Article in press: February 5, 2015
Published online: March 21, 2015

Abstract

Pancreatic ductal adenocarcinoma (PDAC) represents the fourth cause of death in cancer and has a 5-year survival of < 5%. Only about 15% of the patients present with a resectable PDAC with potential to undergo "curative" surgery. After surgery, local and systemic recurrence, is though very common. The median survival of resected patients with adjuvant chemotherapy after surgery is only 20-23 mo. This underscores the significant need to improve PDAC management strategies. Increased survival rate is dependent on new breakthroughs in our understanding of not at least tumor biology. The aim of this review is to update and comment on recent knowledge concerning PDAC biology and new diagnostics and treatment modalities. One fundamental approach to improve survival rates is by earlier and improved diagnosis of the disease. In recent years, novel blood-based biomarkers have emerged based on genetic, epigenetic and protein changes in PDAC with very promising results. For biomarkers to enter clinical practice they need to have been developed using adequate control groups and provide high sensitivity and specificity and by this identify patients at risk already in a pre-symptomatic stage. Another way to improve outcomes, is by employing neoadjuvant treatments thereby increasing the number of resectable cases. Novel systemic treatment regimes like FOLFIRINOX and nab-paclitaxel have demonstrated improvements in prolonging survival in advanced cases, but long-term survival is still scarce. The future improved understanding of PDAC biology will inevitably render new treatment options directed against both the cancer cells and the surrounding microenvironment.

Key words: Pancreatic cancer; Diagnosis; Genetics; Epigenetics; Proteomics; Surgery; Prognostic models; Chemotherapy; Stroma

Core tip: This review updates the current progress in the management of pancreatic cancer with focus on novel modes of diagnosis and treatment. New blood-based biomarkers for early detection based on genetic, epigenetic and protein changes in pancreatic cancer are discussed and new treatment strategies such as stromal depletion are highlighted. Pancreatic cancer is a systemic disease already at diagnosis and demands multimodal managements strategies in order to improve outcomes.

Ansari D, Gustafsson A, Andersson R. Update on the management of pancreatic cancer: Surgery is not enough. *World J Gastroenterol* 2015; 21(11): 3157-3165 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3157.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3157

INTRODUCTION

Pancreatic cancer is a devastating disease. The estimated number of deaths attributed to the disease is over 266000 worldwide every year[1]. It represents the fourth cause of death in cancer but may, by the time of 2030, have moved to the second place if no significant treatment advances are made[2]. The overall 5-year survival rate is < 5% and a majority of patients presents with inoperable and non-curable tumors. Pancreatic cancer refers to adenocarcinoma arising from the ductal epithelium in the exocrine part of the gland. Pancreatic ductal adenocarcinoma (PDAC) accounts for about 90% and is the form mainly addressed in this review.

Much effort has focused to find evidence for causative risk factors but reasonably few are widely established. Tobacco smoking, obesity, longstanding diabetes, family history of PDAC and chronic pancreatitis are risk factors[3]. Overweight (BMI > 25) during adult life is associated with increased risk and earlier onset of the disease[4]. Diabetes mellitus has recently been presented as an independent risk factor and increased risk associated with disease duration, although metformin reduces this risk[5]. Still this connection is somewhat complicated since studies have reported that diabetes might be an early indication of underlying PDAC[6]. Several studies have also provided evidence for the connection between chronic pancreatitis and PDAC. This usually occurs with a delay of one or two decades between the chronic pancreatitis and PDAC[7].

Lack of early biomarkers together with the silent nature of the disease, consequently rendering diagnosis in late, unresectable stages, contributes to the high death rate (Figure 1). To make progress and improve long-term survival, advances in several areas such as biomarkers for early diagnosis, optimized surgical strategies, and new types of systemic therapy are required (Figure 2). This review aims to update and comment on recent knowledge concerning PDAC management. PDAC is a complex disease with different clinical and pathological phenotypes and hence requires multiple modes of intervention to control disease progression.

PATHOPHYSIOLOGY

In 1976 it was suggested that ductal papillary hyperplasia, atypia and carcinoma *in situ* morphologically appear as precursor lesions. This was based on examinations of histological samples from 227 cases of human PDAC[8]. Numerous studies have since observed

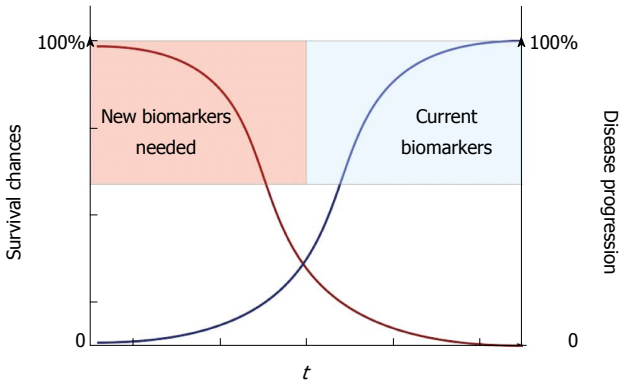


Figure 1 Pancreatic ductal adenocarcinoma and biomarkers. Modified from Herreros-Villanueva *et al*[13].

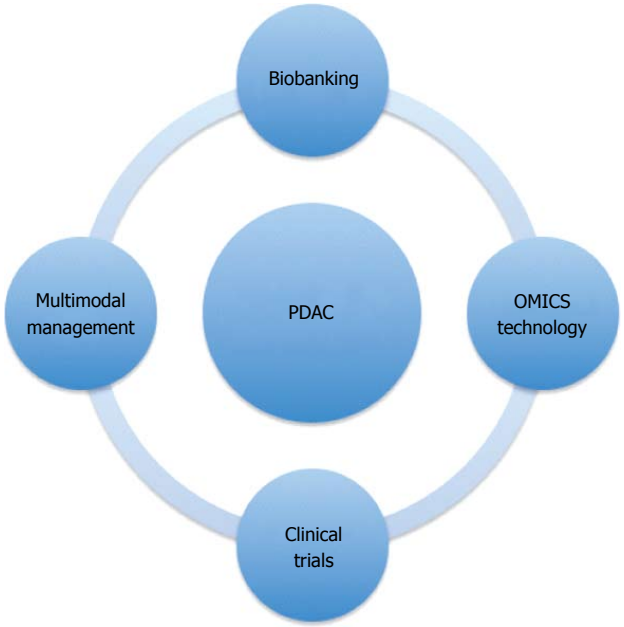


Figure 2 Different potential ways to improve the management of pancreatic ductal adenocarcinoma. PDAC: Pancreatic ductal adenocarcinoma.

and graded histological tissue of these lesions, called pancreatic intraepithelial neoplasia (PanIN) and provided confirmation for a common pathogenesis with similar profiles and progress leading to PDAC[9]. Transformation from a dysplastic epithelium to dysplasia and ultimately invasive carcinoma is parallel with the accumulation of mutations[10]. There have been focused efforts to genetically map out PanINs and to discover collective mutations in order to find clinical useful biomarkers. Commonly found mutations include activation of *KRAS* oncogene, inactivation of tumor suppressor genes *CDKN2A*/*INK4A*, *TP53* and *SMAD4*[11].

KRAS mutation is a critical event and is the most frequently found oncogene in PDAC[12]. This mutation induces degradation of the tumor suppressor protein p53-SNAIL complex and is present in a higher degree in more advanced stages of PanIN[13]. KRAS has been considered for diagnostic purposes, but has a lack in both sensitivity and specificity[14]. The inactivation

of CDKN2A results in loss of the p16 protein, a cell cycle regulator which generates increased cellular proliferation[10]. This mutation occurs in PDAC, but is also seen in several other malignancies. Abnormal TP53 adds genomic instability by permitting cells to bypass DNA checkpoints and this way avoid apoptotic signals. This mutation occurs in approximately 50%-75% of PDAC[10]. Tumor suppressor gene *SMAD4* is mutated in about 55% of PDAC[15]. SMAD4 occurs in various cancers but with a higher sensitivity and specificity in PDAC. It often appears in late stages and loss of SMAD4 expression correlates with a poorer prognosis and potential metastasis[16]. However almost all PDAC contain one of these four mentioned mutations[17].

The stroma, a hallmark for PDAC, is the structural framework surrounding the tumor. The PDAC cells interact with the stromal cells in order to create a unique microenvironment to facilitate tumor progression by promoting tumor growth, local hypoxia and preventing the effects of chemotherapy[18]. Signals to stromal cells, from PDAC cells, modify the structure of the extracellular matrix (ECM) composed of collagens, noncollagen glycoproteins, glycosaminoglycans, growth factors and proteoglycans. These signals (mostly being proinflammatory) increase the recruitment of inflammatory cells, but also stimulate proliferation of fibroblasts, specifically pancreatic stellate cells (PSCs)[19]. PSCs contribute to the excess fibrosis formation around the tumor and promote tumor progression[20]. PSCs seem to facilitate immune evasion, chemoresistance and the recurrence of PDAC, as verified both by in vitro and in vivo studies[21].

SERUM CARBOHYDRATE ANTIGEN 19-9

Currently, carbohydrate antigen 19-9 (CA19-9) is the only biomarker used in the routine management of PDAC. CA19-9 is a sialylated Lewis blood group antigen that can be quantitatively measured in serum, but its use is limited to monitoring response to therapy, not as a diagnostic marker. The sensitivity and specificity of CA19-9 is about 80% for PDAC diagnosis[22]. CA19-9 is elevated in benign conditions, such as cholestasis and chronic pancreatitis, which hampers its specificity for PDAC. Moreover, approximately 5%-10% of the population do not express Lewis antigens. Thus, there is great interest in identifying and developing new markers for PDAC to help detect early stage PDAC and its precursors. The development of useful biomarkers requires well-designed studies that evaluate marker performance in the appropriate clinical setting where the marker will be required. Because blood is more accessible and less invasive to achieve than tissue, the optimal screening strategy for PDAC will likely involve the development of highly accurate and inexpensive blood biomarkers, followed by a second-level, imaging-based test to confirm a positive biomarker result. The recent developments in the detection of biomarkers, such as point mutations, DNA methylation patterns, microRNAs (miR), and proteins in blood from patients with PDAC, have opened up new avenues of research for the early diagnosis and treatment of this lethal disease.

EARLY DETECTION

Detecting resectable disease is the first step in the fight against PDAC[23]. Biomarkers that could increase the proportion of patients that are candidates for surgery could potentially improve survival rates. Of the entire PDAC cohort, only 10%-15% have localized disease. According to the American Cancer Society[24], the 5-year survival is 24% for resectable PDAC, 9% for locally advanced PDAC, and 2% for metastatic PDAC. However, the 5-year survival rate in resectable PDAC is about 50% in tumors < 2 cm and close to 100% in tumors < 1 cm[25], indicating that even earlier detection can improve survival. A recent study[26] modeled the benefits of early diagnosis of PDAC using a blood-based biomarker signature. It was found that the cost-effectiveness depended on the incidence of PDAC within the population and that certain high-risk groups, such as familial cases and new-onset diabetes mellitus, could be screened at an acceptable cost for the society.

Genetic and epigenetic markers

The genetic alterations occurring during PDAC development have been extensively studied. A comprehensive genetic analysis of 24 PDAC found an average of 63 genetic alterations, the majority of which are point mutations[27]. Mathematical analyses of tumor DNA sequence data suggest a broad time window of opportunity for early detection to prevent deaths from metastatic PDAC[28]. One recent study showed that mutations in *KRAS* and *TP53* can be detected using genomic DNA from exosomes derived from serum from patients with PDAC, providing a novel diagnostic tool for PDAC[29].

PDAC is an epigenetic disease in addition to being a genetic disease. Epigenetic biomarkers, such as DNA methylation and microRNAs, may be utilized for diagnosis of PDAC. One study identified two novel genes, BNC1 and ADAMTS1, that showed a high frequency of methylation in PDAC, up to 100% in PanIN-3 and 97% in stage I invasive cancers[30]. These alterations could be detected in serum samples from patients with PDAC. The sensitivity was 79% for BNC1 and 48% for ADAMTS1, whereas specificity was 89% for BNC1 and 92% for ADAMTS1. Overall sensitivity using both markers was 81% and specificity was 85% for PDAC diagnosis. MicroRNAs are small non-coding RNA molecules, about 22 nucleotides, that regulate stability and translation of messenger RNAs. One study investigated differences in microRNA expression in whole blood between patients with PDAC, chronic pancreatitis, and healthy participants[31]. A total of 38

microRNAs were significantly dysregulated in patients with PDAC as compared to controls. Two diagnostic panels were constructed comprising 4 microRNAs in index Ⅰ (miR-145, miR-150, miR-223, miR-636) and 10 in index Ⅱ (miR-26b, miR-34a, miR-122, miR-126*, miR-145, miR-150, miR-223, miR-505, miR-636, miR-885.5p). The performance of the panels was validated in patients with stage ⅠA-ⅡB PDAC, with an index Ⅰ area under the curve (AUC) of 0.80; index Ⅰ and CA19-9 AUC of 0.83; index Ⅱ AUC of 0.91; and index Ⅱ and CA19-9 AUC of 0.91.

Protein markers

Proteomics is defined as the large-scale study of proteins, particularly their abundances, functions, structures, and interacting partners. In the field of proteomics, antibody-based technologies and mass spectrometry are the most common techniques used for biomarker discovery. One study used a recombinant antibody microarray platform, targeting mainly immunoregulatory proteins, in sera from patients with resectable PDAC, chronic pancreatitis, autoimmune pancreatitis, and healthy controls[32]. The results identified serum portraits distinguishing PDAC from chronic pancreatitis (AUC: 0.86), autoimmune pancreatitis (AUC: 0.99) and healthy controls (AUC: 0.95). A 25-serum biomarker signature discriminating PDAC from the combined group of chronic pancreatitis, autoimmune pancreatitis, and healthy controls was determined that had a high diagnostic yield with an AUC of 0.88. Another study applied protein deep sequencing using high-definition mass spectrometry (HDMS^{E}) to serum samples from patients with resectable PDAC, benign pancreatic disease, and healthy controls[33]. A global protein expression comparison of the three study groups was made using label-free quantification and bioinformatic analyses. More than 71000 features were detected within the data revealing 715 unique proteins. Two-way unsupervised hierarchical clustering identified 134 proteins that successfully classified PDAC patients from the controls, and found 40 proteins that showed a significant up-regulation in the PDAC group. This discrimination reliability was further confirmed by principal component analysis. Disease link associations could be made for BAZ2A, CDK13, DAPK1, DST, EXOSC3, INHBE, KAT2B, KIF20B, SMC1B, and SPAG5, by pathway network linkages to TP53, the most frequently altered tumor suppressor in PDAC.

CHALLENGES IN BIOMARKER TRANSLATION

PDAC is generally diagnosed when the disease is at an advanced stage. As a consequence, the majority of samples available for biomarker discovery come from patients with advanced disease. One potential solution is to take advantage of prospective cohort studies, such as European Prospective Investigation into Cancer and Nutrition. In such cohorts, the performance of the biomarkers in the months or even years prior to PDAC diagnosis can be evaluated. Obstructive jaundice is a common complication of PDAC, but few studies include patients with benign causes of obstructive jaundice in their evaluation of tumor markers. Furthermore, many candidate markers of PDAC have been found to clearly distinguish PDAC from healthy controls but fail to distinguish them from chronic pancreatitis. This may be due to the fact that there is an inflammatory component in PDAC and several markers may be shared by both conditions. These observations suggest that choice of adequate controls is important identify the most cancer-specific biomarkers.

TREATMENT OF RESECTABLE PDAC

Surgical resection with radical intent remains the only potential curative treatment option today. PDAC is staged according to the tumor-node-metastasis classification, which categorizes patients into 3 stages: resectable, locally advanced, and metastatic disease[34]. Contrast-enhanced computed tomographyis an established method for staging and provides around 80% accuracy concerning resectability[35]. Magnetic resonance imaging, and endoscopic ultrasound are valuable modalities if diagnostic difficulties persist after[36]. Tumor size, vascular involvement, age and comorbidity are to be considered in the preoperative staging and decision to proceed with surgical resection[37]. A pancreatoduodenectomy (Whipple), distal pancreatectomy or a total pancreatectomy is usually performed, depending on tumor location and type. The hospital mortality rate following surgical resection may be below 2%, while overall morbidity remains up to 60%[38]. Complications include delayed gastric emptying, wound infections, abdominal abscess, and not at least pancreatic fistulas where grades B and C are most problematic[39-41]. It should be emphasized that an uncomplicated postoperative course is associated with a better long-term survival[42]. With regard to endocrine status, progression of disease has a greater impact than the surgical intervention, and diabetes mellitus (especially new-onset) may often be resolved by resection of the pancreatic tumor[43,44].

Surgical results tend to correlate strongly with both institutional and surgical volumes. Several studies have demonstrated significantly decreased mortality and morbidity at high volume centers[38,45,46]. This association also applies to long-term survival even after corrected perioperative mortality[47]. This association between hospital volume and improved results is believed to be multifactorial. One suggested explanation is more experienced medical staff with ability to detect and treat complications at an earlier stage, which may improve the outcome[47]. Superior surgical performance due to higher operation frequency may improve both short- and long-term outcome. Since the treatment is multimodal and also

includes adjuvant therapy, the oncologists are likely to improve their results with increased experience as well. Following the implementation of centralization, the 2-year survival among resected patients has increased with over 10% and is considered a great way to improve surgical outcome[46].

The fast-track (FT) concept is a standardized and coordinated perioperative protocol to handle surgical patients. This method intends to reduce surgical stress, accelerate postoperative recovery and improve safety[48]. Several studies have displayed the effect of implementation of the FT concept in pancreaticoduodenectomy[49-51]. It evidently correlates with early recovery and reduces morbidities, such as delayed gastric emptying[52]. The length of hospital stay will also be significantly shortened by 2-6 d[49]. The FT concept is also beneficial from an economical point of view with reduced hospital costs as demonstrated by Kennedy *et al*[49].

Adjuvant chemotherapy is recommended after pancreatic resection for PDAC based on several randomized controlled trials, including GITSG[53], ESPAC-1[54], ESPAC-3[55], RTOG-9704[56] and CONKO-001[57]. Whether neoadjuvant therapy is superior to adjuvant therapy for PDAC is controversial. The proposed upside of neoadjuvant therapy is early treatment of micrometastases, potential downstaging of borderline resectable tumors, decreasing the percentage of positive lymph nodes, enhanced chemotherapeutic penetrance due to improved vascularization and a higher percentage of accomplished R0 resections[58,59]. It has also been hypothesized that patients receiving neoadjuvant treatment stand a better chance of completing the full multimodal treatment[58]. Another suggested advantage is the selection and the ability to exclude patients developing progressing and metastatic disease, thus avoiding unnecessary surgery[60]. A neoadjuvant treatment requires histological confirmation and this might further increase the detection of patients unlikely to benefit from resection[59,61]. Neoadjuvant therapy is associated with delayed resection, and thus theoretically a possible risk of tumor progression[62]. Previous studies have concluded gemcitabine with radiotherapy as the most effective neoadjuvant treatment with the best effect on overall survival[58,63]. A population based study between 1987-2006 demonstrated a 12 mo survival advantage and lower rate of lymph node positivity between neoadjuvant and adjuvant treatment[59]. Still a recent meta-analysis did not conclude a significant effect on overall survival among resectable patients after receiving neoadjuvant or adjuvant treatments approaches. However, there was a small though not statistically significant effect on survival benefits for neoadjuvant as compared to adjuvant treatment[62]. Thus, with better chemotherapy (see *e.g.*, below) there might be a case for neoadjuvant treatment future on.

Lymph node involvement and resection margin status (R0/R1) remain important prognostic factors after surgical resection for PDAC[64,65]. Artificial neural networks (ANNs) represent a non-linear pattern recognition technique that simulates the analytic learning processes of the human brain. Thus, adapting to changing environment through continuous learning via trial and error, ANN is supplied with information from various sources to detect complicated patterns. The benefit of ANNs is to automatically detect relationships between "inputs" to the network and the "output" by integrating all possible connections between the input variables[66]. One study displayed ANN as a tool in prediction of survival after surgical resection[67]. This was achieved by including clinical risk factors in order to create a survival model. The C-index of ANN was 0.79 compared to Cox regression 0.67 thus, indicating ANNs superior predictive ability. Biomarkers, if available, should also be taken into consideration for prognostic prediction. Unfortunately, there are no validated biomarkers to predict the clinical course[68]. In the future, however promising investigational biomarkers may be incorporated into ANNs in order to improve prognostic performance and help inform clinical decision-making.

TREATMENT OF UNRESECTABLE PDAC

Most PDAC patients present with locally advanced or metastatic disease and are consequently not suitable for surgery. Single therapy with gemcitabine has generally been regarded as first-line therapy for advanced PDAC over the almost two last decades[69]. Numerous patients do not respond to gemcitabine therapy due to chemoresistance[70]. The variation in chemoresistance between individuals is partly attributed the human equilibrative nucleoside transporter-1 (hENT-1)[71]. This transporter is responsible for the intracellular uptake of gemcitabine and studies have demonstrated a relationship between longer median survival and high levels of hENT-1[72,73]. Clinical data have revealed that a majority of patients do not have a high hENT-1 expression and thus an expected decreased response to gemcitabine treatment[70]. To determine expression of hENT-1 and individualize gemcitabine treatment strategies could be both cost efficient and avoid unnecessary treatment, but needs further investigations and ethical considerations[74].

Few combinations of gemcitabine with other cytotoxic agents have yet provided any significantly prolonged overall survival rates[75,76]. Gemcitabine treatment combinations also often involved more toxic effects compared with gemcitabine administration as monotherapy[76]. FOLFIRINOX (*i.e.*, a combination of oxaliplatin, irinotecan, fluorouracil, and leucovorin) has been proved to prolong both survival (from 6.8 mo with gemcitabine alone to 11.1 mo with FOLFIRINOX) and progression free time (3.4 to 6.4 mo) for metastatic patients with good performance status (ECOG 0-1). This treatment does, however, also carry significantly more adverse effects and is only applicable in a

selected group of patients.

Another interesting treatment regime is albumin-bound paclitaxel (nab-paclitaxel) in combination with gemcitabine. Preclinical studies have showed synergistic effects and increased chemotherapeutic levels in the tumor[77]. SPARC has affinity for albumin, and could thus theoretically facilitate delivery of paclitaxel to the tumor stroma. The stroma is thereby broken down (stromal depletion) and tumor cells close in on each other with resulting increased tumor vascularity[78]. Conversely, a study in mice reported that SPARC deficiency did not alter levels of paclitaxel in the tumors[79]. However, recently published data from a phase 3 study reported improved survival, in patients with advanced PDAC treated with gemcitabine and nab-paclitaxel, compared to gemcitabine as a single therapy[80]. The toxicity profile includes neutropenia and neuropathy, but is not worse when compared to gemcitabine treatment alone. Consequently, nab-paclitaxel has become a therapeutic agent with potential to be part of future PDAC treatments[81].

Secreted protein acidic and rich in cysteine (SPARC) or osteonectin is a calcium-binding glycoprotein frequently expressed by stromal cells surrounding the tumor. The biological role of SPARC involves cell-to-matrix interactions, including cell migration and tissue remodeling, mainly in tissues with high ECM turnover. SPARC is tumor-specific and could act as a tumor suppressor, but also have preinvasive characteristics that possibly increase efficiency of chemotherapy[82]. High SPARC expression, as in the pancreatic tumor stroma, has emerged as a clinical and prognostic factor for PDAC[83,84]. Other strategies to target the tumor stroma involve reverting the activated PSC to its quiescent state. It has been found that PSCs express high levels of the vitamin D and that treatment with calcipotriol, a vitamin receptor D ligand, depletes the stroma and increases intratumoral delivery of gemcitabine in mouse models of PDAC[85]. Other therapies aiming to target and block the interaction between tumor cells and PSCs are also being tested, hopefully further improving the poor prognosis of PDAC[19]. These studies provide novel approaches for targeting the pancreatic tumor stroma and suggest that stromal depletion could become the mainstay of PDAC therapy in the future.

CONCLUSION

Many new genetic, epigenetic, and proteomic tumor markers are under investigation for the non-invasive diagnosis of early-stage PDAC. For a cost-effective diagnostic test, screening should be applied in high-risk individuals and the test must have high sensitivity and specificity. The use of adequate controls is mandatory during discovery and validation steps in the biomarker development phase. Future biomarkers need to identify patients already in a pre-symptomatic stage. There are strong data that centralization of surgical services improves outcomes after PDAC surgery. Principles for the fast-track concept are now well recognized as both safe and applying best possible available evidence-based and structured care. The benefits of neoadjuvant treatment strategies warrant further investigation before being fully implemented. ESPAC-5, an ongoing study with comparisons between immediate surgical explorations to neoadjuvant therapy, will hopefully further determine the potential role of neoadjuvant therapy. Increased understanding of the stromal compartment of PDAC, both in terms of tumor progression and as a defense barrier against innate immunity and chemotherapy, will be of outmost relevance for the development of future treatments. Even though improved diagnostics and neoadjuvant/preoperative chemotherapy might increase the number of resectable patients, PDAC is a systemic disease already at the time of diagnosis and surgery alone is not enough.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Rahib L**, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913-2921 [PMID: 24840647 DOI: 10.1158/0008-5472.CAN-14-0155]

3 **Hassan MM**, Bondy ML, Wolff RA, Abbruzzese JL, Vauthey JN, Pisters PW, Evans DB, Khan R, Chou TH, Lenzi R, Jiao L, Li D. Risk factors for pancreatic cancer: case-control study. *Am J Gastroenterol* 2007; **102**: 2696-2707 [PMID: 17764494 DOI: 10.1111/j.1572-0241.2007.01510.x]

4 **Li D**, Morris JS, Liu J, Hassan MM, Day RS, Bondy ML, Abbruzzese JL. Body mass index and risk, age of onset, and survival in patients with pancreatic cancer. *JAMA* 2009; **301**: 2553-2562 [PMID: 19549972 DOI: 10.1001/jama.2009.886]

5 **Wang Z**, Lai ST, Xie L, Zhao JD, Ma NY, Zhu J, Ren ZG, Jiang GL. Metformin is associated with reduced risk of pancreatic cancer in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. *Diabetes Res Clin Pract* 2014; **106**: 19-26 [PMID: 24837144 DOI: 10.1016/j.diabres.2014.04.007]

6 **Chari ST**, Leibson CL, Rabe KG, Ransom J, de Andrade M, Petersen GM. Probability of pancreatic cancer following diabetes: a population-based study. *Gastroenterology* 2005; **129**: 504-511 [PMID: 16083707 DOI: 10.1016/j.gastro.2005.05.007]

7 **Raimondi S**, Lowenfels AB, Morselli-Labate AM, Maisonneuve P, Pezzilli R. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. *Best Pract Res Clin Gastroenterol* 2010; **24**: 349-358 [PMID: 20510834 DOI: 10.1016/j.bpg.2010.02.007]

8 **Cubilla AL**, Fitzgerald PJ. Morphological lesions associated with human primary invasive nonendocrine pancreas cancer. *Cancer Res* 1976; **36**: 2690-2698 [PMID: 1277176]

9 **Bardeesy N**, DePinho RA. Pancreatic cancer biology and genetics. *Nat Rev Cancer* 2002; **2**: 897-909 [PMID: 12459728 DOI: 10.1038/nrc949]

10 **Hidalgo M**. New insights into pancreatic cancer biology. *Ann Oncol* 2012; **23** Suppl 10: x135-x138 [PMID: 22987949 DOI: 10.1093/annonc/mds313]

11 **Aho U**, Zhao X, Löhr M, Andersson R. Molecular mechanisms of pancreatic cancer and potential targets of treatment. *Scand J Gastroenterol* 2007; **42**: 279-296 [PMID: 17354106 DOI: 10.1080/

00365520601106384]
12 **Klimstra DS**, Longnecker DS. K-ras mutations in pancreatic ductal proliferative lesions. *Am J Pathol* 1994; **145**: 1547-1550 [PMID: 7992857]
13 **Herreros-Villanueva M**, Gironella M, Castells A, Bujanda L. Molecular markers in pancreatic cancer diagnosis. *Clin Chim Acta* 2013; **418**: 22-29 [PMID: 23305796 DOI: 10.1016/j.cca.2012.12.025]
14 **Herreros-Villanueva M**, Rodrigo M, Claver M, Muñiz P, Lastra E, García-Girón C, Coma del Corral MJ. KRAS, BRAF, EGFR and HER2 gene status in a Spanish population of colorectal cancer. *Mol Biol Rep* 2011; **38**: 1315-1320 [PMID: 20563851 DOI: 10.1007/s11033-010-0232-x]
15 **Iacobuzio-Donahue CA**, Song J, Parmiagiani G, Yeo CJ, Hruban RH, Kern SE. Missense mutations of MADH4: characterization of the mutational hot spot and functional consequences in human tumors. *Clin Cancer Res* 2004; **10**: 1597-1604 [PMID: 15014009]
16 **Blackford A**, Serrano OK, Wolfgang CL, Parmigiani G, Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Eshleman JR, Goggins M, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Cameron JL, Olino K, Schulick R, Winter J, Herman JM, Laheru D, Klein AP, Vogelstein B, Kinzler KW, Velculescu VE, Hruban RH. SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res* 2009; **15**: 4674-4679 [PMID: 19584151 DOI: 10.1158/1078-0432.CCR-09-0227]
17 **Maitra A**, Hruban RH. Pancreatic cancer. *Annu Rev Pathol* 2008; **3**: 157-188 [PMID: 18039136 DOI: 10.1146/annurev.pathmechdis.3.121806.154305]
18 **Hamada S**, Masamune A, Shimosegawa T. Alteration of pancreatic cancer cell functions by tumor-stromal cell interaction. *Front Physiol* 2013; **4**: 318 [PMID: 24198790 DOI: 10.3389/fphys.2013.00318]
19 **Lunardi S**, Muschel RJ, Brunner TB. The stromal compartments in pancreatic cancer: are there any therapeutic targets? *Cancer Lett* 2014; **343**: 147-155 [PMID: 24141189 DOI: 10.1016/j.canlet.2013.09.039]
20 **Haqq J**, Howells LM, Garcea G, Metcalfe MS, Steward WP, Dennison AR. Pancreatic stellate cells and pancreas cancer: current perspectives and future strategies. *Eur J Cancer* 2014; **50**: 2570-2582 [PMID: 25091797 DOI: 10.1016/j.ejca.2014.06.021]
21 **Xu Z**, Pothula SP, Wilson JS, Apte MV. Pancreatic cancer and its stroma: a conspiracy theory. *World J Gastroenterol* 2014; **20**: 11216-11229 [PMID: 25170206 DOI: 10.3748/wjg.v20.i32.11216]
22 **Goonetilleke KS**, Siriwardena AK. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. *Eur J Surg Oncol* 2007; **33**: 266-270 [PMID: 17097848 DOI: 10.1016/j.ejso.2006.10.004]
23 **Chari ST**. Detecting early pancreatic cancer: problems and prospects. *Semin Oncol* 2007; **34**: 284-294 [PMID: 17674956 DOI: 10.1053/j.seminoncol.2007.05.005]
24 **Siegel R**, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786 DOI: 10.3322/caac.21208]
25 **Ansari D**, Aronsson L, Sasor A, Welinder C, Rezeli M, Marko-Varga G, Andersson R. The role of quantitative mass spectrometry in the discovery of pancreatic cancer biomarkers for translational science. *J Transl Med* 2014; **12**: 87 [PMID: 24708694 DOI: 10.1186/1479-5876-12-87]
26 **Ghatnekar O**, Andersson R, Svensson M, Persson U, Ringdahl U, Zeilon P, Borrebaeck CA. Modelling the benefits of early diagnosis of pancreatic cancer using a biomarker signature. *Int J Cancer* 2013; **133**: 2392-2397 [PMID: 23649606 DOI: 10.1002/ijc.28256]
27 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]
28 **Yachida S**, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, Velculescu VE, Kinzler KW, Vogelstein B, Iacobuzio-Donahue CA. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010; **467**: 1114-1117 [PMID: 20981102 DOI: 10.1038/nature09515]
29 **Kahlert C**, Melo SA, Protopopov A, Tang J, Seth S, Koch M, Zhang J, Weitz J, Chin L, Futreal A, Kalluri R. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. *J Biol Chem* 2014; **289**: 3869-3875 [PMID: 24398677 DOI: 10.1074/jbc.C113.532267]
30 **Yi JM**, Guzzetta AA, Bailey VJ, Downing SR, Van Neste L, Chiappinelli KB, Keeley BP, Stark A, Herrera A, Wolfgang C, Pappou EP, Iacobuzio-Donahue CA, Goggins MG, Herman JG, Wang TH, Baylin SB, Ahuja N. Novel methylation biomarker panel for the early detection of pancreatic cancer. *Clin Cancer Res* 2013; **19**: 6544-6555 [PMID: 24088737 DOI: 10.1158/1078-0432.CCR-12-3224]
31 **Schultz NA**, Dehlendorff C, Jensen BV, Bjerregaard JK, Nielsen KR, Bojesen SE, Calatayud D, Nielsen SE, Yilmaz M, Holländer NH, Andersen KK, Johansen JS. MicroRNA biomarkers in whole blood for detection of pancreatic cancer. *JAMA* 2014; **311**: 392-404 [PMID: 24449318 DOI: 10.1001/jama.2013.284664]
32 **Wingren C**, Sandström A, Segersvärd R, Carlsson A, Andersson R, Löhr M, Borrebaeck CA. Identification of serum biomarker signatures associated with pancreatic cancer. *Cancer Res* 2012; **72**: 2481-2490 [PMID: 22589272 DOI: 10.1158/0008-5472.CAN-11-2883]
33 **Ansari D**, Andersson R, Bauden MP, Andersson B, Connolly JB, Welinder C, Sasor A, Marko-Varga G. Protein deep sequencing applied to biobank samples from patients with pancreatic cancer. *J Cancer Res Clin Oncol* 2015; **141**: 369-380 [PMID: 25216700 DOI: 10.1007/s00432-014-1817-x]
34 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]
35 **Karmazanovsky G**, Fedorov V, Kubyshkin V, Kotchatkov A. Pancreatic head cancer: accuracy of CT in determination of resectability. *Abdom Imaging* 2005; **30**: 488-500 [PMID: 15759205 DOI: 10.1007/s00261-004-0279-z]
36 **Klapman J**, Malafa MP. Early detection of pancreatic cancer: why, who, and how to screen. *Cancer Control* 2008; **15**: 280-287 [PMID: 18813195]
37 **Hartwig W**, Werner J, Jäger D, Debus J, Büchler MW. Improvement of surgical results for pancreatic cancer. *Lancet Oncol* 2013; **14**: e476-e485 [PMID: 24079875 DOI: 10.1016/S1470-2045(13)70172-4]
38 **Ansari D**, Williamsson C, Tingstedt B, Andersson B, Lindell G, Andersson R. Pancreaticoduodenectomy--the transition from a low- to a high-volume center. *Scand J Gastroenterol* 2014; **49**: 481-484 [PMID: 24255988 DOI: 10.3109/00365521.2013.847116]
39 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542]
40 **Bassi C**, Dervenis C, Butturini G, Fingerhut A, Yeo C, Izbicki J, Neoptolemos J, Sarr M, Traverso W, Buchler M. Postoperative pancreatic fistula: an international study group (ISGPF) definition. *Surgery* 2005; **138**: 8-13 [PMID: 16003309 DOI: 10.1016/j.surg.2005.05.001]
41 **Winter JM**, Cameron JL, Campbell KA, Arnold MA, Chang DC, Coleman J, Hodgin MB, Sauter PK, Hruban RH, Riall TS, Schulick RD, Choti MA, Lillemoe KD, Yeo CJ. 1423 pancreaticoduodenectomies for pancreatic cancer: A single-institution experience. *J Gastrointest Surg* 2006; **10**: 1199-1210; discussion 1210-1211 [PMID: 17114007 DOI: 10.1016/j.gassur.2006.08.018]
42 **Howard TJ**, Krug JE, Yu J, Zyromski NJ, Schmidt CM, Jacobson LE, Madura JA, Wiebke EA, Lillemoe KD. A margin-negative R0 resection accomplished with minimal postoperative complications is the surgeon's contribution to long-term survival in pancreatic cancer. *J Gastrointest Surg* 2006; **10**: 1338-1345; discussion 1345-1346 [PMID: 17175452 DOI: 10.1016/j.gassur.2006.09.008]

43 **Bartosch-Härlid A**, Andersson R. Diabetes mellitus in pancreatic cancer and the need for diagnosis of asymptomatic disease. *Pancreatology* 2010; **10**: 423-428 [PMID: 20720443 DOI: 10.1159/000264676]

44 **He XY**, Li JF, Yao WY, Yuan YZ. Resolution of new-onset diabetes after radical pancreatic resection predicts long-term survival in patients with pancreatic ductal cell adenocarcinoma. *Ann Surg Oncol* 2013; **20**: 3809-3816 [PMID: 23943021 DOI: 10.1245/s10434-013-3095-2]

45 **Ghaferi AA**, Birkmeyer JD, Dimick JB. Hospital volume and failure to rescue with high-risk surgery. *Med Care* 2011; **49**: 1076-1081 [PMID: 22002649 DOI: 10.1097/MLR.0b013e3182329b97]

46 **Lemmens VE**, Bosscha K, van der Schelling G, Brenninkmeijer S, Coebergh JW, de Hingh IH. Improving outcome for patients with pancreatic cancer through centralization. *Br J Surg* 2011; **98**: 1455-1462 [PMID: 21717423 DOI: 10.1002/bjs.7581]

47 **Birkmeyer JD**, Sun Y, Wong SL, Stukel TA. Hospital volume and late survival after cancer surgery. *Ann Surg* 2007; **245**: 777-783 [PMID: 17457171 DOI: 10.1097/01.sla.0000252402.33814.dd]

48 **Kehlet H**, Wilmore DW. Multimodal strategies to improve surgical outcome. *Am J Surg* 2002; **183**: 630-641 [PMID: 12095591]

49 **Kennedy EP**, Grenda TR, Sauter PK, Rosato EL, Chojnacki KA, Rosato FE, Profeta BC, Doria C, Berger AC, Yeo CJ. Implementation of a critical pathway for distal pancreatectomy at an academic institution. *J Gastrointest Surg* 2009; **13**: 938-944 [PMID: 19190968 DOI: 10.1007/s11605-009-0803-0]

50 **Balzano G**, Zerbi A, Braga M, Rocchetti S, Beneduce AA, Di Carlo V. Fast-track recovery programme after pancreatico-duodenectomy reduces delayed gastric emptying. *Br J Surg* 2008; **95**: 1387-1393 [PMID: 18844251 DOI: 10.1002/bjs.6324]

51 **Kennedy EP**, Rosato EL, Sauter PK, Rosenberg LM, Doria C, Marino IR, Chojnacki KA, Berger AC, Yeo CJ. Initiation of a critical pathway for pancreaticoduodenectomy at an academic institution--the first step in multidisciplinary team building. *J Am Coll Surg* 2007; **204**: 917-923; discussion 923-924 [PMID: 17481510 DOI: 10.1016/j.jamcollsurg.2007.01.057]

52 **Pillai SA**, Palaniappan R, Pichaimuthu A, Rajendran KK, Sathyanesan J, Govindhan M. Feasibility of implementing fast-track surgery in pancreaticoduodenectomy with pancreaticogastrostomy for reconstruction--a prospective cohort study with historical control. *Int J Surg* 2014; **12**: 1005-1009 [PMID: 25014648 DOI: 10.1016/j.ijsu.2014.07.002]

53 **Kalser MH**, Ellenberg SS. Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection. *Arch Surg* 1985; **120**: 899-903 [PMID: 4015380]

54 **Neoptolemos JP**, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, Beger H, Fernandez-Cruz L, Dervenis C, Lacaine F, Falconi M, Pederzoli P, Pap A, Spooner D, Kerr DJ, Büchler MW. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med* 2004; **350**: 1200-1210 [PMID: 15028824 DOI: 10.1056/NEJMoa032295]

55 **Neoptolemos JP**, Stocken DD, Bassi C, Ghaneh P, Cunningham D, Goldstein D, Padbury R, Moore MJ, Gallinger S, Mariette C, Wente MN, Izbicki JR, Friess H, Lerch MM, Dervenis C, Oláh A, Butturini G, Doi R, Lind PA, Smith D, Valle JW, Palmer DH, Buckels JA, Thompson J, McKay CJ, Rawcliffe CL, Büchler MW. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. *JAMA* 2010; **304**: 1073-1081 [PMID: 20823433 DOI: 10.1001/jama.2010.1275]

56 **Regine WF**, Winter KA, Abrams RA, Safran H, Hoffman JP, Konski A, Benson AB, Macdonald JS, Kudrimoti MR, Fromm ML, Haddock MG, Schaefer P, Willett CG, Rich TA. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. *JAMA* 2008; **299**: 1019-1026 [PMID: 18319412 DOI: 10.1001/jama.299.9.1019]

57 **Oettle H**, Neuhaus P, Hochhaus A, Hartmann JT, Gellert K, Ridwelski K, Niedergethmann M, Zülke C, Fahlke J, Arning MB, Sinn M, Hinke A, Riess H. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. *JAMA* 2013; **310**: 1473-1481 [PMID: 24104372 DOI: 10.1001/jama.2013.279201]

58 **Paulson AS**, Tran Cao HS, Tempero MA, Lowy AM. Therapeutic advances in pancreatic cancer. *Gastroenterology* 2013; **144**: 1316-1326 [PMID: 23622141 DOI: 10.1053/j.gastro.2013.01.078]

59 **Artinyan A**, Anaya DA, McKenzie S, Ellenhorn JD, Kim J. Neoadjuvant therapy is associated with improved survival in resectable pancreatic adenocarcinoma. *Cancer* 2011; **117**: 2044-2049 [PMID: 21523715 DOI: 10.1002/cncr.25763]

60 **Brunner TB**. Neoadjuvant therapy for potentially resectable pancreatic cancer: an emerging paradigm? *Curr Oncol Rep* 2013; **15**: 162-169 [PMID: 23325567 DOI: 10.1007/s11912-012-0291-3]

61 **He J**, Page AJ, Weiss M, Wolfgang CL, Herman JM, Pawlik TM. Management of borderline and locally advanced pancreatic cancer: where do we stand? *World J Gastroenterol* 2014; **20**: 2255-2266 [PMID: 24605025 DOI: 10.3748/wjg.v20.i9.2255]

62 **Xu CP**, Xue XJ, Liang N, Xu DG, Liu FJ, Yu XS, Zhang JD. Effect of chemoradiotherapy and neoadjuvant chemoradiotherapy in resectable pancreatic cancer: a systematic review and meta-analysis. *J Cancer Res Clin Oncol* 2014; **140**: 549-559 [PMID: 24370686 DOI: 10.1007/s00432-013-1572-4]

63 **Zhu CP**, Shi J, Chen YX, Xie WF, Lin Y. Gemcitabine in the chemoradiotherapy for locally advanced pancreatic cancer: a meta-analysis. *Radiother Oncol* 2011; **99**: 108-113 [PMID: 21571383 DOI: 10.1016/j.radonc.2011.04.001]

64 **Collins A**, Bloomston M. Diagnosis and management of pancreatic cancer. *Minerva Gastroenterol Dietol* 2009; **55**: 445-454 [PMID: 19942828]

65 **Raut CP**, Tseng JF, Sun CC, Wang H, Wolff RA, Crane CH, Hwang R, Vauthey JN, Abdalla EK, Lee JE, Pisters PW, Evans DB. Impact of resection status on pattern of failure and survival after pancreaticoduodenectomy for pancreatic adenocarcinoma. *Ann Surg* 2007; **246**: 52-60 [PMID: 17592291 DOI: 10.1097/01.sla.0000259391.84304.2b]

66 **Ramesh AN**, Kambhampati C, Monson JR, Drew PJ. Artificial intelligence in medicine. *Ann R Coll Surg Engl* 2004; **86**: 334-338 [PMID: 15333167 DOI: 10.1308/147870804290]

67 **Ansari D**, Nilsson J, Andersson R, Regnér S, Tingstedt B, Andersson B. Artificial neural networks predict survival from pancreatic cancer after radical surgery. *Am J Surg* 2013; **205**: 1-7 [PMID: 23245432 DOI: 10.1016/j.amjsurg.2012.05.032]

68 **Wolfgang CL**, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, Hruban RH. Recent progress in pancreatic cancer. *CA Cancer J Clin* 2013; **63**: 318-348 [PMID: 23856911 DOI: 10.3322/caac.21190]

69 **Burris HA**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413 [PMID: 9196156]

70 **Andersson R**, Aho U, Nilsson BI, Peters GJ, Pastor-Anglada M, Rasch W, Sandvold ML. Gemcitabine chemoresistance in pancreatic cancer: molecular mechanisms and potential solutions. *Scand J Gastroenterol* 2009; **44**: 782-786 [PMID: 19214867 DOI: 10.1080/00365520902745039]

71 **Damaraju VL**, Damaraju S, Young JD, Baldwin SA, Mackey J, Sawyer MB, Cass CE. Nucleoside anticancer drugs: the role of nucleoside transporters in resistance to cancer chemotherapy. *Oncogene* 2003; **22**: 7524-7536 [PMID: 14576856 DOI: 10.1038/sj.onc.1206952]

72 **Giovannetti E**, Del Tacca M, Mey V, Funel N, Nannizzi S, Ricci S, Orlandini C, Boggi U, Campani D, Del Chiaro M, Iannopollo M, Bevilacqua G, Mosca F, Danesi R. Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. *Cancer Res* 2006; **66**: 3928-3935 [PMID: 16585222 DOI: 10.1158/0008-5472.CAN-05-4203]

73 **Spratlin J**, Sangha R, Glubrecht D, Dabbagh L, Young JD,

Dumontet C, Cass C, Lai R, Mackey JR. The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. *Clin Cancer Res* 2004; **10**: 6956-6961 [PMID: 15501974 DOI: 10.1158/1078-0432.CCR-04-0224]

74 **Ansari D**, Tingstedt B, Andersson R. Pancreatic cancer - cost for overtreatment with gemcitabine. *Acta Oncol* 2013; **52**: 1146-1151 [PMID: 23244671 DOI: 10.3109/0284186X.2012.744140]

75 **Di Marco M**, Di Cicilia R, Macchini M, Nobili E, Vecchiarelli S, Brandi G, Biasco G. Metastatic pancreatic cancer: is gemcitabine still the best standard treatment? (Review). *Oncol Rep* 2010; **23**: 1183-1192 [PMID: 20372829]

76 **Sun C**, Ansari D, Andersson R, Wu DQ. Does gemcitabine-based combination therapy improve the prognosis of unresectable pancreatic cancer? *World J Gastroenterol* 2012; **18**: 4944-4958 [PMID: 23002368 DOI: 10.3748/wjg.v18.i35.4944]

77 **Von Hoff DD**, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, Korn RL, Desai N, Trieu V, Iglesias JL, Zhang H, Soon-Shiong P, Shi T, Rajeshkumar NV, Maitra A, Hidalgo M. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol* 2011; **29**: 4548-4554 [PMID: 21969517 DOI: 10.1200/JCO.2011.36.5742]

78 **Garber K**. Stromal depletion goes on trial in pancreatic cancer. *J Natl Cancer Inst* 2010; **102**: 448-450 [PMID: 20339135 DOI: 10.1093/jnci/djq113]

79 **Neesse A**, Frese KK, Chan DS, Bapiro TE, Howat WJ, Richards FM, Ellenrieder V, Jodrell DI, Tuveson DA. SPARC independent drug delivery and antitumour effects of nab-paclitaxel in genetically engineered mice. *Gut* 2014; **63**: 974-983 [PMID: 24067278 DOI: 10.1136/gutjnl-2013-305559]

80 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]

81 **Hartlapp I**, Müller J, Kenn W, Steger U, Isbert C, Scheurlen M, Germer CT, Einsele H, Kunzmann V. Complete pathological remission of locally advanced, unresectable pancreatic cancer (LAPC) after intensified neoadjuvant chemotherapy. *Onkologie* 2013; **36**: 123-125 [PMID: 23486001 DOI: 10.1159/000348527]

82 **Nagaraju GP**, Dontula R, El-Rayes BF, Lakka SS. Molecular mechanisms underlying the divergent roles of SPARC in human carcinogenesis. *Carcinogenesis* 2014; **35**: 967-973 [PMID: 24675529 DOI: 10.1093/carcin/bgu072]

83 **Infante JR**, Matsubayashi H, Sato N, Tonascia J, Klein AP, Riall TA, Yeo C, Iacobuzio-Donahue C, Goggins M. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. *J Clin Oncol* 2007; **25**: 319-325 [PMID: 17235047 DOI: 10.1200/JCO.2006.07.8824]

84 **Saif MW**. Advancements in the management of pancreatic cancer: 2013. *JOP* 2013; **14**: 112-118 [PMID: 23474549 DOI: 10.6092/1590-8577/1481]

85 **Sherman MH**, Yu RT, Engle DD, Ding N, Atkins AR, Tiriac H, Collisson EA, Connor F, Van Dyke T, Kozlov S, Martin P, Tseng TW, Dawson DW, Donahue TR, Masamune A, Shimosegawa T, Apte MV, Wilson JS, Ng B, Lau SL, Gunton JE, Wahl GM, Hunter T, Drebin JA, O'Dwyer PJ, Liddle C, Tuveson DA, Downes M, Evans RM. Vitamin d receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. *Cell* 2014; **159**: 80-93 [PMID: 25259922 DOI: 10.1016/j.cell.2014.08.007]

P- Reviewer: Fusai GK **S- Editor**: Yu J
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3166

World J Gastroenterol 2015 March 21; 21(11): 3166-3169
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Perioperative management of distal pancreatectomy

Yasuhiro Fujino

Yasuhiro Fujino, Department of Surgery, Hyogo Cancer Center, Akashi 673-8558, Japan
Author contributions: Fujino Y solely contributed to this paper.
Conflict-of-interest: The author declares no conflicts of interest.

Correspondence to: Yasuhiro Fujino, MD, PhD, Department of Surgery, Hyogo Cancer Center, 13-70 Kitaoji-cho, Akashi 673-8558, Japan. yasu120@hp.pref.hyogo.jp
Telephone: +81-78-9291151
Fax: +81-78-9292380
Received: November 25, 2014
Peer-review started: November 26, 2014
First decision: December 26, 2014
Revised: January 19, 2015
Accepted: February 5, 2015
Article in press: February 5, 2015
Published online: March 21, 2015

Abstract

Recent advances in surgical techniques and perioperative management have markedly reduced operative morbidity after distal pancreatectomy (DP). However, some questions remain regarding the protocol for the perioperative management of DP, in particular, with regard to the development of pancreatic fistula (PF). A review of DP was therefore conducted in order to standardize the management of patients for a favorable outcome. Overall, operative technique and perioperative management emerged as two critical factors contributing to favorable outcome in DP patients. As for the operative method, surgical and closure techniques exhibited differences in outcome. Laparoscopic DP generally yields more favorable perioperative outcomes compared to open DP, and is applicable for benign tumors and some ductal carcinomas of the pancreas. Robotic DP is also available for safe pancreatic surgery. *En bloc* celiac axis resection offers a high R0 resection rate and potentially allows for some local control in the case of advanced pancreatic cancer. Following resection, staple closure was not found to reduce the rate of PF when compared to hand-sewn closure. In addition, ultrasonic dissection devices, fibrin glue sealing, and staple closure with mesh reinforcement were shown to significantly reduce PF, although there was some bias in these studies. In perioperative management, both preoperative and postoperative treatment affected outcome. First, preoperative endoscopic pancreatic stenting may be an effective prophylactic measure against fistula development following DP in selected patients. Second, in postoperative management, a multifactorial approach including prophylactic antibiotics improved high surgical site infection rates following complex hepato-pancreato-biliary surgery. Furthermore, although conflicting results have been reported, somatostatin analogues should be administered selectively to patients considered to have a high risk for PF. Finally, careful drain management also facilitates a favorable outcome in patients with PF after DP. The results of the review indicate that laparoscopic DP coupled with perioperative management influences outcome in DP patients.

Key words: Distal pancreatectomy; Pancreatic fistula; Perioperative management

Core tip: Perioperative management of distal pancreatectomy has been reviewed in order to standardize management for a favorable outcome in these patients.

Fujino Y. Perioperative management of distal pancreatectomy. *World J Gastroenterol* 2015; 21(11): 3166-3169 Available from:

URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3166.htm
DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3166

INTRODUCTION

Distal pancreatectomy (DP) is generally performed on patients with benign and malignant neoplasms of the distal pancreas and chronic pancreatitis. Recent advances in surgical techniques and perioperative management have markedly reduced the rates of operative morbidity and mortality after DP[1,2]. However, pancreatic fistula (PF) still remains a major cause of morbidity[3]. The overall incidence of PF in patients undergoing DP is as high as 10%-30%[1,2]. In addition, PF is associated with additional complications such as intra-abdominal abscess or hemorrhage, and leads to a prolonged hospital stay[2]. Some factors have been reported to predispose patients to the development of PF, including the surgical technique, a soft or normal pancreas, pancreatic thickness, age, obesity, and extended lymphadenectomy[1-6]. A review of published results for DP in PubMed Central from January 1998 to October 2014 was conducted using the following terms: "distal pancreatectomy", "pancreas", and "randomized study". Twenty-one articles and the references therein were reviewed. Favorable outcome associated with laparoscopic DP along with clear parameters for perioperative management are discussed.

OPERATIVE METHOD

Resection of the pancreas, closure of the remnant pancreas, and other techniques

Conventional resection of the pancreas was performed with scissors or electric scissors, bleeding points were ligated, and the main pancreatic duct was ligated. The remnant pancreas was closed with hand-sewn sutures[1,2]. In this approach, ligation of the main pancreatic duct was found to be important in preventing PF[7].

Recently, more varied techniques and surgical devices have been introduced into pancreatic surgery for both resection and closure. Resection of the pancreas has been performed with ultrasonic dissection devices[8], saline-coupled bipolar electrocautery[9], and a vessel-sealing system[10]. Ultrasonic dissection devices in particular are easily available and also significantly reduce the occurrence of PF in DP[8]. Seromuscular patches[11], fibrin glue sealing[12], the application of surface-active meshes, and combinations of these techniques[13] were used for closure of the pancreas. Seromuscular patch closure of the pancreatic remnant has been described using either an isolated Roux-n-Y loop[11] or gastric serosa[14]. Hassenpflug *et al*[15] reported that coverage of the pancreatic remnant after DP decreased the occurrence of clinically relevant PF. Suzuki *et al*[12] reported that sealing with fibrin glue also prevented PF. In addition, PF was reduced when the remnant pancreas was tightly patched and sutured vertically with the hepatic ligament[16,17] or an absorbable fibrin sealant patch[18].

Stapling devices can be used at the same time to resect the pancreas[19]. This technique is applied mainly in laparoscopic DP[20]. However, a randomized trial demonstrated that staple closure did not reduce the occurrence of PF compared to hand-sewn closure[21]. Oláh *et al*[22] also reported that closure with a stapler in combination with a seromuscular patch from the jejunum did not reduce the occurrence of PF compared to the use of a stapler alone.

In a systematic review[23], ultrasonic dissection devices[8], fibrin glue sealing[12], and staple closure with mesh reinforcement[24] were shown to significantly reduce the occurrence of PF, although there was some bias in these studies.

Open vs laparoscopic and robotic surgeries

Laparoscopic techniques have been recently applied to hepato-pancreato-biliary surgery[25], so there are many studies reporting on the use of laparoscopic DP. Laparoscopic DP is used for resection of benign tumors and some ductal carcinomas of the pancreas[25]. Systematic reviews have demonstrated that laparoscopic DP leads to significantly more favorable perioperative outcomes[20,25]. Robotic DP is also available and safe for pancreatic surgery, but the influence of the technique on overall survival of oncology patients is still unknown[26].

Extended surgery

DP was performed with various extents of lymphadenectomy based on the disease and stage of cancer. Although tumors invading the celiac axis had been considered unresectable, Hirano *et al*[27] advocated DP with *en bloc* celiac axis resection. This strategy offers a high R0 resection rate and potentially allows for some local control of advanced pancreatic cancer. Although this method is associated with a high frequency of complications, Okada *et al*[28] demonstrated that preservation of the left gastric artery in DP with *en bloc* celiac axis resection reduced postoperative morbidity.

POSTOPERATIVE MANAGEMENT

Prophylactic antibiotics

In general, a prophylactic, intravenous, broad-spectrum antibiotic (cefotiam or cefazolin sodium) was started intraoperatively. Once an infective complication was diagnosed, an appropriate sensitive antibiotic agent was selected and administered[17]. A recent study by Ceppa *et al*[29] reported that a multifactorial approach improved high surgical site infection rates following complex hepato-pancreato-biliary surgery.

Somatostatin analogues

Somatostatin analogues inhibit pancreatic exocrine

secretion, but various groups reported conflicting results for their use in perioperative management of patients undergoing DP. In some studies, perioperative treatment with these compounds was shown to decrease the rate of clinically significant postoperative PF, leak, or abscess[30]. In contrast, other studies failed to demonstrate a benefit in the perioperative use of somatostatin analogues in patients undergoing DP[31,32]. Therefore, the use of somatostatin analogues should be administered selectively to patients considered to have a high risk for PF.

Drain management

Abdominal drains were positioned on the left sub-diaphragm and stump of the remnant pancreas. The drain of the left sub-diaphragm was usually removed on postoperative days 2-3, and the drain of the stump of the remnant pancreas was usually removed within six postoperative days based on clinical symptoms (no sign of infection) and the values of drain amylase and lipase (less than three times the serum amylase and lipase activity)[1,17].

Kawai *et al*[33] also showed that early removal of drains was a critical factor in the reduction of morbidity following pancreaticoduodenectomy. These results support the view that drains are not mandatory and that, if placed, should be removed as soon as possible after DP. Thus, careful drain management also facilitates a favorable outcome in patients with PF after DP[34].

Stent management

Prophylactic transpapillary pancreatic stenting has been proposed as a strategy to prevent PF. However, this technique does not reduce PF when standardized resection of the body and tail of the pancreas is performed[35]. However, Abe *et al*[36] reported that preoperative endoscopic pancreatic stenting might be an effective prophylactic measure against fistula development following DP in select patients.

CONCLUSION

Perioperative management is important for an early favorable outcome in patients undergoing DP. Laparoscopic DP facilitates favorable results.

ACKNOWLEDGMENTS

The author is grateful to Dr. Yoshikazu Kuroda for his advice.

REFERENCES

1 **Lillemoe KD**, Kaushal S, Cameron JL, Sohn TA, Pitt HA, Yeo CJ. Distal pancreatectomy: indications and outcomes in 235 patients. *Ann Surg* 1999; **229**: 693-698; discussion 698-700 [PMID: 10235528 DOI: 10.1097/00000658-199905000-00012]

2 **Kleeff J**, Diener MK, Z'graggen K, Hinz U, Wagner M, Bachmann J, Zehetner J, Müller MW, Friess H, Büchler MW. Distal pancreatectomy: risk factors for surgical failure in 302 consecutive cases. *Ann Surg* 2007; **245**: 573-582 [PMID: 17414606 DOI: 10.1097/01.sla.0000251438.43135.fb]

3 **Goh BK**, Tan YM, Chung YF, Cheow PC, Ong HS, Chan WH, Chow PK, Soo KC, Wong WK, Ooi LL. Critical appraisal of 232 consecutive distal pancreatectomies with emphasis on risk factors, outcome, and management of the postoperative pancreatic fistula: a 21-year experience at a single institution. *Arch Surg* 2008; **143**: 956-965 [PMID: 18936374 DOI: 10.1001/archsurg.143.10.956]

4 **Balcom JH**, Rattner DW, Warshaw AL, Chang Y, Fernandez-del Castillo C. Ten-year experience with 733 pancreatic resections: changing indications, older patients, and decreasing length of hospitalization. *Arch Surg* 2001; **136**: 391-398 [PMID: 11296108 DOI: 10.1001/archsurg.136.4.391]

5 **Yoshioka R**, Saiura A, Koga R, Seki M, Kishi Y, Morimura R, Yamamoto J, Yamaguchi T. Risk factors for clinical pancreatic fistula after distal pancreatectomy: analysis of consecutive 100 patients. *World J Surg* 2010; **34**: 121-125 [PMID: 20020297 DOI: 10.1007/s00268-009-0300-3]

6 **Okano K**, Oshima M, Kakinoki K, Yamamoto N, Akamoto S, Yachida S, Hagiike M, Kamada H, Masaki T, Suzuki Y. Pancreatic thickness as a predictive factor for postoperative pancreatic fistula after distal pancreatectomy using an endopath stapler. *Surg Today* 2013; **43**: 141-147 [PMID: 22782593 DOI: 10.1007/s00595-012-0235-4]

7 **Bilimoria MM**, Cormier JN, Mun Y, Lee JE, Evans DB, Pisters PW. Pancreatic leak after left pancreatectomy is reduced following main pancreatic duct ligation. *Br J Surg* 2003; **90**: 190-196 [PMID: 12555295 DOI: 10.1002/bjs.4032]

8 **Suzuki Y**, Fujino Y, Tanioka Y, Hori Y, Ueda T, Takeyama Y, Tominaga M, Ku Y, Yamamoto YM, Kuroda Y. Randomized clinical trial of ultrasonic dissector or conventional division in distal pancreatectomy for non-fibrotic pancreas. *Br J Surg* 1999; **86**: 608-611 [PMID: 10361178 DOI: 10.1046/j.1365-2168.1999.01120.x]

9 **Makino I**, Kitagawa H, Nakagawara H, Tajima H, Ninomiya I, Fushida S, Fujimura T, Ohta T. Management of remnant pancreatic stump fto prevent the development of postoperative pancreatic fistulas after distal pancreatectomy: current evidence and our strategy. *Surg Today* 2013; **43**: 595-602 [PMID: 23093346 DOI: 10.1007/s00595-012-0370-y]

10 **Chamberlain RS**, Korvick D, Mootoo M, Story S, Dubiel B, Sharpnack D. Can harmonic focus curved shear effectively seal the pancreatic ducts and prevent pancreatic leak? Feasibility evaluation and testing in ex vivo and in vivo porcine models. *J Surg Res* 2009; **157**: 279-283 [PMID: 19765731 DOI: 10.1016/j.jss.2009.05.008]

11 **Moriura S**, Kimura A, Ikeda S, Iwatsuka Y, Ikezawa T, Naiki K. Closure of the distal pancreatic stump with a seromuscular flap. *Surg Today* 1995; **25**: 992-994 [PMID: 8640031 DOI: 10.1007/BF00312391]

12 **Suzuki Y**, Kuroda Y, Morita A, Fujino Y, Tanioka Y, Kawamura T, Saitoh Y. Fibrin glue sealing for the prevention of pancreatic fistulas following distal pancreatectomy. *Arch Surg* 1995; **130**: 952-955 [PMID: 7661678 DOI: 10.1001/archsurg.1995.01430090038015]

13 **Thaker RI**, Matthews BD, Linehan DC, Strasberg SM, Eagon JC, Hawkins WG. Absorbable mesh reinforcement of a stapled pancreatic transection line reduces the leak rate with distal pancreatectomy. *J Gastrointest Surg* 2007; **11**: 59-65 [PMID: 17390188 DOI: 10.1007/s11605-006-0042-6]

14 **Kluger Y**, Alfici R, Abbley B, Soffer D, Aladgem D. Gastric serosal patch in distal pancreatectomy for injury: a neglected technique. *Injury* 1997; **28**: 127-129 [PMID: 9205579 DOI: 10.1016/S0020-1383(96)00157-X]

15 **Hassenpflug M**, Hartwig W, Strobel O, Hinz U, Hackert T, Fritz S, Büchler MW, Werner J. Decrease in clinically relevant pancreatic fistula by coverage of the pancreatic remnant after distal pancreatectomy. *Surgery* 2012; **152**: S164-S171 [PMID: 22819173 DOI: 10.1016/j.surg.2012.05.026]

16 **Iannitti DA**, Coburn NG, Somberg J, Ryder BA, Monchik J, Cioffi WG. Use of the round ligament of the liver to decrease pancreatic fistulas: a novel technique. *J Am Coll Surg* 2006; **203**: 857-864 [PMID: 17116554 DOI: 10.1016/j.jamcollsurg.2006.08.021]

17 **Fujino Y**, Sendo H, Oshikiri T, Sugimoto T, Tominaga M. A novel surgical technique to prevent pancreatic fistula in distal pancreatectomy using a patch of the falciform ligament. *Surg Today* 2015; **45**: 44-49 [PMID: 24909496 DOI: 10.1007/s00595-014-0942-0]

18 **Montorsi M**, Zerbi A, Bassi C, Capussotti L, Coppola R, Sacchi M. Efficacy of an absorbable fibrin sealant patch (TachoSil) after distal pancreatectomy: a multicenter, randomized, controlled trial. *Ann Surg* 2012; **256**: 853-859; discussion 859-860 [PMID: 23095631 DOI: 10.1097/SLA.0b013e318272dec0]

19 **Kajiyama Y**, Tsurumaru M, Udagawa H, Tsutsumi K, Kinoshita Y, Akiyama H. Quick and simple distal pancreatectomy using the GIA stapler: report of 35 cases. *Br J Surg* 1996; **83**: 1711 [PMID: 9038547]

20 **Venkat R**, Edil BH, Schulick RD, Lidor AO, Makary MA, Wolfgang CL. Laparoscopic distal pancreatectomy is associated with significantly less overall morbidity compared to the open technique: a systematic review and meta-analysis. *Ann Surg* 2012; **255**: 1048-1059 [PMID: 22511003 DOI: 10.1097/SLA.0b013e318251ee09]

21 **Diener MK**, Seiler CM, Rossion I, Kleeff J, Glanemann M, Butturini G, Tomazic A, Bruns CJ, Busch OR, Farkas S, Belyaev O, Neoptolemos JP, Halloran C, Keck T, Niedergethmann M, Gellert K, Witzigmann H, Kollmar O, Langer P, Steger U, Neudecker J, Berrevoet F, Ganzera S, Heiss MM, Luntz SP, Bruckner T, Kieser M, Büchler MW. Efficacy of stapler versus hand-sewn closure after distal pancreatectomy (DISPACT): a randomised, controlled multicentre trial. *Lancet* 2011; **377**: 1514-1522 [PMID: 21529927 DOI: 10.1016/S0140-6736(11)60237-7]

22 **Oláh A**, Issekutz A, Belágyi T, Hajdú N, Romics L. Randomized clinical trial of techniques for closure of the pancreatic remnant following distal pancreatectomy. *Br J Surg* 2009; **96**: 602-607 [PMID: 19434697 DOI: 10.1002/bjs.6620]

23 **Cečka F**, Jon B, Subrt Z, Ferko A. Surgical technique in distal pancreatectomy: a systematic review of randomized trials. *Biomed Res Int* 2014; **2014**: 482906 [PMID: 24971333 DOI: 10.1155/2014/482906]

24 **Hamilton NA**, Porembka MR, Johnston FM, Gao F, Strasberg SM, Linehan DC, Hawkins WG. Mesh reinforcement of pancreatic transection decreases incidence of pancreatic occlusion failure for left pancreatectomy: a single-blinded, randomized controlled trial. *Ann Surg* 2012; **255**: 1037-1042 [PMID: 22534422 DOI: 10.1097/SLA.0b013e31825659ef]

25 **Nakamura M**, Nakashima H. Laparoscopic distal pancreatectomy and pancreatoduodenectomy: is it worthwhile? A meta-analysis of laparoscopic pancreatectomy. *J Hepatobiliary Pancreat Sci* 2013; **20**: 421-428 [PMID: 23224732 DOI: 10.1007/s00534-012-0578-7]

26 **Parisi A**, Coratti F, Cirocchi R, Grassi V, Desiderio J, Farinacci F, Ricci F, Adamenko O, Economou AI, Cacurri A, Trastulli S, Renzi C, Castellani E, Di Rocco G, Redler A, Santoro A, Coratti A. Robotic distal pancreatectomy with or without preservation of spleen: a technical note. *World J Surg Oncol* 2014; **12**: 295 [PMID: 25248464 DOI: 10.1186/1477-7819-12-295]

27 **Hirano S**, Kondo S, Hara T, Ambo Y, Tanaka E, Shichinohe T, Suzuki O, Hazama K. Distal pancreatectomy with en bloc celiac axis resection for locally advanced pancreatic body cancer: long-term results. *Ann Surg* 2007; **246**: 46-51 [PMID: 17592290 DOI: 10.1097/01.sla.0000258608.52615.5a]

28 **Okada K**, Kawai M, Tani M, Hirono S, Miyazawa M, Shimizu A, Kitahata Y, Yamaue H. Preservation of the left gastric artery on the basis of anatomical features in patients undergoing distal pancreatectomy with celiac axis en-bloc resection (DP-CAR). *World J Surg* 2014; **38**: 2980-2985 [PMID: 25104543 DOI: 10.1007/s00268-014-2702-0]

29 **Ceppa EP**, Pitt HA, House MG, Kilbane EM, Nakeeb A, Schmidt CM, Zyromski NJ, Lillemoe KD. Reducing surgical site infections in hepatopancreatobiliary surgery. *HPB* (Oxford) 2013; **15**: 384-391 [PMID: 23557410 DOI: 10.1111/j.1477-2574.2012.00604.x]

30 **Allen PJ**, Gönen M, Brennan MF, Bucknor AA, Robinson LM, Pappas MM, Carlucci KE, D'Angelica MI, DeMatteo RP, Kingham TP, Fong Y, Jarnagin WR. Pasireotide for postoperative pancreatic fistula. *N Engl J Med* 2014; **370**: 2014-2022 [PMID: 24849084 DOI: 10.1056/NEJMoa1313688]

31 **Ramos-De la Medina A**, Sarr MG. Somatostatin analogues in the prevention of pancreas-related complications after pancreatic resection. *J Hepatobiliary Pancreat Surg* 2006; **13**: 190-193 [PMID: 16708293]

32 **Sarr MG**. The potent somatostatin analogue vapreotide does not decrease pancreas-specific complications after elective pancreatectomy: a prospective, multicenter, double-blinded, randomized, placebo-controlled trial. *J Am Coll Surg* 2003; **196**: 556-564; discussion 564-565; author reply 565 [PMID: 12691930]

33 **Kawai M**, Tani M, Terasawa H, Ina S, Hirono S, Nishioka R, Miyazawa M, Uchiyama K, Yamaue H. Early removal of prophylactic drains reduces the risk of intra-abdominal infections in patients with pancreatic head resection: prospective study for 104 consecutive patients. *Ann Surg* 2006; **244**: 1-7 [PMID: 16794381 DOI: 10.1097/01.sla.0000218077.14035.a6]

34 **Balzano G**, Zerbi A, Cristallo M, Di Carlo V. The unsolved problem of fistula after left pancreatectomy: the benefit of cautious drain management. *J Gastrointest Surg* 2005; **9**: 837-842 [PMID: 15985241]

35 **Frozanpor F**, Lundell L, Segersvärd R, Arnelo U. The effect of prophylactic transpapillary pancreatic stent insertion on clinically significant leak rate following distal pancreatectomy: results of a prospective controlled clinical trial. *Ann Surg* 2012; **255**: 1032-1036 [PMID: 22584629 DOI: 10.1097/SLA.0b013e318251610f]

36 **Abe N**, Sugiyama M, Suzuki Y, Yamaguchi Y, Yanagida O, Masaki T, Mori T, Atomi Y. Preoperative endoscopic pancreatic stenting for prophylaxis of pancreatic fistula development after distal pancreatectomy. *Am J Surg* 2006; **191**: 198-200 [PMID: 16442945]

P- Reviewer: Cecka F, Limongelli P, Michalopoulos N
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3170

World J Gastroenterol 2015 March 21; 21(11): 3170-3173
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Contra-lateral liver lobe hypertrophy after unilobar Y90 radioembolization: An alternative to portal vein embolization?

Jin-Yao Teo, Brian K.P. Goh

Jin-Yao Teo, Brian K.P. Goh, Department of Hepatopancreatobiliary and Transplantation Surgery, Singapore General Hospital, Singapore 169856, Singapore
Brian K.P. Goh, Duke-NUS Graduate Medical School, Singapore 169857, Singapore
Author contributions: Teo JY performed the literature search, retrieved data and wrote the paper; Goh BKP was responsible for conception and design of the paper, wrote the paper and made critical revisions.
Conflict-of-interest: Goh BKP has received travel grants from Sirtex Medical.

Correspondence to: Brian K.P. Goh, MBBS, MMed, MSc, FRCSEd, Senior Consultant and Associate Professor (Adj), Department of Hepatopancreatobiliary and Transplantation Surgery, Singapore General Hospital, 20 College Road, Academia Level 5, Singapore 169856, Singapore. bsgkp@hotmail.com
Telephone: +65-63265564
Fax: +65-62209323
Received: October 16, 2014
Peer-review started: October 18, 2014
First decision: October 29, 2014
Revised: November 11, 2014
Accepted: February 12, 2015
Article in press: February 13, 2015
Published online: March 21, 2015

Abstract

Liver resection (LR) with negative margins confers survival advantage in many patients with hepatic malignancies. However, an adequate future liver remnant (FLR) is imperative for safe LR. Presently, in patients with an inadequate FLR; the 2 most established clinical techniques performed to induce liver hypertrophy are portal vein embolization (PVE) and portal vein ligation. More recently, it has been observed that patients who undergo treatment *via* Y90 radioembolization experience hypertrophy of the contra-lateral untreated liver lobe. Based on these observations, several investigators have proposed the potential use of this modality as an alternative technique for increasing the FLR prior to liver resection. Y90 radioembolization induces hypertrophy at a slower rate than PVE but has the added advantage of concomitant local disease control and tumour down-staging.

Key words: Liver hypertrophy; Y90; Radioembolization; Portal vein embolization; Selective internal radiation therapy

Core tip: Both portal vein embolization and Y90 radioembolization induce significant hypertrophy of the contralateral lobe. Y90 radioembolization induces hypertrophy at a slower rate than PVE but has the added advantage of concomitant local disease control and tumour down-staging.

Teo JY, Goh BKP. Contra-lateral liver lobe hypertrophy after unilobar Y90 radioembolization: An alternative to portal vein embolization? *World J Gastroenterol* 2015; 21(11): 3170-3173 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3170.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3170

LIVER HYPERTROPHY

Liver resection (LR) with negative margins confers consistent survival advantage in patients with both primary (hepatocellular carcinoma/cholangiocarcinoma) or secondary malignant disease[1]. In patients with well-preserved liver function, curative liver resection remains the standard of care. An adequate future liver remnant (FLR) is imperative for safe LR. Presently, in patients with a normal liver function, a FLR of at least 25% is deemed sufficient by most clinicians to avoid liver failure. However, in patients with an impaired liver function (*e.g*., cirrhosis), a larger FLR of up to 40% should be preserved[2-4]. An inadequate FLR is a major reason why otherwise suitable patients are precluded from potentially curative LR.

Presently, the 2 most well-established clinical techniques performed to induce liver hypertrophy in patients with an inadequate FLR are portal vein embolization (PVE) and portal vein ligation (PVL). In head-to-head comparisons, both these techniques have been shown to result in equivalent degrees of hypertrophy[5,6], estimated to be between 10%-46% at 2 to 8 wk[7]. PVE is preferentially utilised usually in view of its minimally invasive nature, and the avoidance of a laparotomy. However, a major drawback of both PVE and PVL is that tumour growth continues unabated while awaiting hypertrophy, which may eventually preclude resection especially in tumours which are in close proximity to major bilio-vascular structures. This is far from being a merely theoretical concern as increased tumour growth rates after PVE have been reported in both animal models[8,9] and humans[10].

Based on these concerns, a sequential approach combining transarterial chemoembolization (TACE) and PVE has been advocated, with proponents claiming both a significant rate of FLR hypertrophy as well as increased local tumour control. This approach was first shown to result in good FLR hypertrophy, with no increased risk of liver failure, as might be expected after occlusion of the liver's dual blood supply[11]. These findings were replicated in subsequent larger studies, which also showed an improvement in both overall and disease-free survival in patients undergoing sequential treatment as opposed to PVE alone[12,13]. However, in these studies, the mean increase in percentage of FLR achieved in the PVE + TACE arms was only 7.3%-22%, which was significantly less than that reported with PVE in the rest of the literature.

Y90 RADIOEMBOLIZATION

The first series to report the phenomenon of contralateral liver lobe hypertrophy after Y90 radioembolization was published in 2008[14]. Subsequently, several groups have also published similar results from their retrospective experience[15-22]. The main limitations of these retrospective studies are that the patient cohorts were vastly heterogenous in terms of pathology treated, underlying liver disease, dosage and delivery of Y90, number of treatment sessions and time to measurement of hypertrophy. However, it was clear that unilobar Y90 radioembolization resulted in significant hypertrophy of the contralateral lobe - the reported average hypertrophy achieved ranged from 21%-47% at 44 d-9 mo. The degree of hypertrophy reported is thus comparable with that achieved with PVE/PVL, although the time to hypertrophy is clearly heterogenous, and precludes any meaningful direct comparison. To date, there have been no prospective trials directly comparing the efficacy of Y90 radioembolization to PVE/PVL in achieving liver hypertrophy.

Only one series[21] has attempted a direct head-to-head comparison between these two modalities. In this study, a matched-pair analysis of patients with secondary liver malignancy confined to the right hemiliver was performed. Patients were well matched for: (1) baseline FLR; (2) history of platinum-based chemotherapy; (3) platelet count; and (4) extent of embolization. Although subject to the usual biases inherent in such a study, the authors demonstrated that PVE produced significantly more hypertrophy (61.5% *vs* 29.0%) within a shorter time frame (median 33 d *vs* 46 d). Another recent study[18] attempted to study the relationship between the degree of hypertrophy with duration from treatment. In this study, median FLR growth progressed from 7% at one month to 45% at 9 mo post-radioembolization. Hence, based on current evidence it can be concluded that the kinetics of hypertrophy may differ between the two modalities, with post Y90 radioembolization causing a slower, more gradual increase in volume compared to PVE. Hence, the potential advantage of Y90 radioembolization in inducing liver hypertrophy would therefore lie in its ability to provide concomitant local tumour control and even down-staging. Tumour response to Y90 according to the RECIST criteria had been reported to range between 42%-70%[23]. This decrease in tumour size, coupled with hypertrophy of the FLR holds great promise in potentially rendering previously unresectable disease curable.

It is worth mentioning here the recent development of another novel technique for inducing liver hypertrophy, *i.e*., associating liver partition with portal vein ligation for staged hepatectomy (ALPPS). This technique allows for extremely rapid hypertrophy of the FLR, at the expense of increased morbidity and a significant mortality rate. A recent review of the literature[24] concluded that a mean FLR hypertrophy of 80% at 7-10 d was achievable, but at the risk of a 35% significant morbidity rate and a 30-d mortality of 6%. In view of the significant morbidity and mortality, ALPPS is therefore best considered to be an experimental technique at present. It is to be used in highly selected patients in a clinical trial setting.

In light of current evidence, we therefore propose that instead of being an alternative to PVE, the technique of Y90 radioembolization is instead complementary. The former is best utilised in the setting where the tumour is technically resectable except for a concern over the adequacy of the FLR. PVE would then result in a greater degree of hypertrophy over a shorter time frame. However, in situations where a large, bulky tumour abuts major vascular and/or biliary structures which must be conserved or when the ability to achieve adequate oncological margins are a concern, then Y90 radioembolisation would provide the added advantage of both tumour control/downsizing while increasing the FLR.

REFERENCES

1 **Goh BK**, Chow PK, Teo JY, Wong JS, Chan CY, Cheow PC, Chung AY, Ooi LL. Number of nodules, Child-Pugh status, margin positivity, and microvascular invasion, but not tumor size, are prognostic factors of survival after liver resection for multifocal hepatocellular carcinoma. *J Gastrointest Surg* 2014; **18**: 1477-1485 [PMID: 24855028 DOI: 10.1007/s11605-014-2542-0]

2 **Tanaka K**, Shimada H, Matsuo K, Ueda M, Endo I, Togo S. Remnant liver regeneration after two-stage hepatectomy for multiple bilobar colorectal metastases. *Eur J Surg Oncol* 2007; **33**: 329-335 [PMID: 17140759 DOI: 10.1016/j.ejso.2006.10.038]

3 **Hemming AW**, Reed AI, Howard RJ, Fujita S, Hochwald SN, Caridi JG, Hawkins IF, Vauthey JN. Preoperative portal vein embolization for extended hepatectomy. *Ann Surg* 2003; **237**: 686-691; discussion 691-693 [PMID: 12724635 DOI: 10.1097/01.SLA.0000065265.16728.C0]

4 **Goh BK**. Re: Measured Versus Estimated Total Liver Volume to Preoperatively Assess the Adequacy of Future Liver Remnant: Which Method Should We Use? *Ann Surg* 2014; Epub ahead of print [PMID: 24509213 DOI: 10.1097/SLA.0000000000000548]

5 **Capussotti L**, Muratore A, Baracchi F, Lelong B, Ferrero A, Regge D, Delpero JR. Portal vein ligation as an efficient method of increasing the future liver remnant volume in the surgical treatment of colorectal metastases. *Arch Surg* 2008; **143**: 978-982; discussion 982 [PMID: 18936377 DOI: 10.1001/archsurg.143.10.978]

6 **Aussilhou B**, Lesurtel M, Sauvanet A, Farges O, Dokmak S, Goasguen N, Sibert A, Vilgrain V, Belghiti J. Right portal vein ligation is as efficient as portal vein embolization to induce hypertrophy of the left liver remnant. *J Gastrointest Surg* 2008; **12**: 297-303 [PMID: 18060468 DOI: 10.1007/s11605-007-0410-x]

7 **Liu H**, Zhu S. Present status and future perspectives of preoperative portal vein embolization. *Am J Surg* 2009; **197**: 686-690 [PMID: 19249737 DOI: 10.1016/j.amjsurg.2008.04.022]

8 **Ikeda Y**, Matsumata T, Takenaka K, Sasaki O, Soejima K, Sugimachi K. Preliminary report of tumor metastasis during liver regeneration after hepatic resection in rats. *Eur J Surg Oncol* 1995; **21**: 188-190 [PMID: 7720894 DOI: 10.1016/S0748-7983(95)90468-9]

9 **Mizutani J**, Hiraoka T, Yamashita R, Miyauchi Y. Promotion of hepatic metastases by liver resection in the rat. *Br J Cancer* 1992; **65**: 794-797 [PMID: 1616850 DOI: 10.1038/bjc.1992.170]

10 **Pamecha V**, Levene A, Grillo F, Woodward N, Dhillon A, Davidson BR. Effect of portal vein embolisation on the growth rate of colorectal liver metastases. *Br J Cancer* 2009; **100**: 617-622 [PMID: 19209170 DOI: 10.1038/sj.bjc.6604872]

11 **Aoki T**, Imamura H, Hasegawa K, Matsukura A, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Sequential preoperative arterial and portal venous embolizations in patients with hepatocellular carcinoma. *Arch Surg* 2004; **139**: 766-774 [PMID: 15249411 DOI: 10.1001/archsurg.139.7.766]

12 **Ogata S**, Belghiti J, Farges O, Varma D, Sibert A, Vilgrain V. Sequential arterial and portal vein embolizations before right hepatectomy in patients with cirrhosis and hepatocellular carcinoma. *Br J Surg* 2006; **93**: 1091-1098 [PMID: 16779884 DOI: 10.1002/bjs.5341]

13 **Yoo H**, Kim JH, Ko GY, Kim KW, Gwon DI, Lee SG, Hwang S. Sequential transcatheter arterial chemoembolization and portal vein embolization versus portal vein embolization only before major hepatectomy for patients with hepatocellular carcinoma. *Ann Surg Oncol* 2011; **18**: 1251-1257 [PMID: 21069467 DOI: 10.1245/s10434-010-1423-3]

14 **Jakobs TF**, Saleem S, Atassi B, Reda E, Lewandowski RJ, Yaghmai V, Miller F, Ryu RK, Ibrahim S, Sato KT, Kulik LM, Mulcahy MF, Omary R, Murthy R, Reiser MF, Salem R. Fibrosis, portal hypertension, and hepatic volume changes induced by intra-arterial radiotherapy with 90yttrium microspheres. *Dig Dis Sci* 2008; **53**: 2556-2563 [PMID: 18231857 DOI: 10.1007/s10620-007-0148-z]

15 **Gaba RC**, Lewandowski RJ, Kulik LM, Riaz A, Ibrahim SM, Mulcahy MF, Ryu RK, Sato KT, Gates V, Abecassis MM, Omary RA, Baker TB, Salem R. Radiation lobectomy: preliminary findings of hepatic volumetric response to lobar yttrium-90 radioembolization. *Ann Surg Oncol* 2009; **16**: 1587-1596 [PMID: 19357924 DOI: 10.1245/s10434-009-0454-0]

16 **Ahmadzadehfar H**, Meyer C, Ezziddin S, Sabet A, Hoff-Meyer A, Muckle M, Logvinski T, Schild HH, Biersack HJ, Wilhelm K. Hepatic volume changes induced by radioembolization with 90Y resin microspheres. A single-centre study. *Eur J Nucl Med Mol Imaging* 2013; **40**: 80-90 [DOI: 10.1007/s00259-012-2253-2]

17 **Edeline J**, Lenoir L, Boudjema K, Rolland Y, Boulic A, Le Du F, Pracht M, Raoul JL, Clément B, Garin E, Boucher E. Volumetric changes after (90)y radioembolization for hepatocellular carcinoma in cirrhosis: an option to portal vein embolization in a preoperative setting? *Ann Surg Oncol* 2013; **20**: 2518-2525 [PMID: 23494107 DOI: 10.1245/s10434-013-2906-9]

18 **Vouche M**, Lewandowski RJ, Atassi R, Memon K, Gates VL, Ryu RK, Gaba RC, Mulcahy MF, Baker T, Sato K, Hickey R, Ganger D, Riaz A, Fryer J, Caicedo JC, Abecassis M, Kulik L, Salem R. Radiation lobectomy: time-dependent analysis of future liver remnant volume in unresectable liver cancer as a bridge to resection. *J Hepatol* 2013; **59**: 1029-1036 [PMID: 23811303 DOI: 10.1016/j.jhep.2013.06.015]

19 **Theysohn JM**, Ertle J, Müller S, Schlaak JF, Nensa F, Sipilae S, Bockisch A, Lauenstein TC. Hepatic volume changes after lobar selective internal radiation therapy (SIRT) of hepatocellular carcinoma. *Clin Radiol* 2014; **69**: 172-178 [PMID: 24209871 DOI: 10.1016/j.crad.2013.09.009]

20 **Fernández-Ros N**, Silva N, Bilbao JI, Iñarrairaegui M, Benito A, D'Avola D, Rodriguez M, Rotellar F, Pardo F, Sangro B. Partial liver volume radioembolization induces hypertrophy in the spared hemiliver and no major signs of portal hypertension. *HPB* (Oxford) 2014; **16**: 243-249 [PMID: 23530966 DOI: 10.1111/hpb.12095]

21 **Garlipp B**, de Baere T, Damm R, Irmscher R, van Buskirk M, Stübs P, Deschamps F, Meyer F, Seidensticker R, Mohnike K, Pech M, Amthauer H, Lippert H, Ricke J, Seidensticker M. Left-liver hypertrophy after therapeutic right-liver radioembolization is substantial but less than after portal vein embolization. *Hepatology* 2014; **59**: 1864-1873 [PMID: 24259442 DOI: 10.1002/hep.26947]

22 **Teo JY**, Goh BK, Cheah FK, Allen JC, Lo RH, Ng DC, Goh AS, Khor AY, Sim HS, Ng JJ, Chow PK. Underlying liver disease influences volumetric changes in the spared hemiliver after selective internal radiation therapy with 90Y in patients with hepatocellular carcinoma. *J Dig Dis* 2014; **15**: 444-450 [PMID: 24828952 DOI: 10.1111/1751-2980.12162]

23 **Kulik LM**, Carr BI, Mulcahy MF, Lewandowski RJ, Atassi B, Ryu RK, Sato KT, Benson A, Nemcek AA, Gates VL, Abecassis M, Omary RA, Salem R. Safety and efficacy of 90Y radiotherapy for hepatocellular carcinoma with and without portal vein thrombosis. *Hepatology* 2008; **47**: 71-81 [PMID: 18027884 DOI: 10.1002/hep.21980]

24 **Ielpo B**, Caruso R, Ferri V, Quijano Y, Duran H, Diaz E, Fabra I, Oliva C, Olivares S, Plaza JC, Vicente E. ALPPS procedure: our experience and state of the art. *Hepatogastroenterology* 2013; **60**: 2069-2075 [PMID: 24719949]

P- Reviewer: Anil G, Edeline J, Oklu R, van den Hoven AF
S- Editor: Yu J **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3174

World J Gastroenterol 2015 March 21; 21(11): 3174-3183
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Nuclear factor kappa B role in inflammation associated gastrointestinal malignancies

Sahil Gambhir, Dinesh Vyas, Michael Hollis, Apporva Aekka, Arpita Vyas

Sahil Gambhir, Dinesh Vyas, Michael Hollis, Apporva Aekka, Department of Surgery, College of Human Medicine, Michigan State University, East Lansing, MI 48912, United States
Arpita Vyas, Department of Pediatrics, College of Human Medicine - Michigan State University, East Lansing, MI 48912, United States
Author contributions: Gambhir S and Vyas D contributed equally to this work; Gambhir S and Vyas D designed research; analyzed data and wrote the paper; Hollis M contributed in research and editing; Vyas A contributed in data and conceptualization; Aekka A contributed in editing.
Conflict-of-interest: No potential conflicts of interest relevant to this article were reported.

Correspondence to: Dinesh Vyas, MD, MS, FICS, FACS, Department of Surgery, College of Human Medicine, Michigan State University, 1200 East Michigan Avenue, Suite 655, East Lansing, MI 48912, United States. dinesh.vyas@hc.msu.edu
Telephone: +-517-2672491
Fax: +1-517-2672488
Received: June 15, 2014
Peer-review started: June 16, 2014
First decision: August 15, 2014
Revised: August 29, 2014
Accepted: February 12, 2015
Article in press: February 13, 2015
Published online: March 21, 2015

Abstract

Nuclear factor kappa B (NF-κB) has an established role in the regulation of innate immunity and inflammation. NF-κB is also involved in critical mechanisms connecting inflammation and cancer development. Recent investigations suggest that the NF-κB signaling cascade may be the central mediator of gastrointestinal malignancies including esophageal, gastric and colorectal cancers. This review will explore NF-κB's function in inflammation-associated gastrointestinal malignancies, highlighting its oncogenic contribution to each step of carcinogenesis. NF-κB's role in the inflammation-to-carcinoma sequence in gastrointestinal malignancies warrants stronger emphasis upon targeting this pathway in achieving greater therapeutic efficacy.

Key words: Gastrointestinal cancer; Inflammation; Nuclear factor kappa B

Core tip: Inflammation has a critical role in cancer, its metastasis and angiogenesis. One of the critical mediator is nuclear transcription factor nuclear factor kappa B. This article will be critically useful for basic scientist and clinicians in understanding the the importance of this transcription factor.

Gambhir S, Vyas D, Hollis M, Aekka A, Vyas A. Nuclear factor kappa B role in inflammation associated gastrointestinal malignancies. *World J Gastroenterol* 2015; 21(11): 3174-3183 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3174.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3174

INTRODUCTION

Gastrointestinal cancers, comprised of esophageal, gastric and colorectal cancers, are some of the most common and fatal cancers worldwide. However, mortality rates have declined due to improved efforts in cancer prevention, earlier detection and a growing number of treatment options, especially in the development of therapies targeted to specific signaling pathways. These

therapies are founded upon the body of basic scientific research that has implicated various signaling pathways in different gastrointestinal (GI) malignancies. Recent literature suggests that the nuclear factor kappa B (NF-κB) signaling cascade in particular may serve as a central mediator of carcinogenesis. The NF-κB signaling pathway has numerous physiological roles, including regulation of innate immunity and inflammation, and therefore may be involved in carcinogenesis in multiple ways[1,2]. The primary function of this signaling cascade is to protect the cell from harm, but if it becomes aberrant, then the transition from inflammation to cancerous growth can be elicited. In our previous study, we explored gastrointestinal epithelial response to injury and by extension intend to scrutinize NF-κB's effects on the gut in this review[3].

Recent epidemiological investigations have shown that chronic inflammation, including infection, is associated with 15%-20% of all human malignancies[4]. Numerous genetic studies have also exposed NF-κB's potential role in inflammation-related cancers[4-7]. For example, NF-κB's constitutive activation has been demonstrated in various human solid tumors, including those of ovarian, lung, breast, melanoma, hepatocellular, thyroid, pancreatic, prostate, colon, esophageal, gastric, laryngeal, parathyroid, bladder, endometrial, retinoblastoma, astrocytoma and squamous cell carcinoma of the head and neck[8-17]. Moreover, aberrant NF-κB regulation has been observed in hematopoietic malignancies, such as multiple myeloma, mantle cell lymphoma, MALT lymphoma, diffuse large B-cell lymphoma, Hodgkin's lymphoma, myelodysplastic syndrome, adult T - cell leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, and chronic myeloid leukemia[8,11,18,19].

NF-κB is a pleiotropic transcriptional factor that is encoded by a family of five main genes[5,20]. These genes contain the homologous domain Rel homology domain (RHD), which includes RelA (p65), RelB, CRel (REI), p50 and p52. NF-κB1 (p100) and NF-κB2 (p102) are processed to produce p52 and p50 respectively[21]. The RHD allows these proteins to homo- and heterodimerize, localize to the nucleus, bind to DNA and interact with the inhibitor of NF-κB (IκB)[22]. NF-κB homodimers or heterodimers remain inhibited in the cytoplasm bound to ankyrin rich regions of IκB proteins, which prevent NF-κB translocation to the nucleus and thus transcription[22]. The IκB family consists of IκBα, IκBβ, IκBγ, IκBε, BCL-3 and p100 and p105. It is through IκB kinase dependent (IKK-dependent) phosphorylation, polyubiquitination and proteasomal degradation of IκB proteins that the NF-κB proteins are liberated[22]. The IKK family consists of two catalytic subunits, IKKα and IKKβ, complexed to the regulatory subunit IKKγ/NEMO (NF-κB essential modulator)[22].

The tight regulation and activation of NF-κB is controlled by two principal signaling pathways, modulated by divergent but specific stimuli: the classical or canonical pathway and the alternative pathway[1,22]. With regards to the former, inflammatory stimuli, including TNF-α, IL-1β, TLRs and viruses, promote phosphorylation at two serine sites of IκB through the activation of IKKα, IKKβ and NEMO[21]. This leads to polyubiquitination at adjacent lysine residues and eventually proteolysis, releasing NF-κB dimers-most commonly p50/p65 heterodimers-and degradation of IκB[5]. The alternative pathway is characterized by its independence from IKKβ and NEMO and reliance on IKKα[22]. Following stimulation by TNF-α receptor superfamily members (lymphotoxin (LT) βR, B-cell activating factor receptor (BAFFR), receptor activator of NF-κB (RANK), and CD40), IKK mediates the phosphorylation of NF-κB2/p100:RelB complexed with an IκB[22]. The complex is subsequently polyubiquinated, leading to the proteasome-dependent processing of RelB's inhibitor NF-κB2 p100, which results in the liberation of the ReIB-p50 heterodimer[1,21]. In both pathways, the dimers proceed to translocate to the nucleus, bind to NF-κB DNA sites and activate transcription. It should be noted that one of the first proteins transcribed in this process is IκBα, which then translocates to the nucleus and binds to the dimer to inhibit further transcription[5]. The classical pathway is implicated in preventing apoptosis and in innate immunity and inflammation[1,21]. The alternative pathway is involved in secondary lymphoid organogenesis and B cell maturation and survival[21]. Thus, both are responsible for cell survival and ultimately play a role in carcinogenesis.

NF-κB AND CANCER

Virchow first postulated and proposed the causal relationship between chronic inflammation and cancer. Chronic inflammation due to irritants is thought to lead to increased cell multiplication, predisposing cells to neoplastic DNA changes. An important mechanistic link may include NF-κB, as recent studies have suggested that NF-κB promotes tumor growth by inducing downstream proteins that have oncogenic effects[22]. Initial evidence of NF-κB's link to cancer was the identification of the p50 subunit and RelA and its close homology with the oncoprotein v-REL of the avian REL retrovirus[23]. The onco-virus constitutively activates the v-REL oncoprotein, which is an important catalyst in the progression of lymphomas[23]. Similarly, the human T-cell leukemia virus's oncoprotein TAX has been shown to constitutively activate the IKK complex, stimulating both pathways and again leading to upregulation of NF-κB's downstream proteins[24].

Hanahan and Weinberg's postulation of each step of tumorigenesis includes self-sufficiency in growth signals, insensitivity to growth inhibitor signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis[25-28]. NF-κB can exert its effects on each of these aspects of tumorigenesis through the induction of downstream protein expression, and thus might be the basis of the transition from inflammation to cancer growth.

Essentially, NF-κB controls cell proliferation by activating growth factors, including IL-2, granulocyte monocyte colony stimulator factor and CD40L[25,26]. It also serves as a positive regulator of cell cycle progression as it can activate c-myc and cyclin D1[26]. NF-κB inhibition of programmed cell death, through its regulation of the anti-apoptotic proteins ciAPS, c-FLP and members of the bcl-2 family, is also well documented[25,26]. These proteins are vital in sustaining genetically altered precancerous cells, and their effects therefore increase the probability of malignant changes. NF-κB activity can also lead to increased angiogenesis and metastasis through the upregulation of chemokines, such as IL-8 (migration), VEGF (angiogenesis) and MMP (spread). The altered expression of these genes, with NF-κB as a key mediator, is associated with tumor growth and spread[25,26]. Finally, the heavily scrutinized pro-inflammatory gene COX-2, which is under direct control of NF-κB, has been demonstrated to be closely involved in the process of carcinogenesis[25-30].

This review article will explore NF-κB's role in cancers of the gastrointestinal tract, including esophageal, gastric and colonic. Discussion of each cancer will begin with a presentation of inflammatory stimuli, including infection and other inciting factors that affect NF-κB's expression. Evidence will then be provided in support of the fact that NF-κB activity increases through progression of inflammation to cancer on a continuum that culminates in constitutive expression. The documented upregulation of NF-κB's downstream proteins in each specific GI malignancy will be discussed next, with emphasis of COX-2 expression. This will be followed by an illustration of the importance of targeting the NF-κB pathway for greater therapeutic efficacy, including addressing chemoresistance. In essence, the amalgamation of the data presented in this review will uncover NF-κB as the mechanistic link between inflammation and tumor growth, specifically in gastrointestinal cancers. Finally, common themes will be clarified in emphasizing the connection between the two entities.

NF-κB'S ROLE IN ESOPHAGEAL CARCINOGENESIS

Esophageal cancer is responsible for roughly 16000 deaths in the United States per year; mortality depends on cell type (adenocarcinoma *vs* squamous), risk factors and location of pathology[31]. There is increasing evidence that the NF-κB pathway mediates the progression of inflamed esophageal epithelium through carcinogenesis[30,31]. This begins when the upper GI tract is exposed to noxious stimuli that promote inflammation and foster a propensity for cancerous growth[30,31]. Examples of such stimuli include tobacco smoke, alcohol, reflux, corrosive injury, nitrosamines, HPV and thermal injury[31]. Moreover, multiple studies link exposure to upregulation of NF-κB. One study conducted by Abdel-Latif *et al*[32] showed that bile acids and low pH induced expression of NF-κB in esophageal cell lines. Similarly, another study illustrated that physiological levels of bile acid not only activated NF-κB, but also led to increased expression of the downstream protein IL-8[33]. It is suggested that after epithelial cells are exposed to inflammatory stimuli, they defensively resort to activating the NF-κB pathway in order to prevent apoptosis and sustain existence by DNA repair[34]. This persistence predisposes the cells to malignancy as they continue to proliferate due to genetic alterations[35].

Involvement of NF-κB in promoting inflammatory changes to metaplasia and the progression to esophageal cancer has been demonstrated in the literature[36]. Current evidence supports constitutive NF-κB activity in cancerous growth of both adenocarcinoma and squamous cell carcinoma in *in vivo* and *in vitro* studies[11,36-38]. In one study, nuclear immunologic staining revealed increased nuclear expression of p50, p52 and ReI in patient biopsies of esophageal cancer cells compared to normal squamous cells[25]. Abdel-Latif *et al*[38] showed that overexpression of NF-κB in tumor tissues correlated with higher expression of Rel-A and lower expression of IκB when compared to expression levels in non-tumor cells. This was further supported by the finding that NF-κB expression was increased in patients of Barrett's epithelium, however there was virtually no expression in patients with normal esophageal epithelium[38,39]. In another study, 40% NF-κB expression was noted in Barrett's tissues, but markedly increased to 76% in adenocarcinomas[36]. In addition to demonstrating NF-κB's presence in esophageal malignancy, studies have also correlated NF-κB activity with metastasis[40].

It is the downstream proteins that are responsible for exerting oncogenic effects, however, and these are vital in inflammation-related tumorigenesis. A number of studies have examined this increase in inflammatory proteins specific to esophageal malignancy. O'Riordan *et al*[37] demonstrated the elevation of proinflammatory cytokines IL-8 and IL-1B in esophagitis, Barrett's epithelium and adenocarcinoma in human histological specimens. These cytokines contribute to tumor progression by regulating angiogenesis, sustaining cancer cell growth and promoting tumor cell migration. Investigators further found a significant association of NF-κB activation and cytokine upregulation in adenocarcinoma. Similar results have been found in other studies with regard to increased pro-inflammatory cytokines in esophageal cancerous growth[11,38]. Recent evidence also supports NF-κB's role in invasion and metastasis in esophageal carcinomas as it leads to the upregulation of MMP-9 and reduction of E cadherin, two proteins involved in cell migration[41].

Another example illustrating NF-κB's involvement in neoplastic progression is the overexpression of COX-2, an enzyme important in regulating prostaglandin synthesis[42]. In the context of cancer, increased COX-2 activity has been shown to be associated with key pathways that control cell proliferation, migration,

apoptosis, and angiogenesis[11]. Previous studies have revealed increased simultaneous expression of NF-κB and COX-2 in cells exposed to inflammation and cells of malignant growth when compared to normal esophageal mucosa[29]. Studies exploring the effect of COX-2 inhibitors have also shed light upon the relationship between COX-2 and progression to malignancy. Liu *et al*[43] studied the effects of aspirin at different concentrations and different times in esophageal squamous cell carcinoma cell lines and found that aspirin significantly reduced COX-2 mRNA and protein expression and prostaglandin synthesis. The study's results demonstrate a dose-dependent response to aspirin in reducing cell proliferation and inducing apoptosis. In a recent meta-analysis, use of aspirin and other COX inhibitors was shown to be associated with reduced risk of esophageal adenocarcinoma among patients. They noted that the use of COX inhibitors decreased the risk of transition from Barrett's esophagus to esophageal cancer. Many *in vivo* and *in vitro* studies have reported similar results and advocate for aspirin use in both preventing and treating abnormal esophageal changes.

Patients with esophageal cancer often have a poor prognosis due to late presentation of the disease. Therefore, it is important to explore new treatments, such as those that target NF-κB[44,45]. In esophageal adenocarcinoma, elevated NF-κB expression is associated with advanced stages and inversely correlated with response to neoadjuvant chemotherapy and radiation[46,47]. NF-κB expression in esophageal cancers is indicative of poor prognosis and may be implicated in multidrug resistance and treatment failure. In a recent study, NF-κB activity in patients with esophageal squamous and adenocarcinoma correlated with TMN staging, increased susceptibility to spread, chemoresistance and overall poor survival rate[48]. Interestingly, their data also demonstrated patients who were established to have NF-κB negative cancer prior to treatment became positive after receiving chemotherapy, suggesting chemotherapy as a potential initiator of tumor resistance through upregulation of NF-κB[48]. Treatment with chemotherapy may induce resistance to the therapy, and therefore, result in poorer prognosis. In addition, Izzo *et al*[48] showed that after constitutive NF-κB activity was established, exposure to NF-κB inhibitors, such as Bay11-7082 and sulfasalazine, reduced cancer cell proliferation and induced apoptosis. They also found that treatment with these inhibitors in combination with chemotherapeutic drugs 5-fluorouracil and cisplatin led to a synergistic effect on inhibiting cell growth, decreasing chemoresistance[11]. This investigation also illustrated that Bay 11 was associated with decreased tumor growth and angiogenesis in animal studies[11]. Tian *et al*[49] found similar results with siRNA molecules of p65. They first found p65 expression was decreased in ESCC cell lines when exposed to siRNA p65 after establishing constitutive activity of the NF-κB signaling pathway. Moreover, they too found a synergistic effect of siRNA with 5FU as ESCC cells became more sensitive to 5-FU after exposure to siRNA p65. Finally, utilization of neoadjuvant treatment with NF-κB inhibitors in the management of esophageal carcinomas was shown to help in a specific cohort of refractory treatment cases[46,47].

NF-κB'S ROLE IN GASTRIC CARCINOGENESIS

Gastric cancer further elucidates the role of NF-κB as a central mediator of carcinogenesis and as an important link between inflammation and cancer[50,51]. Gastric cancer, especially the intestinal type, entails the progression from chronic gastritis to chronic atrophic gastritis to intestinal metaplasia, then dysplasia and finally adenocarcinoma[52]. The initiation of chronic inflammation typically begins with the presence of such risk factors as *Helicobacter pylori* (*H. pylori*) infection, pernicious anemia, smoking or high salt diet. This leads to longstanding chronic superficial gastritis, which eventually progresses to intestinal metaplasia followed by adenocarcinoma[53,54]. *H. pylori*'s detrimental effect on gastric mucosa has been well established in the literature through clinical data, animal studies and cell cultures[52,53]. However, only certain *H. pylori* strains have been shown to be involved in malignancy and these have the pathogenic island, CagA[54]. Current studies have established that CagA-positive *H. pylori* significantly contributes to gastric cancer cell tissue invasion[55,56]. Wu *et al*[57] visualized the link between CagA-positive *H. pylori* and NF-κB by using NF-κB blockers. Exposing gastric cancer cells infected by CagA-positive strains to NF-κB blockers significantly reduced tissue invasiveness. This not only supported CagA's effects as a pathogenic factor, but demonstrated the significant role of NF-κB. These data support the idea that *H. pylori* becomes a potent NF-κB activator if it carries CagA pathogenicity. The exact molecular pathway between CagA and NF-κB cannot be addressed due to a paucity of evidence. Some studies speculate the connection lies in the protein transforming growth factor-beta-activated kinase 1 (TAK1)[54].

Constitutive activation of the NF-κB pathway and its increased production in gastric cancer cells of human tissue and cell lines has been demonstrated through various laboratory techniques, including IHC, EMSA and Western blotting[5,57,58]. Constitutive NF-κB activity has also been documented in *H. pylori* gastritis and diffuse gastric cancer[11,59]. Although chronic irritants initiate mucosal inflammation, it is the NF-κB pathway which is vital in sustaining the gastritis-metaplasia-carcinoma sequence. Studies have shown that NF-κB is notably higher in patients with *H. pylori* gastritis, and this is significantly correlated with histological scores of gastritis[60]. In intestinal type gastric cancer, increased NF-κB expression is strongly associated with CagA strain-induced metaplasia, dysplasia and gastric

cancer itself[56]. This is currently under debate for diffuse type gastric cancer. Yamanaka *et al*[61] identified a major function of NF-κB in carcinogenesis when they found that patients with higher NF-κB activity had a shorter survival rate and concluded that NF-κB was a prognostic indicator in gastric cancer. Interestingly, NF-κB expression is also associated with stage, depth of invasion, WHO classification and Lauren's histological classification[11,51,52,58,62]. Levidou *et al*[11] further concluded through multivariate survival analysis that NF-κB1 expression was an independent predictor of gastric cancer prognosis.

For the gastritis-to-carcinoma sequence to be successful, each step requires a pattern of oncoprotein expression to promote carcinogenesis. As in esophageal cancer, NF-κB regulates these oncoproteins in gastric cancer as well in terms of endorsing tumor growth, preventing apoptosis, and initiating tumor angiogenesis and migration. Keates *et al*[63] demonstrated an increased activation of NF-κB and its downstream proteins by infecting gastric epithelial cells with *H. pylori* and then observing increases in p50/p65 heterodimers and p50 dimers, followed by increased IL-8 mRNA and protein synthesis. Yin *et al*[64] found that IL-6 and VEGF were significantly increased in NF-κB positive gastric cancer cells compared to their levels in normal mucosa. Like IL-8, IL-6 is also associated with malignancy-both facilitate cancer cell apoptosis and stimulation of angiogenesis. VEGF is a well-known growth factor implicated in angiogenesis and therefore is crucial in carcinogenesis[65]. Other studies have shown that gastric cell lines exposed to *H. pylori* have displayed upregulation of important tumorigenesis markers including MMP-9, VEGF and COX-2[50].

NF-κB is also an important regulator of COX-2 in gastric cancer as it is in esophageal cancer. Expression of NF-κB and COX-2 in the same neoplastic mucosa has been established in previous studies[66,67]. Studies exploring COX-2 inhibitors also shed light upon the specific actions of COX-2 and its potential applications[58,68]. In one study, p50 positive cells treated with COX inhibitors showed a dose-dependent suppression of cell growth in gastric cells[66]. A large population-based case-control study conducted by Farrow *et al*[69] revealed that patients who took aspirin or NSAIDs had a decreased risk of gastric and esophageal cancer compared to never users. Evidence provides support that COX-2 inhibitors may prevent gastric carcinogenesis and can aid in treating gastric malignancy[69]. COX-2 expression is, in part, facilitated by NF-κB expression; if the central regulator can be inhibited, then all downstream proteins, including COX-2, may be suppressed, thereby resulting in reduced oncogenesis[50].

Despite advances in current therapeutic approaches, gastric cancer is still one the most prevalent cancers and the second leading cause of death due to cancer, worldwide. Therefore, new treatment options should be explored by scrutinizing the molecular pathway of the gastritis- adenocarcinoma sequence. The NF-κB pathway provides a link between inflammation and cancer and can explain many of the hallmarks of cancer[50,52]. Moreover, literature has illustrated the dysregulation of the NF-κB signaling pathway in gastric cancer, with its presence indicating poor prognosis[61]. Studies have shown in CagA-mediated gastric cancer that cell migration is attenuated by NF-κB inhibitors. These results were expanded upon in a study conducted by Keates *et al*[63], which found that pretreatment with PDTC, a potent NF-κB inhibitor, reduced *H. pylori*-activated p65 and IL-8. In one study, parthenolide, another NF-κB inhibitor, was used on three gastric cancer cell lines and significantly induced apoptosis in all three[70]. They also found a synergistic effect when NF-κB inhibitors were combined with chemotherapeutic drugs[71]. This finding is analogous to that of combining NF-κB inhibitors and chemotherapy in addressing esophageal cancers.

NF-κB'S ROLE IN COLORECTAL CARCINOGENESIS

The development of colorectal cancer (CRC) is a multistep process and there is growing evidence in favor of the connection between inflammation and carcinogenesis. NF-κB has become the main focus of this neoplastic transformation. CRC can be divided into sporadic CRC, hereditary CRC and colitis-associated carcinoma (CAC). CAC primarily stems from the two major forms of inflammatory bowel disease (IBD): ulcerative colitis and Crohn's disease. NF-κB's role as the mechanistic link between inflammation and cancer has been intensively investigated regarding constitutive activity of NF-κB in both IBDs and CAC[71-74]. CAC is one of the most well-known examples of an inflammation-dysplasia-carcinoma sequence[73,75]. IBD prompts a chronic inflammatory state leading to the constant production of noxious compounds including reactive oxygen species (ROS) and other cytokines (TNF-α, IL-6, and IL-1). In the long term, ROS are detrimental as they repress regulators of DNA damage and induce mutagenic enzymes, such as activation-induced cytidine deaminase[74]. Furthermore, studies indicate that both ROS and cytokines activate the NF-κB pathway[74]. These cytokines activate signal transducer and activator of transcription 3 (STAT3) in intestinal epithelial cells. This, in turn, contributes to further inflammation, perpetuating noxious stimuli and ultimately creating a positive feedback loop. The combination of DNA damaging agents and inflammatory cytokines results in constant activation of the NF-κB pathway, setting up the progression to carcinogenesis.

Overexpression of NF-κB has been demonstrated in colon cancer cell lines and human tumor specimens, including those of sporadic CRC and CAC[74,76]. This is synonymous with genetic syndromes including familial adenomatous polyposis (FAP) and hereditary non-polyposis cancer (HNPCC)[72]. The expression of

NF-κB was determined to be significantly higher in the adenocarcinoma tissue compared to expression levels in tissue specimens earlier in the inflammation-adenocarcinoma sequence[77,78]. Furthermore, studies have shown that NF-κB activity increases in association with histological tumor progression[74,79]. This indicates that NF-κB activity correspondingly increases from the initiation of inflammation to malignant proliferation. Notably, one study concluded that NF-κB activation in CRC served as a poor prognostic indicator[78].

As in esophageal and gastric cancer, NF-κB regulates expression of various oncoproteins in colorectal cancer as well to sustain inflammation and support cell proliferation, inhibition of apoptosis, angiogenesis, invasion and metastasis. Overexpression of NF-κB and its downstream effectors are well recognized in CRC[56,79]. For example, IL-8 upregulation was also described in CRC and was identified in various ways including microarray and protein array analyses[28]. In addition, another cytokine, IL-6, has been found to be weakly correlated with poor prognosis in CRC. In a well-known study, Greten *et al*[24] investigated the role of NF-κB in inflammation-associated tumor growth using a mouse model and found that IKKβ deletion led to a direct decrease in tumor incidence. They attributed these results to decreased expression of anti-apoptotic proteins. CRC also has a significant association between NF-κB overexpression and VEGF[24].

Another emerging theme is the relationship between NF-κB and its downstream protein COX-2. Studies of CRC have shown an upregulation of both NF-κB and COX-2 and the literature also supports their individual and coexistent overexpression[23,24,27,75,78-82]. Maihöfner *et al*[83] studied surgically resected tissues of CRC patients and found a high expression of p65, IL-6 and COX-2 compared to controls[81,82]. It is well established that regular aspirin use is associated with a significant reduction in the risk of COX-2-positive CRC[80]. The relative risk of CRC is reduced by 40%-50% when patients consume aspirin or NSAIDs over a period of 10-15 years[24]. Moreover, epidemiologic evidence shows that NSAID usage protects against CRC to a greater extent than against other GI malignancies. In one study, aspirin exposure resulted in apoptosis of colorectal cancer cell lines in a concentration-dependent manner[83]. Specifically, given that CAC is the result of inflammatory processes, it follows that anti-inflammatory medication would reduce the risk of cancer.

Inhibition of the master regulator NF-κB will shut off the different steps of carcinogenesis. NF-κB inhibitors, such as sulfasalazine, mesalamine, and glucocorticoids, have already shown promise in treating IBD[23]. Gan *et al*[84] explored the effects of sulfasalazine on the NF-κB signaling pathway. After establishing that patients with ulcerative colitis (UC) had higher NF-κB and downstream proteins including IL-6 and IL-8, those who were exposed to sulfasalazine demonstrated a decrease in all three proteins[84]. Corticosteroids strongly inhibit NF-κB activation *in vivo* and *in vitro*, and clinical trials have found dexamethasone to be useful in inhibiting CRC metastasis. One study illustrated this by using NF-κB inhibitors and displaying that blocking the action of NF-κB led to inhibition of angiogenesis in CRC[79]. Another study reported these same inhibitors prompted apoptosis, as NF-κB could not overcome the anti-apoptotic machinery to exert pro-survival activity[72]. After establishing constitutive activity of NF-κB in CRC, Lind *et al*[72] further showed that NF-κB binding was increased in CRC cell lines after exposure to the chemotherapeutic agent. In contrast, pretreating cells with NF-κB inhibitors prior to administration of gemcitabine showed a decrease in tumor size. The common theme of chemoresistance resurfaces and NF-κB may be involved in its development. With regards to CRC, NF-κB inhibitors are already showing clinical applications. Further research should be employed, as this pathway is involved in many innate processes and therefore may have a large range of systemic side effects.

CONCLUSION

This review showed support for NF-κB's role in inflammation-associated cancers. NF-κB activation is elicited by various inflammatory stimuli (Figure 1). Chronic irritation induces constitutive NF-κB activity, promoting carcinogenesis (Figure 1). NF-κB's mechanistic link to cancer is best displayed through its pleiotropic effects, as it leads to upregulation of important proteins that promote tumor progression (Figure 1). Upregulation of COX-2 by NF-κB in GI malignancies is consistently supported in the literature and the efficacy of COX-2 inhibitors is still being scrutinized. This review also confirmed the presence of NF-κB in advanced gastrointestinal malignancies. Additionally, this review noted that NF-κB may play a role in developing chemo-resistance in gastrointestinal malignancies. Recent investigations by Vyas *et al*[85] postulate that chemotherapies may pose therapy-induced resistance by stimulating various signaling pathways including the NF-κB cascade. The authors clearly delineated literature supporting that first-line chemotherapy agents such as doxorubicin, 5-FU, cisplatin and paclitaxel commonly induce NF-κB production. They showed that this led to increased expression of downstream proteins that promote proliferation, anti-apoptosis and angiogenesis to sustain tumor growth[85]. These findings demonstrated the need for further research in discovering common themes among the gastrointestinal malignancies. Interestingly, Kim *et al*[86] recently reported a significant increase in the protein caspase-associated recruitment domain 6 (CARD6), a NF-κB activator in esophageal, gastric and colorectal tissues[87]. Finally, targeted therapy focusing upon NF-κB inhibition and its outcomes for the individual cancers was also discussed. The amalgamation of all the literature presented in this review emphasizes the importance of exploring molecular targeted therapy to hone in on NF-κB. In addition, although NF-κB is the central mediator,

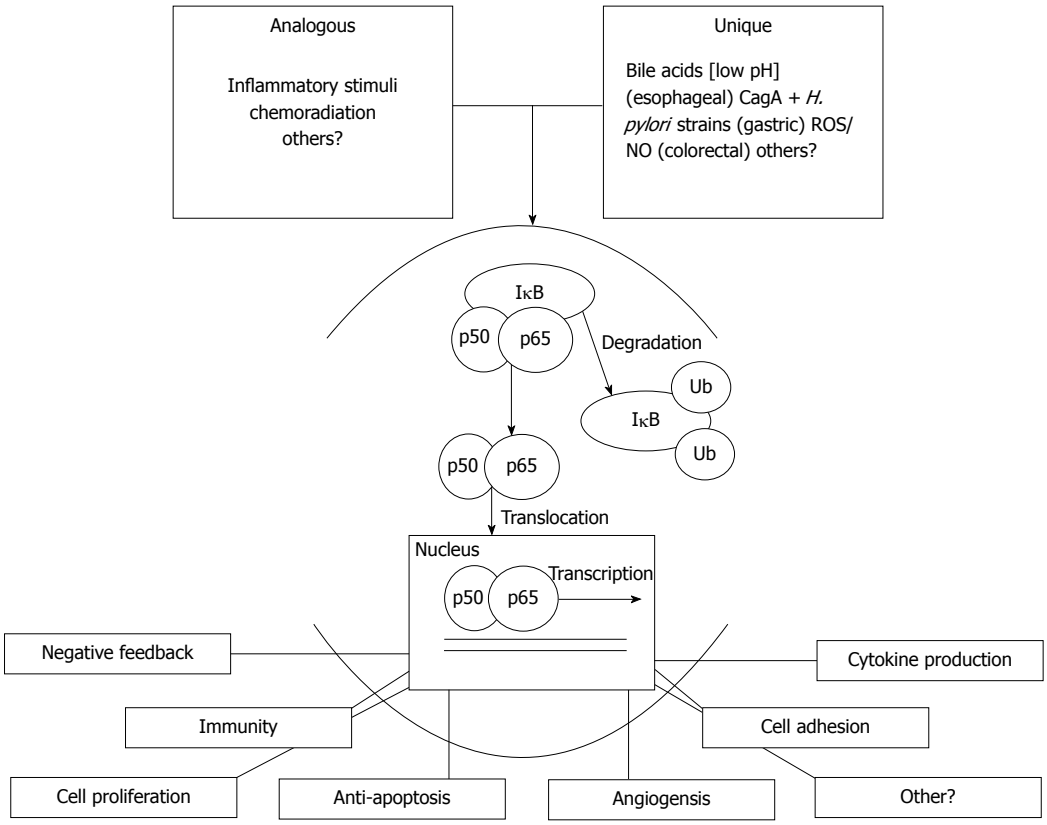


Figure 1 Summary model representing stimulation of the NF-κB pathway leading to the classical pathway, followed by expression of NF-κB dependent proteins. Analogous inflammatory stimuli are common amongst esophageal, gastric and colorectal tissue. The unique stimuli commonly induce inflammation. The classic pathway is displayed and this model would be consistent with the alternate pathway. An array of genes are transcribed and translated contributing to the carcinogenesis. ROS: Reactive oxygen species.

targeting the regulators of the actual pathway itself could also prove fruitful. For instance, the use of histone deacetylases (HDACs) has gained popularity as an emerging strategy in inhibiting the NF-κB pathway. Histone acetyltransferases and deacetylases modulate NF-κB activity through their actions on the Re1A/p65 subunit[87]. Yun *et al*[88] showed acetylation of Re1A/p65 subsequently increased NF-κB activation and deacetylation lead to diminished levels of Re1A/p65 and thus NF-κB. This was further explored when Yeung *et al*[89] demonstrated that the use of sirtuins, a group of nicotinamide adenosine dinucleotide-dependent HDACs, led to increased apoptosis as these compounds decreased NF-κB levels; this is yet another potential strategy for intervention in the future.

NF-κB may induce inflammatory-associated gastrointestinal carcinomas which are often refractory to current treatments. We, therefore, urge greater exploration and development of therapies specifically targeting the NF-κB pathway as these may prove more successful for patients than existing therapeutic options.

REFERENCES

1 **Baud V**, Karin M. Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. *Nat Rev Drug Discov* 2009; **8**: 33-40 [PMID: 19116625 DOI: 10.1038/nrd2781]

2 **Bonizzi G**, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004; **25**: 280-288 [PMID: 15145317 DOI: 10.1016/j.it.2004.03.008]

3 **Vyas D**, Robertson CM, Stromberg PE, Martin JR, Dunne WM, Houchen CW, Barrett TA, Ayala A, Perl M, Buchman TG, Coopersmith CM. Epithelial apoptosis in mechanistically distinct methods of injury in the murine small intestine. *Histol Histopathol* 2007; **22**: 623-630 [PMID: 17357092]

4 **Karin M**, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2002; **2**: 301-310 [PMID: 12001991 DOI: 10.1038/nrc780]

5 **Karin M**. NF-kappaB and cancer: mechanisms and targets. *Mol Carcinog* 2006; **45**: 355-361 [PMID: 16673382 DOI: 10.1002/mc.20217]

6 **DiDonato JA**, Mercurio F, Karin M. NF-κB and the link between inflammation and cancer. *Immunol Rev* 2012; **246**: 379-400 [PMID: 22435567 DOI: 10.1111/j.1600-065X.2012.01099.x]

7 **Ditsworth D**, Zong WX. NF-kappaB: key mediator of inflammation-associated cancer. *Cancer Biol Ther* 2004; **3**: 1214-1216 [PMID: 15611628 DOI: 10.4161/cbt.3.12.1391]

8 **Bours V**, Dejardin E, Goujon-Letawe F, Merville MP, Castronovo V. The NF-kappa B transcription factor and cancer: high expression of NF-kappa B- and I kappa B-related proteins in tumor cell lines. *Biochem Pharmacol* 1994; **47**: 145-149 [PMID: 8311838 DOI: 10.1016/0006-2952(94)90448-0]

9 **Claudio E**, Brown K, Park S, Wang H, Siebenlist U. BAFF-induced NEMO-independent processing of NF-kappa B2 in maturing B cells. *Nat Immunol* 2002; **3**: 958-965 [PMID: 12352969 DOI: 10.1038/ni842]

10 **Howe LR**. Inflammation and breast cancer. Cyclooxygenase/prostaglandin signaling and breast cancer. *Breast Cancer Res* 2007; **9**: 210 [PMID: 17640394 DOI: 10.1186/bcr1678]

11 **Levidou G**, Korkolopoulou P, Nikiteas N, Tzanakis N, Thymara I, Saetta AA, Tsigris C, Rallis G, Vlasis K, Patsouris E. Expression of Nuclear Factor κB in Human Gastric Carcinoma: Relationship

with IκBa and Prognostic Significance. *Virchows Archiv* 2007; **450**: 519-527 [PMID: 17429689 DOI: 10.1007/s00428-007-0396-5]
12 **Mukhopadhyay T**, Roth JA, Maxwell SA. Altered expression of the p50 subunit of the NF-kappa B transcription factor complex in non-small cell lung carcinoma. *Oncogene* 1995; **11**: 999-1003 [PMID: 7675461]
13 **Nakshatri H**, Bhat-Nakshatri P, Martin DA, Goulet RJ, Sledge GW. Constitutive activation of NF-kappaB during progression of breast cancer to hormone-independent growth. *Mol Cell Biol* 1997; **17**: 3629-3639 [PMID: 9199297]
14 **Shattuck-Brandt RL**, Richmond A. Enhanced degradation of I-kappaB alpha contributes to endogenous activation of NF-kappaB in Hs294T melanoma cells. *Cancer Res* 1997; **57**: 3032-3039 [PMID: 9230219]
15 **Tai DI**, Tsai SL, Chang YH, Huang SN, Chen TC, Chang KS, Liaw YF. Constitutive activation of nuclear factor kappaB in hepatocellular carcinoma. *Cancer* 2000; **89**: 2274-2281 [PMID: 11147598 DOI: 10.1002/1097-0142]
16 **Visconti R**, Cerutti J, Battista S, Fedele M, Trapasso F, Zeki K, Miano MP, de Nigris F, Casalino L, Curcio F, Santoro M, Fusco A. Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires NFkappaB p65 protein expression. *Oncogene* 1997; **15**: 1987-1994 [PMID: 9365245 DOI: 10.1038/sj.onc.1201373]
17 **Wang W**, Abbruzzese JL, Evans DB, Larry L, Cleary KR, Chiao PJ. The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. *Clin Cancer Res* 1999; **5**: 119-127 [PMID: 9918209]
18 **Bargou RC**, Leng C, Krappmann D, Emmerich F, Mapara MY, Bommert K, Royer HD, Scheidereit C, Dörken B. High-level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. *Blood* 1996; **87**: 4340-4347 [PMID: 8639794]
19 **Feinman R**, Koury J, Thames M, Barlogie B, Epstein J, Siegel DS. Role of NF-kappaB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2. *Blood* 1999; **93**: 3044-3052 [PMID: 10216101]
20 **Bassères DS**, Baldwin AS. Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression. *Oncogene* 2006; **25**: 6817-6830 [PMID: 17072330 DOI: 10.1038/sj.onc.1209942]
21 **Dolcet X**, Llobet D, Pallares J, Matias-Guiu X. NF-kB in development and progression of human cancer. *Virchows Arch* 2005; **446**: 475-482 [PMID: 15856292 DOI: 10.1007/s00428-005-1264-9]
22 **Brown KD**, Estefania Claudio, and Ulrich Siebenlist. The Roles of the Classical and Alternative Nuclear Factor-kappaB Pathways: Potential Implications for Autoimmunity and Rheumatoid Arthritis. *Arthritis Research Therapy* 2008; **10**: 212 [PMID: 18771589 DOI: 10.1186/ar2457]
23 **Greten FR**, Karin M. The IKK/NF-kappaB activation pathway-a target for prevention and treatment of cancer. *Cancer Lett* 2004; **206**: 193-199 [PMID: 15013524 DOI: 10.1016/j.canlet.2003.08.029]
24 **Greten FR**, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 2004; **118**: 285-296 [PMID: 15294155 DOI: 10.1016/j.cell.2004.07.013]
25 **Kang MR**, Kim MS, Kim SS, Ahn CH, Yoo NJ, Lee SH. NF-kappaB signalling proteins p50/p105, p52/p100, RelA, and IKKepsilon are over-expressed in oesophageal squamous cell carcinomas. *Pathology* 2009; **41**: 622-625 [PMID: 20001340 DOI: 10.3109/00313020903257756]
26 **Karin M**, Lin A. NF-kappaB at the crossroads of life and death. *Nat Immunol* 2002; **3**: 221-227 [PMID: 11875461 DOI: 10.1038/ni0302-221]
27 **Rogler G**, Brand K, Vogl D, Page S, Hofmeister R, Andus T, Knuechel R, Baeuerle PA, Schölmerich J, Gross V. Nuclear Factor κB Is Activated in Macrophages and Epithelial Cells of Inflamed Intestinal Mucosa. *Gastroenterology* 1998; **115**: 357-369 [PMID: 9679041 DOI: 10.1016/S0016-5085(98)70202-1]
28 **Zubair A**, Frieri M. Role of nuclear factor-κB in breast and colorectal cancer. *Curr Allergy Asthma Rep* 2013; **13**: 44-49 [PMID: 22956391 DOI: 10.1007/s11882-012-0300-5]
29 **Konturek PC**, Nikiforuk A, Kania J, Raithel M, Hahn EG, Mühldorfer S. Activation of NFkappaB represents the central event in the neoplastic progression associated with Barrett's esophagus: a possible link to the inflammation and overexpression of COX-2, PPARgamma and growth factors. *Digest Dis Sci* 2004; **49**: 1075-1083 [PMID: 15387324]
30 **Chan AT**, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. *N Engl J Med* 2007; **356**: 2131-2142 [PMID: 17522398 DOI: 10.1056/NEJMoa067208]
31 **Gibson MK**, Dhaliwal AS, Clemons NJ, Phillips WA, Dvorak K, Tong D, Law S, Pirchi ED, Räsänen J, Krasna MJ, Parikh K, Krishnadath KK, Chen Y, Griffiths L, Colleypriest BJ, Farrant JM, Tosh D, Das KM, Bajpai M. Barrett's esophagus: cancer and molecular biology. *Ann N Y Acad Sci* 2013; **1300**: 296-314 [PMID: 24117650 DOI: 10.1111/nyas.12252]
32 **Abdel-Latif MM**, O'Riordan J, Windle HJ, Carton E, Ravi N, Kelleher D, Reynolds JV. NF-kappaB activation in esophageal adenocarcinoma: relationship to Barrett's metaplasia, survival, and response to neoadjuvant chemoradiotherapy. *Ann Surg* 2004; **239**: 491-500 [PMID: 15024310 DOI: 10.1097/01.sla.0000118751.95179.c6]
33 **Jenkins GJ**, Harries K, Doak SH, Wilmes A, Griffiths AP, Baxter JN, Parry JM. The bile acid deoxycholic acid (DCA) at neutral pH activates NF-kappaB and induces IL-8 expression in oesophageal cells in vitro. *Carcinogenesis* 2004; **25**: 317-323 [PMID: 14656946 DOI: 10.1152/ajpgi.00092.2011]
34 **Luo JL**, Kamata H, Karin M. IKK/NF-kappaB signaling: balancing life and death--a new approach to cancer therapy. *J Clin Invest* 2005; **115**: 2625-2632 [PMID: 16200195 DOI: 10.1172/JCI26322]
35 **Hormi-Carver K**, Zhang X, Zhang HY, Whitehead RH, Terada LS, Spechler SJ, Souza RF. Unlike Esophageal Squamous Cells, Barrett's Epithelial Cells Resist Apoptosis by Activating the Nuclear Factor- B Pathway. *Cancer Res* 2009; **69**: 672-677 [PMID: 19147583 DOI: 10.1158/0008-5472.CAN-08-3703]
36 **Jenkins GJ**, Mikhail J, Alhamdani A, Brown TH, Caplin S, Manson JM, Bowden R, Toffazal N, Griffiths AP, Parry JM, Baxter JN. Immunohistochemical Study of Nuclear Factor- B Activity and Interleukin-8 Abundance in Oesophageal Adenocarcinoma; a Useful Strategy for Monitoring These Biomarkers. *J Clin Pathol* 2007; **60**: 1232-237 [PMID: 17220207 DOI: 10.1136/jcp.2006.043976]
37 **O'Riordan JM**, Abdel-latif MM, Ravi N, McNamara D, Byrne PJ, McDonald GS, Keeling PW, Kelleher D,Reynolds JV. Proinflammatory cytokine and nuclear factor kappa-B expression along the inflammation-metaplasia-dysplasia-adenocarcinoma sequence in theesophagus. *Am J Gastroenterol* 2005; **100**: 1257-1264 [PMID: 15929754 DOI: 10.1111/j.1572-0241.2005.41338.x]
38 **Abdel-Latif MMMJ**, O'riordan M, Ravi N, Kelleher D, Reynolds JV. Activated Nuclear Factor-kappa B and Cytokine Profiles in the Esophagus Parallel Tumor Regression following Neoadjuvant Chemoradiotherapy. *Dis Esoph* 2005; **18**: 246-252 [DOI: 10.1111/j.1442-2050.2005.00497.x]
39 **Szachnowicz S**, Cecconello I, Iriya K, Marson AG, Takeda FR, Gama-Rodrigues JJ. Origin of adenocarcinoma in Barrett's esophagus: p53 and Ki67 expression and histopathologic background. *Clinics* (Sao Paulo) 2005; **60**: 103-112 [PMID: 15880245]
40 **Izzo JG**, Correa AM, Wu TT, Malhotra U, Chao CK, Luthra R, Ensor J, Dekovich A, Liao Z, Hittelman WN, Aggarwal BB, Ajani JA. Pretherapy Nuclear Factor- B Status, Chemoradiation Resistance, and Metastatic Progression in Esophageal Carcinoma. *Mole Cancer Therap* 2006; **5**: 2844-850 [DOI: 10.1158/1535-7163.MCT-06-0351]
41 **Wang F**, He W, Fanghui P, Wang L, Fan Q. NF-κBP65 promotes invasion and metastasis of oesophageal squamous cell cancer by regulating matrix metalloproteinase-9 and epithelial-to-mesenchymal transition. *Cell Biol Int* 2013; **37**: 780-8 [PMID: 23504993 DOI: 10.1002/cbin.10089]
42 **Wang F**, He W, Fanghui P, Wang L, Fan Q. NF-κBP65 promotes invasion and metastasis of oesophageal squamous cell cancer

by regulating matrix metalloproteinase-9 and epithelial-to-mesenchymal transition. *Cell Biol Int* 2013; **37**: 780-788 [PMID: 23504993 DOI: 10.1136/gut.2004.047100]

43 **Liu JF**, Jamieson GG, Drew PA, Zhu GJ, Zhang SW, Zhu TN, Shan BE, Wang QZ. Aspirin Induces Apoptosis in Oesophageal Cancer Cells by Inhibiting the Pathway of NF-kappaB Downstream Regulation of Cyclooxygenase-2. *ANZ J Surg* 2005; **75**: 1011-1016 [PMID: 16336399 DOI: 10.1111/j.1445-2197.2005.03596.x]

44 **Lin A**, Karin M. NF-kappaB in cancer: a marked target. *Semin Cancer Biol* 2003; **13**: 107-114 [PMID: 12654254]

45 **Monks NR**, Biswas DK, Pardee AB. Blocking anti-apoptosis as a strategy for cancer chemotherapy: NF-kappaB as a target. *J Cell Biochem* 2004; **92**: 646-650 [PMID: 15211562 DOI: 10.1002/jcb.20080]

46 **Li B**, Li YY, Tsao SW, Cheung ALM. Targeting NF- B Signaling Pathway Suppresses Tumor Growth, Angiogenesis, and Metastasis of Human Esophageal Cancer. *Mole Cancer Therap* 2009; **8**: 2635-2644 [PMID: 19723887 DOI: 10.1158/1535-7163.MCT-09-0162]

47 **Li J**, Wang K, Chen X, Meng H, Song M, Wang Y, Xu X, Bai Y. Transcriptional activation of microRNA-34a by NF-kappa B in human esophageal cancer cells. *BMC Mol Biol* 2012; **13**: 4 [PMID: 22292433 DOI: 10.1186/1471-2199-13-4]

48 **Izzo JG**, Malhotra U, Wu TT, Ensor J, Luthra R, Lee JH, Swisher SG, Liao Z, Chao KS, Hittelman WN, Aggarwal BB, Ajani JA. Association of Activated Transcription Factor Nuclear Factor B With Chemoradiation Resistance and Poor Outcome in Esophageal Carcinoma. *J Clini Oncol* 2006; **24**: 748-754 [PMID: 16401681 DOI: 10.1200/JCO.2005.03.8810]

49 **Tian F**, Zang WD, Hou WH, Liu HT, Xue LX. Nuclear Factor-κB Signaling Pathway Constitutively Activated in EsophagealSquamous Cell Carcinoma Cell Lines and Inhibition of Growth of Cells by Small Interfering RNA. *Acta Biochim Biophys Sin* 2006; **38**: 318-326 [PMID: 16680372 DOI: 10.1111/j.1349-7006.2011.02025.x]

50 **Han JC**, Kai-Li Zhang, Xiao-Yan Chen, Hai-Feng Jiang, Qing-You Kong, Yuan Sun, Mo-Li Wu, Lei Huang, Hong Li, and Jia Liu. Expression of Seven Gastric Cancer-associated Genes and Its Relevance for Wnt, NF-κB and Stat3 Signaling. *Apmis* 2007; **115**: 1331-343 [PMID: 18184402 DOI: 10.1111/j.1600-0643.2007.00695.x]

51 **Kwon HC**, Kim SH, Oh SY, Lee S, Lee JH, Jang JS, Kim MC, Kim KH, Kim SJ, Kim SG, Kim HJ.. Clinicopathologic Significance of Expression of Nuclear Factor-κB RelA and Its Target Gene Products in Gastric Cancer Patients. *World J Gastroenterol* 2012; **18**: 4744 [PMID: 23002344 DOI: 10.3748/wjg.v18.i34.4744]

52 **Peek RM**, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2002; **2**: 28-37 [PMID: 11902583 DOI: 10.1038/nrc703]

53 **Isomoto H**, Mizuta Y, Miyazaki M, Takeshima F, Omagari K, Murase K, Nishiyama T, Inoue K, Murata I, Kohno S. Implication of NF-kappaB in Helicobacter pylori-associated gastritis. *Am J Gastroenterol* 2000; **95**: 2768-2776 [PMID: 11051346 DOI: 10.1111/j.1572-0241.2000.02304.x]

54 **Lamb A**, Yang XD, Tsang YHN, Li JD, Higashi H, Hatakeyama M, Peek RM, Blanke SR, Chen LF. Helicobacter Pylori CagA Activates NF-κB by Targeting TAK1 for TRAF6-mediated Lys 63 Ubiquitination. *EMBO Rep* 2009; **10**: 1242-1249 [PMID: 19820695 DOI: 10.1038/embor.2009.210]

55 **Kang DW**, Hwang WC, Park MH, Ko GH, Ha WS, Kim KS, Lee YC, Choi KY, Min DS. Rebamipide Abolishes Helicobacter Pylori CagA-induced Phospholipase D1 Expression via Inhibition of NFκB and Suppresses Invasion of Gastric Cancer Cells. *Oncogene* 2012; **32**: 3531-3542 [PMID: 22890316 DOI: 10.1038/onc.2012.358]

56 **Yang GF**, Deng CS, Xiong YY, Gong LL, Wang BC, Luo J. Expression of nuclear factor-kappa B and target genes in gastric precancerous lesions and adenocarcinoma: association with Helicobactor pylori cagA (+) infection. *World J Gastroenterol* 2004; **10**: 491-496 [PMID: 14966904]

57 **Wu LF**, Pu Z, Feng J, Li G, Zheng Z, Shen W. The Ubiquitin-proteasome Pathway and Enhanced Activity of NF-κB in Gastric Carcinoma. *J Surg Oncol* 2008; **97**: 439-444 [PMID: 18163448 DOI: 10.1002/jso.20952]

58 **Yu BC**, Jiang XH, Fan XM, Lin MCM, Jiang SH, Lam SK, Kung HF. Suppression of RelA/p65 Nuclear Translocation Independent of IκB-α Degradation by Cyclooxygenase-2 Inhibitor in Gastric Cancer. *Oncogene* 2003; **22**: 1189-197 [PMID: 12606945 DOI: 10.1038/sj.onc.1206234]

59 **Sasaki N**, Morisaki T, Hashizume K, Yao T, Tsuneyoshi M, Noshiro H, Nakamura K, Yamanaka T, Uchiyama A, Tanaka M, Katano M. Nuclear factor-kappaB p65 (RelA) transcription factor is constitutively activated in human gastric carcinoma tissue. *Clin Cancer Res* 2001; **7**: 4136-4142 [PMID: 11751513]

60 **Ooi CH**, Ivanova T, Wu J, Lee M, Tan IB, Tao J, Ward L, Koo JH, Gopalakrishnan V, Zhu Y, Cheng LL, Lee J, Rha SY, Chung HC, Ganesan K, So J, Soo KC, Lim D, Chan WH, Wong WK, Bowtell D, Yeoh KG, Grabsch H, Boussioutas A, Tan P. Oncogenic Pathway Combinations Predict Clinical Prognosis in Gastric Cancer. *PLoS Genetics* 2009; **5**: e1000676 [PMID: 19798449 DOI: 10.1371/journal.pgen.100067]

61 **Yamanaka N**, Sasaki N, Tasaki A, Nakashima H, Kubo M, Morisaki T, Noshiro H, Yao T, Tsuneyoshi M, Tanaka M, Katano M. Nuclear factor-kappaB p65 is a prognostic indicator in gastric carcinoma. *Anticancer Res* 2004; **24**: 1071-1075 [PMID: 15154625]

62 **Long YM**, Ye S, Rong J, Xie WR. Nuclear factor kappa B: a marker of chemotherapy for human stage IV gastric carcinoma. *World J Gastroenterol* 2008; **14**: 4739-4744 [PMID: 18720533 DOI: 10.3748/wjg.14.4739]

63 **Keates S**, Hitti YS, Upton M, Kelly CP. Helicobacter pylori infection activates NF-kappa B in gastric epithelial cells. *Gastroenterology* 1997; **113**: 1099-1109 [PMID: 9322504]

64 **Yin Y**, Si X, Gao Y, Gao L, Wang J. The nuclear factor-κB correlates with increased expression of interleukin-6 and promotes progression of gastric carcinoma. *Oncol Rep* 2013; **29**: 34-38 [PMID: 23117246 DOI: 10.3892/or.2012.2089]

65 **Guo Y**, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. *Cancer Treat Rev* 2012; **38**: 904-910 [PMID: 22651903 DOI: 10.1016/j.ctrv.2012.04.007]

66 **Lim JW**, Kim H, Kim KH. Expression of Ku70 and Ku80 mediated by NF-kappa B and cyclooxygenase-2 is related to proliferation of human gastric cancer cells. *J Biol Chem* 2002; **277**: 46093-46100 [PMID: 12324457 DOI: 10.1074/jbc.M206603200]

67 **Liu XJ**, Chen ZF, Li HL, Hu ZN, Liu M, Tian AP, Zhao D, Wu J, Zhou YN, Qiao L. Interaction between cyclooxygenase-2, Snail, and E-cadherin in gastric cancer cells. *World J Gastroenterol* 2013; **19**: 6265-6271 [PMID: 24115825 DOI: 10.3748/wjg.v19.i37.6265]

68 **Wu CY**, Wang CJ, Tseng CC, Chen HP, Wu MS, Lin JT, Inoue H, Chen GH. Helicobacter pylori promote gastric cancer cells invasion through a NF-kappaB and COX-2-mediated pathway. *World J Gastroenterol* 2005; **11**: 3197-3203 [PMID: 15929167]

69 **Farrow DC**, Vaughan TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoenberg JB, West AB, Rotterdam H, Fraumeni JF, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 1998; **7**: 97-102 [PMID: 9488582]

70 **Aranha MM**, Borralho PM, Ravasco P, Moreira da Silva IB, Correia L, Fernandes A, Camilo ME, Rodrigues CM. NF-kappaB and apoptosis in colorectal tumourigenesis. *Eur J Clin Invest* 2007; **37**: 416-424 [PMID: 17461988 DOI: 10.1111/j.1365-2362.2007.01801.x]

71 **Sohma I**, Fujiwara Y, Sugita Y, Yoshioka A, Shirakawa M, Moon JH, Takiguchi S, Miyata H, Yamasaki M, Mori M, Doki Y. Parthenolide, an NF-κB inhibitor, suppresses tumor growth and enhances response to chemotherapy in gastric cancer. *Cancer Genomics Proteomics* 2011; **8**: 39-47 [PMID: 21289336]

72 **Lind D**, Hochwald SN, Malaty J, Rekkas S, Hebig P, Mishra G, Moldawer LL, Copeland EM, Mackay S. Nuclear Factor-κB Is

Upregulated in Colorectal Cancer. *Surgery* 2001; **130**: 363-369 [PMID: 11490372 DOI: 10.1093/abbs/gmt071]

73 **Shanahan F**. Review article: colitis-associated cancer -- time for new strategies. *Aliment Pharmacol Ther* 2003; **18** Suppl 2: 6-9 [PMID: 12950414 DOI: 10.1046/j.1365-2036.18.s2.5.x]

74 **Wang S**, Liu Z, Wang L, Zhang X. NF-kappaB signaling pathway, inflammation and colorectal cancer. *Cell Mol Immunol* 2009; **6**: 327-334 [PMID: 19887045 DOI: 10.1038/cmi.2009.43]

75 **Grivennikov SI**. Inflammation and colorectal cancer: colitis-associated neoplasia. *Semin Immunopathol* 2013; **35**: 229-244 [PMID: 23161445 DOI: 10.1007/s00281-012-0352-6]

76 **Vaiopoulos AG**, Athanasoula KCh, Papavassiliou AG. NF-κB in colorectal cancer. *J Mol Med* (Berl) 2013; **91**: 1029-1037 [PMID: 23636511 DOI: 10.1007/s00109-013-1045-x]

77 **Horst D**, Budczies J, Brabletz T, Kirchner T, Hlubek F. Invasion associated up-regulation of nuclear factor kappaB target genes in colorectal cancer. *Cancer* 2009; **115**: 4946-4958 [PMID: 19658179 DOI: 10.1002/cncr.24564]

78 **Kojima M**, Morisaki T, Sasaki N, Nakano K, Mibu R, Tanaka M, Katano M. Increased nuclear factor-kB activation in human colorectal carcinoma and its correlation with tumor progression. *Anticancer Res* 2004; **24**: 675-681 [PMID: 15161011]

79 **Sakamoto K**, Maeda S, Hikiba Y, Nakagawa H, Hayakawa Y, Shibata W, Yanai A, Ogura K, Omata M. Constitutive NF- B Activation in Colorectal Carcinoma Plays a Key Role in Angiogenesis, Promoting Tumor Growth. *Clin Cancer Res* 2009; **15**: 2248-258 [PMID: 19276252 DOI: 10.1158/1078-0432.CCR-08-138]

80 **Din FV**, Dunlop MG, Stark LA. Evidence for colorectal cancer cell specificity of aspirin effects on NF kappa B signalling and apoptosis. *Br J Cancer* 2004; **91**: 381-388 [PMID: 15188000 DOI: 10.1038/sj.bjc.6601913]

81 **Charalambous MP**, Maihöfner C, Bhambra U, Lightfoot T, Gooderham NJ. Upregulation of cyclooxygenase-2 is accompanied by increased expression of nuclear factor-kappa B and I kappa B kinase-alpha in human colorectal cancer epithelial cells. *Br J Cancer* 2003; **88**: 1598-1604 [PMID: 12771929 DOI: 10.1038/sj.bjc.6600927]

82 **Shao J**, Fujiwara T, Kadowaki Y, Fukazawa T, Waku T, Itoshima T, Yamatsuji T, Nishizaki M, Roth JA, Tanaka N. Overexpression of the Wild-type P53 Gene Inhibits NF-κB Activity and Synergizes with Aspirin to Induce Apoptosis in Human Colon Cancer Cells. *Oncogene* 2000; **19**: 726-736 [PMID: 10698490 DOI: 10.1038/sj.onc.1203383]

83 **Maihöfner C**, Charalambous MP, Bhambra U, Lightfoot T, Geisslinger G, Gooderham NJ. Expression of cyclooxygenase-2 parallels expression of interleukin-1beta, interleukin-6 and NF-kappaB in human colorectal cancer. *Carcinogenesis* 2003; **24**: 665-671 [PMID: 12727794 DOI: 10.1093/carcin/bgg006]

84 **Gan HT**, Chen YQ, Ouyang Q. Sulfasalazine inhibits activation of nuclear factor-kappaB in patients with ulcerative colitis. *J Gastroenterol Hepatol* 2005; **20**: 1016-1024 [PMID: 15955209 DOI: 10.1111/j.1440-1746.2005.03862.x]

85 **Vyas D**, Laput G, Vyas AK. Chemotherapy-enhanced inflammation may lead to the failure of therapy and metastasis. *Onco Targets Ther* 2014; **7**: 1015-1023 [PMID: 24959088]

86 **Kim SS**, Ahn CH, Kang MR, Kim YR, Kim HS, Yoo NJ, Lee SH. Expression of CARD6, an NF-kappaB activator, in gastric, colorectal and oesophageal cancers. *Pathology* 2010; **42**: 50-53 [PMID: 20025480 DOI: 10.3109/00313020903434421]

87 **Kawahara TL**, Michishita E, Adler AS, Damian M, Berber E, Lin M, McCord RA, Ongaigui KC, Boxer LD, Chang HY, Chua KF. SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. *Cell* 2009; **136**: 62-74 [PMID: 19135889 DOI: 10.1016/j.cell.2008.10.052]

88 **Yun D**, Rahmani M, Dent P Grant S. Blockade of Histone Deacetylase Inhibitor-Induced RelA/p65 Acetylation and NF-κB Activation Potentiates Apoptosis in Leukemia Cells through a Process Mediated by Oxidative Damage, XIAP Downregulation, and c-Jun N-Terminal Kinase 1 Activation. *Mol Cell Biol* 2005; **25**: 1016-1024 [DOI: 10.1128/MCB.25.13.5429-5444.2005]

89 **Yeung F**, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 2004; **23**: 2369-2380 [PMID: 15152190 DOI: 10.1038/sj.emboj.7600244]

P- Reviewer: Bashashati M, Passi A **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3184

World J Gastroenterol 2015 March 21; 21(11): 3184-3196
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Updates in vaccination: Recommendations for adult inflammatory bowel disease patients

Khadija Chaudrey, Michelle Salvaggio, Aftab Ahmed, Sultan Mahmood, Tauseef Ali

Khadija Chaudrey, Tauseef Ali, Department of Medicine, Section of Gastroenterology, Oklahoma University Health Sciences Center, Oklahoma, OK 73104, United States
Michelle Salvaggio, Department of Medicine, Section of Infectious Diseases, Oklahoma University Health Sciences Center, Oklahoma, OK 73104, United States
Aftab Ahmed, Sultan Mahmood, Department of Internal Medicine, Oklahoma University Health Sciences Center, Oklahoma, OK 73104, United States
Tauseef Ali, Inflammatory Bowel Disease Center, Oklahoma University Physicians, Oklahoma, OK 73104, United States
Author contributions: Chaudrey K contributed to literature search and wrote the manuscript; Mahmood S and Ahmed A contributed to literature review and proof reading of the manuscript; Ali T and Salvaggio M performed proof reading of the manuscript.
Conflict-of-interest: Dr. Khadija Chaudrey has neither received fees for serving as a speaker nor has received research funding from any organization for this review article. Dr. Khadija Chaudrey is an employee of University of Oklahoma Health Sciences Center and the institute has no conflict of interest with the article. Dr. Khadija Chaudrey does not own stocks and/or shares and/or patents in any organization that would have conflict of interest with this review article.

Correspondence to: Khadija Chaudrey, MD, Department of Medicine, Section of Gastroenterology, Oklahoma University Health Sciences Center, Oklahoma, OK 73104, United States. drkchaudrey@gmail.com
Telephone: +1-347-4395656
Received: September 29, 2014
Peer-review started: September 29, 2014
First decision: October 29, 2014
Revised: November 16, 2014
Accepted: January 30, 2015
Article in press: January 30, 2015
Published online: March 21, 2015

Abstract

Treatment regimens for inflammatory bowel disease (IBD) incorporate the use of a variety of immunosuppressive agents that increase the risk of infections. Prevention of many of these infections can be achieved by the timely and judicious use of vaccinations. IBD patients tend to be under-immunized. Some of the contributing factors are lack of awareness regarding the significance of vaccinating IBD patients, misperception about safety of vaccinations in immunocompromised patients, ambiguity about the perceived role of the gastroenterologist in contrast to the primary care physician and unavailability of vaccination guidelines focused on IBD population. In general, immunocompetent IBD patients can be vaccinated using standard vaccination recommendations. However there are special considerations for IBD patients receiving immunosuppressive therapy, IBD travelers and pregnant women with IBD. This review discusses current vaccination recommendations with updates for adult IBD patients. Centers for Disease Control and Prevention 2013 vaccination guidelines with 2014 updates and the Advisory Committee on Immunization Practices recommendations have been highlighted as a primary source of recommendations.

Key words: Inflammatory bowel disease; Vaccination; Immunocompromised; Influenza; Pneumococcal; Centers for Disease Control and Prevention

Core tip: Patients with inflammatory bowel disease (IBD) are at increased risk of infection because of use of immunosuppressive agents for treatment in many of them. While immunocompetent IBD patients can be vaccinated using standard vaccination schedule, special guidelines need to be followed for IBD patients getting immunosuppressive therapy. In this review article the focus is on current vaccination recommendations for adult IBD patients. This is a much needed dis-

cussion as lack of awareness and misperceptions about vaccination safety is a major cause of under immunization in IBD patients.

Chaudrey K, Salvaggio M, Ahmed A, Mahmood S, Ali T. Updates in vaccination: Recommendations for adult inflammatory bowel disease patients. *World J Gastroenterol* 2015; 21(11): 3184-3196 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3184.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3184

INTRODUCTION

Inflammatory bowel disease (IBD) is an immunologically mediated disease often necessitating the use of immunosuppressive therapies as treatment. Maintenance therapy can involve long term use of immunomodulators, biologic agents or the combination of both. Immunosuppression leads to increased susceptibility to many infectious diseases as hepatitis B, pneumococcal sepsis and disseminated zoster. Several of these infections are preventable with the timely and diligent use of vaccination[1,2]. There has been a longstanding debate about the ability of immunosuppressed patients to mount an adequate antibody response to vaccinations. Currently available knowledge has led to the general consensus that IBD patients including those on immunosuppressive therapy will likely respond adequately to vaccinations[3]. However, even if the response is suboptimal in some cases, it may still be sufficient to render immunity. Studies evaluating the safety profile and impact of vaccinations on disease activity have shown reassuring results even in immunocompromised patients. Table 1 demonstrates a widely accepted expert consensus statement that defines an immunocompromised IBD patient. IBD patients can be vaccinated following the standard guidelines applicable to general population. Routine vaccination schedules are recommended to be followed for most IBD patients. Live vaccinations are contraindicated in immunocompromised patients. An approach to vaccination of adult IBD patients including those on immunosuppressive medications is presented in this review article.

PNEUMOCOCCAL VACCINE

Pneumococcal Infection

Streptococcus pneumoniae (pneumococcus) remains a leading cause of serious illness, including bacteremia, meningitis, and pneumonia among adults in the United States[4].

Pneumococcal Infection and IBD

Major risk factors for pneumococcal disease include immunocompromising conditions, chronic medical conditions, functional or anatomic asplenia, CSF leaks and cochlear implants. They are listed in detail in Table 2. Salient factors applicable to IBD patients are age 65 years and older, smoking and use of immunosuppressive agents[5-7].

Table 1 Definition of the immune compromised inflammatory bowel disease patient[1]

Treatment with glucocorticoids: > prednisone 20 mg/d equivalent for 2 wk or more
Ongoing treatment with effective doses of 6-mercaptopurine, AZT, Methotrexate and anti tumor necrosis factor therapy
Within 3 mo of stopping the above listed immunosuppressive therapies
Significant protein-calorie malnutrition

Table 2 Risk factors for pneumococcal disease

All adults 65 and older
Symptomatic or asymptomatic human immunodeficiency virus
Chronic lung disease (COPD, emphysema, and asthma)
Chronic cardiovascular diseases
Diabetes mellitus
Chronic renal failure
Nephrotic syndrome
Chronic liver disease (including cirrhosis)
Alcoholism
Cochlear implants
Cerebrospinal fluid leaks
Immunocompromising conditions
Functional or anatomic asplenia
Residents of nursing homes or long-term care facilities
Smokers

COPD: Chronic obstructive pulmonary diseases.

Pneumococcal vaccination recommendations

For an adult who has already received 4 doses during childhood , the first revaccination is to be given 5 years after the last dose administered, followed by a lifetime revaccination dose at age 65 years or above[5]. For an adult who has not been vaccinated as a child, two doses are given 8 wk apart. This is followed by first revaccination to be given 5 years since the last dose administered, followed by a lifetime revaccination at age 65 years or above. There are two types of pneumococcal vaccines available: Pneumococcal polysaccharide (PPSV23) vaccination (Brand name Pneumovax) and Pneumococcal conjugate 13-valent (PCV13) vaccination (Brand name Prevnar). PPSV23 is the commonly used and recommended vaccine for all adults. However for a subset of adults including immunocompromised IBD patients, at least one dose of PCV13 is recommended to be included in their vaccination regimen. Whenever PCV13 is indicated, it is preferred to be administered before PPSV23 is administered. CDC and ACIP recommend that PCV13 be given in addition to, not instead of, PPSV23 to all immunocompromised adults of all ages. For an adult who has an indication for PCV13 but has not previously received PPSV23, should receive a single dose of PCV13 followed by PPSV23 at least 8

wk later if indicated. If one or more doses of PPSV23 have previously been administered then a dose of PCV13 should be administered at least one year after the last PPSV23 dose was received. Interestingly, PCV13 is not FDA approved for ages less than 50[5]. When immunosuppressive therapy is being considered, the interval between pneumococcal vaccination and initiation of immunosuppressive therapy should be at least 2 wk. PCV13 and PPSV23 is now recommended for all adults 65 years or older. If not previously vaccinated, PCV13 should be given first followed by PPSV23 6-12 mo later. If PPSV23 has been given previously, PCV13 should be given ⩾ 12 mo after[8].

Pneumococcal vaccination and IBD

Studies by Melmed *et al*[9] and Dotan *et al*[10] provided evidence that neither IBD nor monotherapy with immunomodulators impair vaccine response, however the combined use of anti- tumor necrosis factors (TNF) agents with immunomodulators may diminish response to pneumococcal vaccine 23. Fiorino *et al*[11] have demonstrated significantly dampened response to pneumococcal vaccination in IBD patients receiving an anti-TNF agent alone or in combination with azathioprine. Melmed *et al*[9] assessed serological responses to PPSV23 in 21 IBD patients on combined immunomodulator and biologic therapy *vs* 25 non-immunosuppressed patients. Patients on combined therapy had a significantly lower response rate compared to non-immunosuppressed patients (45% *vs* 85%, $P = 0.01$). Serologic response rates were similar between non-immunosuppressed patients and 19 healthy controls (80% *vs* 85%). In a prospective cohort study, Dotan *et al*[10] found that 75% of 28 IBD patients vaccinated with Pneumovax had at least a 2-fold increase between pre- and post-vaccination titers to at least 4 out of 14 serotypic determinants. All patients initiated thiopurine therapy at or near the time of vaccination. Fiorino *et al*[11] evaluated the response rates to pneumococcal vaccination in four different treatment groups: mesalamine, azathioprine, infliximab, and infliximab plus azathioprine. Patients administered infliximab or the combination immunosuppressive therapy had significantly lower response rates (57.6% and 62.5%, respectively) compared with the group on mesalamine (88.6%; $P < 0.05$ for both comparisons). Azathioprine alone did not influence the response rate to vaccination (78.9%; $P = 0.43$ *vs* mesalamine group).

INFLUENZA VACCINE

Influenza infection

Influenza is a highly infectious viral illness that can be fatal as primary infection and may also be complicated by superimposed bacterial infections.

Influenza infection and IBD

Currently there is no knowledge of increased predisposition to influenza infection in non-immunocompromised IBD patients compared to general population. However, morbidity and mortality are both increased in individuals who are immunocompromised. A multicenter, prospective study was conducted in Tokyo to investigate the age distribution associated with H1N1 influenza in immunocompromised IBD patients. A significantly higher incidence of H1N1 influenza infections in patients aged less than 20 years was noted, however this was comparable to the trend seen in the general population[12].

Influenza vaccination recommendations

Annual vaccination against influenza is recommended for all adult IBD patients. Intranasal live attenuated influenza vaccine (LAIV) and inactive influenza vaccine (IIV)/trivalent inactivated vaccine (TIV) that can be administered intramuscularly or intranasally. All non-pregnant and non-immunocompromised IBD patients can receive either form of vaccine[8]. Pregnant, immunocompromised IBD patients and household contacts of immunocompromised patients should not receive live vaccine. Adults aged 65 years and older can receive the standard dose IIV or the high-dose IIV (Fluzone High-Dose). Due to known antigenic drift, a new vaccine is produced annually. As of 2010 the annual influenza vaccine also contains the H1N1 component.

Influenza vaccination and IBD

Based on the currently available data, influenza vaccine is safe and well tolerated in IBD patients. Annual vaccination appears to impart adequate immunogenic response against strain A, irrespective of immunosuppression status. However, seroprotection against strain B strain is impaired and is further blunted due to immunosuppression. In a prospective open label study, Lu *et al*[13] found more children with IBD were seroprotected against strains influenza A/H1N1 and influenza A/H3N2 than influenza B strain ($P < 0.02$), regardless of immunosuppression status. Further sub-analysis showed patients on anti-TNF were less protected against B strain compared to non-immunosuppressed IBD patients (14% *vs* 39%, $P = 0.025$). In their prospective cohort study, deBruyn *et al*[14], children with IBD achieved appropriate immunogenicity to influenza A, immunogenicity to influenza B appears to be diminished, especially with immunosuppressive therapy. For influenza B, 53% children with IBD mounted an immunogenic response compared to 81% in controls ($P = 0.0009$) and 79% immunosuppressed IBD children achieved serologic protection compared to 100% non-immunosuppressed children with IBD ($P = 0.02$). Cullen *et al*[15] conducted an observational prospective open-label study and found decreased postvaccine response in patients on combination immunosuppression as compared to non-immunosuppressed patients (36% *vs* 64%, $P = 0.02$), particularly to 2009 H1N1 influenza strain. Also patients receiving combined immunosuppression had a significantly less increase in geometric mean titers than those on monotherapy immunosuppression (3.5

vs 11.5, *P* = 0.03). A multicenter observational cohort by Rahier *et al*[16], found H1N1 vaccine to be well tolerated by IBD patients, regardless of therapy and the risk of IBD related-flare was concluded to be low.

TETANUS, DIPHTHERIA, ACELLULAR PERTUSSIS VACCINE

Tetanus and diphtheria

Neurotoxin released by *Clostridium tetani* causes neuromuscular excitability leading to prolonged muscle contractions. Those exposed to trauma with contaminated wounds are at risk for this condition[17]. Diptheria is an acute, toxin-mediated respiratory tract illness caused by the corynebacterium diphtheriae.

Tetanus, diphtheria and IBD

All IBD patients are at risk for tetanus after exposure through a contaminated wound[1,2,18,19]. There has been a marked decline in the incidence of Diphtheria due to vaccinations[20]. A case report of severe infection with a non-toxigenic strain of *C. diphtheria* in immunocompromised patients was reported by Wojewoda *et al*[21].

Tetanus and diphtheria vaccination recommendations

Tetanus and diphtheria (Td) is recommended as part of the childhood DTaP 5 series injection[22], followed by Td once every 10 years[22-26]. All adults with unknown or incomplete history of vaccination should complete a 3-dose primary vaccination series and all pregnant women need to be vaccinated during each pregnancy (preferred during 27-36 wk gestation), regardless of number of years since prior Td or tetanus, diphtheria, acellular, pertussis (TDAP) vaccination[27].

Tetanus, diphtheria vaccine and IBD

Data to date examining efficacy of tetanus vaccination in IBD patients is inconsistent, however tetanus vaccine be administered to all IBD patients irrespective of their immunization status. Nielsen *et al*[3], revealed that post vaccination increase in antitetanus antibody levels in 10 patients with clinically inactive Crohn's disease (CD) was comparable to 12 healthy controls. Dotan *et al*[10], found that in 37 patients with IBD who initiated thiopurine therapy at or around the time of Td administration, 73% had seroconversion. Brogan *et al*[28], suggested an impaired response to the booster vaccination in patients with IBD. Dezfoli *et al*[29], categorized 59 patients based on their level of immunosuppression (*i.e.*, no therapy, immunomodulator monotherapy, biologic monotherapy, or combined immunomodulator and biologic therapy). Booster response rates with serum antibody levels and geometric mean titers (GMTs) were measured at baseline and approximately 4 wk after vaccination. Protective tetanus titers were achieved in all patients either on an anti-TNF or an immunosuppressant alone compared to 78% of those on combined therapy (*P* = 0.01).

Pertussis

"Whooping Cough", a highly contagious upper respiratory infection caused by Bordetella pertussis can be associated with sequelae including pneumonia, encephalopathy and seizures[19,30]. A pertussis epidemic was reported in state of Washington by the Secretary of Health in 2012. About 2520 (37.5 cases per 100000 residents) were reported, a 300% increase compared with the same period in 2011 and the highest number of cases reported in any year since 1942[31].

Pertussis and IBD

A steady increase in risk of pertussis infection in the years after completion of the 5-dose DTaP series has been reported[32]. The risk likely continues through adulthood and is attributable, in part, to waning immunity from DTaP vaccines. This increased risk is applicable to IBD patients.

Pertussis vaccination recommendations

For all adults, replacing 1 scheduled Td booster with Tdap is recommended. In an adult with unknown vaccination status, administer the first 2 doses at least 4 wk apart and then third dose 6-12 mo after the second with at least one injection being Tdap[25]. For an incompletely vaccinated adult *i.e.,* less than 3 doses, administer remaining doses.

Pertussis vaccination and IBD

IBD patients should receive the Tdap vaccine according to current guidelines, preferably before initiation of immunomodulator therapy. Dezfoli *et al*[29], prospectively examined 59 consecutive adults with IBD who received a booster vaccination for tetanus, diptheria and acellular pertussis. Patients were categorized based on their level of immunosuppression (*i.e.*, no therapy, immunomodulator monotherapy, biologic monotherapy, or combined immunomodulator and biologic therapy). Outcomes for these patient groups were compared with a control group of IBS patients receiving mesalamine. Serum antibody titers against pertussis toxoid (PT) and pertussis filamentous hemagglutinin (FHA) were measured at baseline and 4 wk after vaccination. Response rates to pertussis toxoid were 68% in the mesalamine group, 67% in the biologic monotherapy group and 44% in both the immunosuppressive monotherapy and combination therapy groups. Similarly, 84%, 87%, 69% and 67% of the four groups, respectively, achieved response to pertussis filamentous hemagglutinin. No difference in response rates between patients off medications or on biologic monotherapy was noted, as mentioned above. However, response to PT was lower in patients on immunomodulator monotherapy, and postvaccination

Table 3 Risk factors for meningococcal disease

College freshman living in dormitories
Microbiologists routinely exposed to *Neisseria meningitidis*
Military recruits
Persons who travel to or reside in countries where *Neisseria meningitidis* is hyper-endemic or epidemic particularly if contact with the local population will be prolonged
Persons with persistent complement component deficiency
Persons with anatomic or functional asplenia
Persons with human immunodeficiency virus infection

titers were lowest to FHA in those on combined immunomodulator and biologic therapy[29].

HUMAN PAPILLOMAVIRUS

Human papillomavirus (HPV) is the most common sexually transmitted infection in the world[33]. An estimated 20 million persons are currently infected, and an estimated 6.2 million new HPV infections occur annually[34]. HPV is known to cause genital warts, cervical, vulvar, vaginal, penile, anal and oropharyngeal cancers. High-risk types of HPV (*e.g.*, types 16 and 18) are associated with 70% of all cervical and anogenital cancers.

HPV and IBD

Diagnosis of IBD in women is related to an increased risk of abnormal Pap smear[35-37]. Immunosuppressive therapy and smoking have been shown to exhibit an association between IBD and cervical dysplasia rather than just the diagnosis of IBD[38,39].

HPV vaccination recommendations

Physician vigilance is especially warranted for IBD patients transitioning from pediatric age group. Misconception that HPV is not an adult vaccine and also assumption that patients might have been vaccinated already, may lead to HPV under vaccination. Two vaccines are licensed for use in females, bivalent HPV vaccine (HPV2) and quadrivalent HPV vaccine (HPV4), and one HPV vaccine for use in males (HPV4). Vaccination can be used for all IBD patients including immunocompromised patients. A complete series for either HPV4 or HPV2 consists of 3 doses. The second dose should be administered 1-2 mo after the first dose; the third dose should be administered 6 mo after the first dose (at least 24 wk after the first dose). For females, either HPV4 or HPV2 is recommended in a 3-dose series for routine vaccination at age 11 or 12 years, and for those aged 13 through 26 years, if not previously vaccinated. HPV vaccination should not be administered during pregnancy, but a pregnancy test is not required before giving vaccination. If woman is found to be pregnant after vaccination, no intervention is needed and rest of the series should be delayed till after delivery[8]. For males, HPV4 is recommended in a 3-dose series for routine vaccination at age 11 or 12 years, and for those aged 13 through 21 years, if not previously vaccinated. Males aged 22 through 26 years may be vaccinated[40-44].

HPV vaccination and IBD

HPV4 vaccine is safe and immunogenic in most women with IBD including those on immunosuppressive therapy. Jacobson *et al*[45], administered 3-dose HPV vaccine to 37 IBD females aged 9 to 26 years on immunosuppressive therapy. Geometric mean titers (GMTs) were determined before dose 1 and 1 mo after dose 3. Seropositivity after dose 3 was 100% and GMTs were qualitatively comparable to healthy females. No serious adverse events were attributable to the vaccine.

MENINGOCOCCAL VACCINE

Neisseria meningitidis causes meningitis and sepsis. Risk factors for the development of meningococcal disease include antecedent viral infection, household crowding, chronic underlying illness, and both active and passive smoking[46]. They are listed in Table 3.

Meningococcal disease and IBD

IBD patients with the above risk factors are at increased risk for developing meningococcal disease.

Meningococcal vaccine recommendations

The two forms of the meningococcal vaccine include polysaccharide vaccine MPSV4 as well as a conjugate vaccine MCV4. The MCV4 vaccine is the vaccine of choice where indicated because it elicits improved primary immune response, as well as strong anamnestic response[47]. Administer 2 doses of MCV4 at least 2 mo apart to adults with functional asplenia or persistent complement component deficiencies. First-year college students up through age 21 years who are living in residence halls should be vaccinated if they have not received a dose on or after their 16th birthday. For immunocompromised IBD patients, revaccination with MCV4 every 5 years is recommended[46]. Human immunodeficiency virus (HIV) infection is not an indication for MCV4, if vaccinated two doses at least 2 mo apart should be given[8]. MPSV4 is preferred for people aged 56 and older who have not received MCV4 previously and who only need a single dose, *e.g.*, travelers.

Meningococcal vaccine and IBD

No studies have evaluated the immunogenic profile of the meningococcal vaccine in the IBD population.

HEPATITIS B VACCINE

Hepatitis B

Hepatitis B is one of the most common infections in the world with approximately two billion people

Table 4 Risk factors for hepatitis

Risk factors for hepatitis A	Risk factors for hepatitis B
18 yr and older who care for an international adopted child	Polygamous relationship (*e.g.*, persons with more than one sex partner during the previous 6 mo)
IV and non IV illicit drug users	Persons seeking evaluation or treatment for a sexually transmitted disease
Homosexual males	Current or recent injection-drug users
Chronic liver disease patient	Homosexual male
Patients awaiting transplant	Health-care personnel and public-safety workers who are potentially exposed to blood or other infectious body fluids
Occupational exposure to Hep A	All diabetics younger than age 60 yr
Persons who receive clotting factor concentrates	Diabetics 60 yr or older at the discretion of the treating clinician
Travel to endemic areas	ESRD, HD
	Human immunodeficiency virus chronic liver disease
	Household contacts and sex partners of hepatitis B surface antigen positive persons; clients and staff members of institutions for persons with developmental disabilities
	International travelers to countries with high or intermediate prevalence of chronic HBV infection

ESRD: End-stage renal disease.

showing an evidence of prior or current infection and approximately 1.5 million dying annually from sequelae such as cirrhosis and hepatocellular carcinoma[48].

Hepatitis B and IBD

Prior studies comparing the prevalence of HBV in healthy controls and IBD patients showed higher prevalence of HBV in IBD patients. This was attributed to the increased number of blood transfusions, endoscopic and surgical interventions for diagnostic and therapeutic purposes during the course of the disease. However relatively newer studies have demonstrated equal prevalence of HBV in IBD patients as compared to the general population[49-51]. Reactivation of chronic HBV also remains a concern in IBD patients on immunosuppressants especially those on dual immunosupression[52]. Fulminant or fatal infections have been reported in patients with IBD receiving immunosuppressive treatment[51].

Hepatitis B vaccination recommendations

HBV vaccination is recommended in all IBD adults not vaccinated against HBV in childhood or who fall in the CDC identified susceptible group shown in Table 4. CDC 2014 standard recommendation is 0, 1, 4 mo regimen[8]. If the combined hepatitis A and hepatitis B vaccine (Twinrix) is used, then 3 doses at 0, 1, and 6 mo are indicated. Alternatively, a 4-dose Twinrix schedule, administered on days 0, 7, and 21-30 followed by a booster dose at month 12 may be used. In immunocompromised IBD adults, 3 doses at 0, 1, 6 mo schedule with Recombivax HB 40 μg or 4 doses at 0, 1, 2, 6 mo schedule with 2 doses of 20 μg/mL Engerix is advised. Routine serology testing for immunity is not required after vaccination in healthy individuals. Post vaccination titer testing is recommended for person whose subsequent clinical management depends on knowledge of their immune status, including immunocompromised IBD patients[53]. Serologic testing of immunocompromised persons with quantitative anti-HBs is recommended 1-2 mo after administration of the final dose of the primary vaccine series to determine the need for revaccination. A concentration of anti-HBs $\geqslant$ 10 mIU/mL establishes immunity. Revaccination of immunocompromised patients is achieved by administering Recombivax HB 40 μg/mL on a 3-dose schedule at 0, 1, and 6 mo or 2 doses of Engerix-B 20 μg/mL administered simultaneously on a 4-dose schedule at 0, 1, 2, and 6 mo. Persons who do not have a protective concentration of anti-HBs after revaccination should be tested for HBsAg. If the HBsAg test result is positive, the person should receive appropriate management. Persons who test negative for HBsAg should be considered susceptible to HBV infection and should be counseled about precautions to prevent HBV infection and the need to obtain HBIG post exposure prophylaxis for any known or likely parenteral exposure to HBsAg-positive blood[53]. Immunocompromised persons might need annual testing to assess anti-HBs concentrations. For immunocompromised persons the need for booster doses has not been determined. When anti-HBs levels decline to < 10 mIU/mL, annual booster doses should be considered for persons with an ongoing risk for exposure[53]. Whenever possible, vaccination is recommended before starting treatment with immunosuppressive agents, preferably at the time of diagnosis.

HBV vaccine and IBD

Unlike healthy adults where the 3-dose vaccine series produces a protective antibody response in > 90% subjects[53], immunogenicity in IBD patients, particularly those on immunosuppressive therapy, has been reported to be low in several studies. Melmed *et al*[18], Vida *et al*[54], and Altunöz *et al*[55], respectively detected anti-HBs antibody in only 33%, 36% and 76% of immunized IBD patients. Gisbert *et al*[48] assessed the effectiveness of HBV vaccine with a double dose at 0, 1 and 2 mo in 241 patients with IBD. Response was achieved in only 59% of patients. Nyström *et al*[56] successfully re-vaccinated HBV vaccine non-responders using a double dose of the combined HAV and HBV vaccine. Forty-four patients who failed to mount an appropriate post vaccination response to a standard hepatitis B vaccination schedule were revaccinated with double-dose combined hepatitis A and B vaccine. An adequate rise in anti-HBs antibody titers was seen in 95% of previous non-responders.

HEPATITIS A VACCINE

Hepatitis A

Hepatitis A is a common worldwide infection commonly transmitted *via* feco-oral route.

HAV and IBD

Risk factors for hepatitis A virus infection among individuals with IBD are the same as for those without IBD[3] (Table 4).

HAV vaccine recommendations

All IBD adults not vaccinated against Hepatitis A in childhood who seek protection against this preventable disease or are categorized to be in the CDC identified susceptible group, should be offered the vaccine. Single-antigen vaccine formulations should be administered in a 2-dose schedule at either 0 and 6-12 mo (Havrix), or 0 and 6-18 mo (Vaqta). If the combined hepatitis A and hepatitis B vaccine (Twinrix) is used, administer 3 doses at 0, 1, and 6 mo; alternatively, a 4-dose schedule may be used, administered on days 0, 7, and 21-30, followed by a booster dose at month 12[22].

HAV vaccination and IBD

Hepatitis A vaccine is an inactivated vaccine that is safe and well tolerated in IBD patients. Data about the immunogeninicity of the vaccine primarily comes from pediatric IBD patients. Radzikowski *et al*[57] conducted an open, prospective, and controlled study on anti-HAV-negative IBD patients aged 2-18 years with IBD. HAV vaccine was administered at 0 mo and at 6-12 mo. Seroconversion and GMTs were measured after each vaccine dose. A total of 134 subjects (66 patients and 68 controls) completed the whole study course consisting of two doses of vaccine and six serum samples. There was no significant difference in the rate of seroconversion between 66 IBD patients and 68 controls when measured after the second dose of vaccine (97% *vs* 100%, $P = 0.2407$). 6-Mercaptopurine (6-MP) or azathioprine (AZA) treatment with and without steroids did not affect seroconversion rates. There were no serious adverse events related to HAV vaccination during the study[57].

Twelve anti HAV-negative patients with IBD were vaccinated using 0 and 6-12 mo schedule. An overall seroconversion rate of 92% was reported. All of the patients were receiving infliximab at the time of vaccination. Two of the patients were receiving concurrent methotrexate, both of whom responded to the HAV vaccine. The authors concluded that pediatric IBD patients on a wide variety of medications for control of their disease are likely able to respond adequately to the HAV vaccine[58]. This seems to be questioned in a recent open prospective study that evaluated the efficacy of HAV vaccination in 419 anti-HAV-negative adult patients with IBD. It was concluded that although HAV vaccination is generally effective in patients with IBD, the seroconversion rates are noted to be lower in patients receiving anti-TNF agents[59].

VARICELLA: CHICKEN POX VACCINE

Varicella: Chicken pox

Primary infection with Varicella Zoster virus (VZV) is highly contagious condition. Immunocompromised patients are especially at risk for severe infection, severe disease occurs in approximately 30% of such persons with primary infection[60].

VZV and IBD

Most IBD adults are generally considered to have acquired immunity to VZV either from childhood infection or vaccination. Interestingly, a 5 year retrospective review of charts of the newly diagnosed 163 IBD patients at University of Buffalo found that a total of 66% of all of the patients had a history of disease or vaccination. Measurable titers against varicella were found in only 77% of all of the patients[61]. There have been several reports of severe, disseminated and occasionally fatal primary varicella infection in immunosuppressed IBD patients. Corticosteroids and combination immunosuppression appeared to be a particular risk for contracting this infection[62].

VZV vaccine recommendations

CDC recommends that all adults without evidence of immunity to varicella should receive 2 doses of single-antigen varicella vaccine or a second dose if they have received only 1 dose. Adult IBD patients should be evaluated for immunity to VZV as soon as the diagnosis is made. Table 5 explains how to establish evidence of immunity to varicella in adults. Unimmunized, immunocompetent IBD adults should receive immunization with a two-dose series of live varicella vaccine as above at least 3 wk before the start of immunosupressive therapy. For immunocompromised patients, live-virus varicella vaccine is contraindicated until immunosuppressive therapy has been discontinued for at least 3 mo[1,22,63].

VZV vaccination in IBD

Data about the immunogenicity and safety of this live vaccine especially in immunocompromised IBD patients remains scarce. In 2008, Levin[64] analyzed clinical trials of varicella vaccine administration to immunocompromised children that were reported since 1975. It was suggested that varicella vaccine is safe and effective in immunocompromised patients. This has been most successful when vaccination occurs during periods of limited immune suppression, such as before treatment with immunosuppressive therapy, when therapy is stopped temporarily, or when maintenance immune suppression is low. However,

Table 5 Evidence of immunity to varicella in adults includes any of the following

Documentation of 2 doses of varicella vaccine at least 4 wk apart
United States-born before 1980 except health-care personnel and pregnant women
History of varicella based on diagnosis or verification of varicella disease by a health-care provider
History of herpes zoster based on diagnosis or verification of herpes zoster disease by a health-care provider
Laboratory evidence of immunity or laboratory confirmation of disease

in patients with IBD receiving immunosuppressants, temporary withdrawal from immunosuppression might pose considerable risks of disease recurrence or flare. Therefore in immunocompromised IBD patients at increased risk of exposure to varicella *e.g.*, primary school teachers, health-care workers and patients with no prior immunity, the risks of acquiring the infection need to be weighed against the potential risks and benefits of vaccinations[18,51].

ZOSTER: SHINGLES VACCINE

Herpes zoster

After primary infection, VZV persists as a latent infection in sensory nerve ganglia. The virus can reactivate after a period of latency, causing herpes zoster (HZ) especially in the elderly and those who are immunocompromised. The most common complication of shingles is postherpetic neuralgia. Other less common complications include meningoencephalitis, cerebellitis, herpes zoster ophthalmicus, and Ramsay-Hunt syndrome. In immunocompromised individuals, reactivation can be complicated by disseminated infection and can be potentially fatal[65].

HZ and IBD

Patients with IBD, especially those on immunosuppression, are at increased risk for herpes zoster. Gupta *et al*[66], in their retrospective cohort and nested case-control study demonstrated that patients with IBD, especially those on immunosuppressive medications, are at higher risk for herpes zoster compared with the general population. In another large retrospective cohort and nested case-control study including more than 100000 patients, Long *et al*[67], found an increased risk of HZ among IBD patients as compared to non-IBD patients. Use of thiopurines, anti-TNF agents, combination therapy and corticosteroids increases HZ risk.

HZ vaccine recommendation

A single dose of zoster vaccine for all IBD adults 60 years and older, regardless of previous shingles. HZ vaccine is contraindicated in immunosuppressed patients. However, the current ACIP recommendations report that patients receiving short-term (*i.e.*, < 14 d) or low-to-moderate dose (*i.e.*, < 20 mg/d) corticosteroid therapy are not considered to be sufficiently immunosuppressed to justify avoiding the live zoster vaccine. This is also applicable to patients on low-dose methotrexate (*i.e.*, ⩽ 0.4 mg/kg per week), azathioprine (⩽ 3.0 mg/kg per day), or 6-mercaptopurine (⩽ 1.5 mg/kg per day)[65]. This opinion does not extend to other live vaccines and patients on anti-TNF therapy[68].

HZ vaccine and IBD

Zhang *et al*[69], examined the association between HZ vaccine and HZ incidence within and beyond 42 d after vaccination in patients with selected immune-mediated diseases and in relation to biologics and other therapies used to treat these conditions. Retrospective cohort study of 463541 Medicare beneficiaries 60 years and older with 66751 patients with inflammatory bowel disease were included in the study. Receipt of HZ vaccine was not associated with a short-term increase in HZ incidence among Medicare beneficiaries with selected immune-mediated diseases, including those exposed to biologics. The vaccine was associated with a lower HZ incidence over a median of 2 years of follow-up.

MEASLES, MUMPS, RUBELLA VACCINE

Measles, mumps, rubella

The developed world has seen a remarkable drop in the incidence of measles, mumps, rubella (MMR) after the introduction of universal vaccination protocols[70].

MMR and IBD

Even though immunocompetent IBD patients do not appear to be at higher risk than general patients, measles can be severe and prolonged among immunocompromised persons[71]. Naganuma *et al*[72], in their study of IBD patients found a significant number of patients seronegative for rubella, measles and mumps (30%, 34%, and 37% respectively). Almost 30% of the patients with a past history of rubella or measles did not have seropositive antibody levels and a total of 54% of the patients being treated with immunosuppressant displayed seronegative levels of antibodies specific for at least one of the viruses.

MMR vaccine recommendations

Individuals born before 1957 are considered immune to measles and mumps. Immunity is established by documenting lab titers, clinically diagnosed disease is not acceptable as an evidence of MMR immunity. CDC 2014 guidelines recommend that all adults born in 1957 or later should have documentation of 1 or more doses of MMR vaccine (unless vaccine is contraindicated) if they have not already been vaccinated in childhood. MMR vaccine is contraindicated in immunocompromised IBD patients and considering the risk for prolonged viremia, vaccine should not be

Table 6 Live attenuated vaccines with recommended times of administration[2]

Vaccine	Before initiation of immunosuppressive therapy	Already on immunosuppressive therapy
MMR	Contraindicated if plans to start therapy in 6 wk	Contraindicated
Zoster	Contraindicated if plans to start therapy in 1-3 mo	Contraindicated-could consider if: On short-term corticosteroids (< 14 d) On Methotrexate (< 0.4 mg/kg per week) On Azathioprine (< 3.0 mg/kg per day) On 6-mercaptopurine (< 1.5 mg/kg per day)
Varicella	Contraindicated if plans to start therapy in 1-3 mo	Contraindicated

MMR: Measles, mumps, rubella.

Table 7 Inflammatory bowel disease traveler

Vaccine	Type	Travel related indication
Yellow fever	Live	Parts of South America and Sub-Saharan Africa
Typhoid polio influenza	Live and inactivated	Asia, Africa, Latin America, The Caribbean, and Oceania
BCG vaccine	Live	Highly endemic area > 1 yr
Hepatitis A	inactivated	Central or South America, Mexico, Asia (except Japan), Africa, and Eastern Europe
Meningococcal vaccine	Inactivated	Africa
Japanese encephalitis virus	Inactivated	Rural Japan

BCG: Bacillus Calmette-Gue'rin.

given to patients expected to start immunosuppressive agents in < 6 wk[73]. The MMR vaccine is considered safe for household contacts of immunosuppressed persons with IBD[74].

MMR vaccine and IBD

Early on there was concern about a possible link between measles virus containing vaccines and inflammatory bowel disease (IBD). This was raised by Thompson *et al*[75] in 1995 when their study in United Kingdom suggested that measles virus containing vaccine recipients had an up to 3-fold increased risk for subsequently developing Crohn's disease and ulcerative colitis. However several larger studies have now demonstrated that there is no increased risk of IBD with MMR vaccination[76,77] ACIP concurs with the conclusion[71].

TIMING OF LIVE VACCINE IN PATIENTS ON IMMUNOSUPPRESSIVE THERAPY OR CONSIDERING INITIATION

Timing of live vaccines is particularly important when dealing with IBD patients on immunosuppressants or those with plans to start immunosuppression. Table 6 provides general considerations for timing of live immunization in IBD patients[2].

SPECIAL SITUATIONS SUCH AS PREGNANCY, HOUSEHOLD CONTACTS AND THE TRAVELER WITH IBD

Within IBD patients, special population groups such as pregnant patients, household contacts of immunocompromised patients and travelers pose special challenges. In general, it is recommended that the household contacts of immunocompromised IBD patients be vaccinated according to recommended guidelines. However if a live vaccine is administered, an immunocompromised patient may be predisposed to exposure from the vaccinated family member. If the vaccinated household contact is noticed to have a rash that developed after a live vaccine as varicella, standard contact precautions should be observed. An IBD traveler may warrant evaluation by an infectious disease specialist or travel medicine specialist. Table 7 details recommended vaccines for IBD traveler.

Pregnancy safety categories are applicable to all vaccinations and are a helpful guide to a physician when administering vaccines to this subgroup. Table 8 summarizes pregnancy safety categories of different vaccines. An immunocompromised mother caring for a newborn should be aware of live vaccines that are administered to newborns and can inadvertently expose her to live pathogens as Rotavirus vaccines. Administration of one dose of Tdap vaccine to pregnant women during each pregnancy (preferred during 27-36 wk gestation), regardless of number of years since prior Td or Tdap vaccination is recommended. HPV vaccines are not recommended for use in pregnant women. Pregnant women should be assessed for evidence of varicella immunity. Women who do not have evidence of immunity should receive the first dose of varicella vaccine upon completion or termination of pregnancy and before discharge from the health-care facility. The second dose should be administered 4-8 wk after the first dose[23]. For women of childbearing age, regardless of birth year, rubella immunity should be determined. Pregnant women who do not have evidence of immunity should receive MMR vaccine

Table 8 Vaccination in pregnancy

Category B	Category C	Category X
Influenza (LAIV)	PPSV23	Varicella; non-immune
	Adacel(Tdap)	1st dose. Upon completion or termination of pregnancy and before discharge from the health care facility
Influenza (IIV)	1 dose of Tdap vaccine during each pregnancy regardless of immunization status	2nd dose. 4-8 wk later
	Zoster	
Boostrix (Tdap)	Meningococcus	
1 dose of Tdap vaccine during each pregnancy regardless of immunization status	Hep A and B	
	MMR. Non-immune	
HPV 4, HPV 2	1st dose. Upon completion or termination of pregnancy and before discharge from the health care facility	
PCV 13	2nd dose. 4-8 wk later	

Newborns of immunosuppressed mothers must not receive any live vaccination up to first 6 mo. Tdap: Tetanus, diphtheria, acellular, pertussis; HPV: Human papillomavirus vaccine.

Table 9 Vaccinations in inflammatory bowel disease summary (quick reference)

Vaccine	How often	Live vaccine	Patients on immunosuppressive therapy
Influenza (Flu Vaccine)	1 dose every year	Nasal spray	Use flu shot only
Varicella (Chicken Pox)	If no documented immunity: 2 doses 4-8 wk apart	Yes	Contraindicated
Measles, mumps, rubella	If no documented immunity: 2 doses, 4 wk apart	Yes	Contraindicated
Zoster (Shingles)	1 dose starting at age 60 yr or older	Yes	Contraindicated
Tetanus, Diphtheria, Acellular Pertussis (Td/Tdap)	If no prior vaccination: 3 doses (0, 1, 6-12). Then 1 dose of Tdap followed by a booster of Td every 10 yr	No	Follow recommended regimen
Human papilloma virus	Female: 3 doses through age 26 (0, 2 and 6 mo)	No	Follow recommended regimen
Pneumococcal (pneumonia vaccine) for subset of patients	Male: 3 doses through age 21 (0, 2 and 6 mo) If no prior vaccination: (0, 2 then 5 yr) 1 dose at 65 If had prior vaccination: 1 dose 5 yr after the last dose and 1 dose at age 65	No	Follow recommended regimen
Meningococcal (meningitis vaccine) for subset of patients	2 doses, 2 mo apart	No	Follow recommended regimen
Hepatitis A	2 doses, 6 mo apart	No	Follow recommended regimen
Hepatitis B	3 doses (0, 1 and 6 mo)	No	Follow recommended regimen

Centers for Disease Control and Prevention recommended vaccines for adults 2014, modified for inflammatory bowel disease patients.

upon completion or termination of pregnancy and before discharge from the health-care facility.

CONCLUSION

Vaccinations offer immunity against preventable diseases. A diligent effort should be made to vaccinate all IBD patients. Immunompromised IBD patients are at a higher risk of infection with vaccine preventable diseases. Optimally, these patients should be vaccinated before immunosuppressive therapy is initiated. Live vaccines are contraindicated in immunocompromised patient due to risks of vaccine-associated infection. Despite the concerns for impaired immune response in immunocompromised IBD patients, most of these patients develop adequate response after vaccination. Table 9 provides a quick reference guide for vaccinating IBD patients.

REFERENCES

1 **Sands BE**, Cuffari C, Katz J, Kugathasan S, Onken J, Vitek C, Orenstein W. Guidelines for immunizations in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**: 677-692 [PMID: 15472534 DOI: 10.1097/00054725-200409000-00028]

2 **Melmed GY**, Ippoliti AF, Papadakis KA, Tran TT, Birt JL, Lee SK, Frenck RW, Targan SR, Vasiliauskas EA. Patients with inflammatory bowel disease are at risk for vaccine-preventable illnesses. *Am J Gastroenterol* 2006; **101**: 1834-1840 [PMID: 16817843 DOI: 10.1111/j.1572-0241.2006.00646.x]

3 **Nielsen HJ**, Mortensen T, Holten-Andersen M, Brünner N, Sørensen S, Rask-Madsen J. Increased levels of specific leukocyte- and platelet-derived substances during normal anti-tetanus antibody synthesis in patients with inactive Crohn disease. *Scand J Gastroenterol* 2001; **36**: 265-269 [PMID: 11305513 DOI: 10.1080/003655201750074537]

4 **Centers for Disease Control and Prevention (CDC)**. Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease--United States, 1998-2003. *MMWR Morb*

Mortal Wkly Rep 2005; **54**: 893-897 [PMID: 16163262]
5 **Centers for Disease Control and Prevention (CDC)**. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine for adults with immunocompromising conditions: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2012; **61**: 816-819 [PMID: 23051612]
6 **Black H**, Mendoza M, Murin S. Thoracic manifestations of inflammatory bowel disease. *Chest* 2007; **131**: 524-532 [PMID: 17296657 DOI: 10.1378/chest.06-1074]
7 **Stobaugh DJ**, Deepak P, Ehrenpreis ED. Hospitalizations for vaccine preventable pneumonias in patients with inflammatory bowel disease: a 6-year analysis of the Nationwide Inpatient Sample. *Clin Exp Gastroenterol* 2013; **6**: 43-49 [PMID: 23818801 DOI: 10.2147/ceg.s42514]
8 **Bridges CB**, Coyne-Beasley T. Advisory committee on immunization practices recommended immunization schedule for adults aged 19 years or older: United States, 2014. *Ann Intern Med* 2014; **160**: 190 [PMID: 24658695 DOI: 10.7326/M13-2826]
9 **Melmed GY**, Agarwal N, Frenck RW, Ippoliti AF, Ibanez P, Papadakis KA, Simpson P, Barolet-Garcia C, Ward J, Targan SR, Vasiliauskas EA. Immunosuppression impairs response to pneumococcal polysaccharide vaccination in patients with inflammatory bowel disease. *Am J Gastroenterol* 2010; **105**: 148-154 [PMID: 19755964 DOI: 10.1038/ajg.2009.523]
10 **Dotan I**, Werner L, Vigodman S, Agarwal S, Pfeffer J, Horowitz N, Malter L, Abreu M, Ullman T, Guzner-Gur H, Halpern Z, Mayer L. Normal response to vaccines in inflammatory bowel disease patients treated with thiopurines. *Inflamm Bowel Dis* 2012; **18**: 261-268 [PMID: 21438101 DOI: 10.1002/ibd.21688]
11 **Fiorino G**, Peyrin-Biroulet L, Naccarato P, Szabò H, Sociale OR, Vetrano S, Fries W, Montanelli A, Repici A, Malesci A, Danese S. Effects of immunosuppression on immune response to pneumococcal vaccine in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2012; **18**: 1042-1047 [PMID: 21674732 DOI: 10.1002/ibd.21800]
12 **Naganuma M**, Fujii T, Kunisaki R, Yoshimura N, Takazoe M, Takeuchi Y, Saito E, Nagahori M, Asakura K, Takebayashi T, Watanabe M. Incidence and characteristics of the 2009 influenza (H1N1) infections in inflammatory bowel disease patients. *J Crohns Colitis* 2013; **7**: 308-313 [PMID: 22819592 DOI: 10.1016/j.crohns.2012.06.019]
13 **Lu Y**, Jacobson DL, Ashworth LA, Grand RJ, Meyer AL, McNeal MM, Gregas MC, Burchett SK, Bousvaros A. Immune response to influenza vaccine in children with inflammatory bowel disease. *Am J Gastroenterol* 2009; **104**: 444-453 [PMID: 19174786 DOI: 10.1038/ajg.2008.120]
14 **deBruyn JC**, Hilsden R, Fonseca K, Russell ML, Kaplan GG, Vanderkooi O, Wrobel I. Immunogenicity and safety of influenza vaccination in children with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 25-33 [PMID: 21472826 DOI: 10.1002/ibd.21706]
15 **Cullen G**, Bader C, Korzenik JR, Sands BE. Serological response to the 2009 H1N1 influenza vaccination in patients with inflammatory bowel disease. *Gut* 2012; **61**: 385-391 [PMID: 21757451 DOI: 10.1136/gutjnl-2011-300256]
16 **Rahier JF**, Papay P, Salleron J, Sebastian S, Marzo M, Peyrin-Biroulet L, Garcia-Sanchez V, Fries W, van Asseldonk DP, Farkas K, de Boer NK, Sipponen T, Ellul P, Louis E, Peake ST, Kopylov U, Maul J, Makhoul B, Fiorino G, Yazdanpanah Y, Chaparro M. H1N1 vaccines in a large observational cohort of patients with inflammatory bowel disease treated with immunomodulators and biological therapy. *Gut* 2011; **60**: 456-462 [PMID: 21270121 DOI: 10.1136/gut.2010.233981]
17 **Gellin BG**, Curlin GT, Rabinovich NR, La Montagne JR. Adult immunization: principles and practice. *Adv Intern Med* 1999; **44**: 327-352 [PMID: 9929715]
18 **Melmed GY**. Vaccination strategies for patients with inflammatory bowel disease on immunomodulators and biologics. *Inflamm Bowel Dis* 2009; **15**: 1410-1416 [PMID: 19462435 DOI: 10.1002/ibd.20943]
19 **Dezfoli S**, Melmed GY. Vaccination issues in patients with inflammatory bowel disease receiving immunosuppression. *Gastroenterol Hepatol* (N Y) 2012; **8**: 504-512 [PMID: 23293563]
20 **Vitek CR**. Diphtheria. *Curr Top Microbiol Immunol* 2006; **304**: 71-94 [PMID: 16989265 DOI: 10.1007/3-540-36583-4_5]
21 **Wojewoda CM**, Koval CE, Wilson DA, Chakos MH, Harrington SM. Bloodstream infection caused by nontoxigenic Corynebacterium diphtheriae in an immunocompromised host in the United States. *J Clin Microbiol* 2012; **50**: 2170-2172 [PMID: 22493337 DOI: 10.1128/JCM.00237-12]
22 **ACIP Childhood/Adolescent Immunization Work Group**, Akinsanya-Beysolow I, Bridges CB, Coyne-Beasley T, Jenkins R, Meissner HC, Woods L. Advisory Committee on Immunization Practices (ACIP) Recommended Immunization Schedules for Persons Aged 0 Through 18 Years and Adults Aged 19 Years and Older--United States, 2013: US Department of Health and Human Services, Centers for Disease Control and Prevention. *MMWR Morb Mortal Wkly Rep* 2013; **62**: Supplement
23 **Bridges CB**, Woods L, Coyne-Beasley T. Advisory Committee on Immunization Practices (ACIP) recommended immunization schedule for adults aged 19 years and older--United States, 2013. *MMWR Surveill Summ* 2013; **62** Suppl 1: 9-19 [PMID: 23364303]
24 **Kadivar K**, Malloch L, Adonsou-Hoyi Y, Ng D, Lavoie S, Pulido K, Kim J. Would CLSI M53-A have helped in the diagnosis of HIV in Canada? Results of the performance of Canadian laboratories participating in a recent NLHRS proficiency testing panel containing HIV-1 antigen positive (antibody negative) and HIV-2 samples. *J Clin Virol* 2013; **58**: 303-305 [PMID: 23890809 DOI: 10.1016/j.jcv.2013.04.009]
25 **Centers for Disease Control and Prevention (CDC)**. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine from the Advisory Committee on Immunization Practices, 2010. *MMWR Morb Mortal Wkly Rep* 2011; **60**: 13-15 [PMID: 21228763]
26 **(ACIP) ACoIP**. Use of DTaP (Diphtheria Toxoid-Tetanus Toxoid-Acellular Pertussis) Vaccine as a Five-Dose Series. *MMWR Morb Mortal Wkly Rep* 2000; **49**: RR13
27 **Bridges CB**, Woods ML, Coyne-Beasley T. Advisory Committee on Immunization Practices (ACIP) Recommended Immunization Schedule for Adults Aged 19 Years and Older-United States, 2013. *MMWR Morb Mortal Wkly Rep* 2002; **62**: 9-19
28 **Brogan MD**, Shanahan F, Oliver M, Stevens RH, Targan SR. Defective memory B cell formation in patients with inflammatory bowel disease following tetanus toxoid booster immunization. *J Clin Lab Immunol* 1987; **24**: 69-74 [PMID: 3437440]
29 **Dezfoli S HH**, Brer D. Immunomodulators, but not anti-TNF monotherapy, impair pertussis and tetanus booster vaccine responses in adults with inflammatory bowel disease (IBD). Presented at Digestive Disease Week; May 19-22; San Diego: California, 2012: Abstract Su2081
30 **Marconi GP**, Ross LA, Nager AL. An upsurge in pertussis: epidemiology and trends. *Pediatr Emerg Care* 2012; **28**: 215-219 [PMID: 22344207 DOI: 10.1097/PEC.0b013e318248b0cd]
31 **Centers for Disease Control and Prevention (CDC)**. Pertussis epidemic--Washington, 2012. *MMWR Morb Mortal Wkly Rep* 2012; **61**: 517-522 [PMID: 22810264]
32 **Spector TB**, Maziarz EK. Pertussis. *Med Clin North Am* 2013; **97**: 537-552, ix [PMID: 23809713 DOI: 10.1016/j.mcna.2013.02.004]
33 **Palefsky J**. Human papillomavirus infection in HIV-infected persons. *Top HIV Med* 2007; **15**: 130-133 [PMID: 17720998]
34 **Trottier H**, Franco EL. The epidemiology of genital human papillomavirus infection. *Vaccine* 2006; **24** Suppl 1: S1-15 [PMID: 16406226 DOI: 10.1016/j.vaccine.2005.09.054]
35 **Kane S**, Khatibi B, Reddy D. Higher incidence of abnormal Pap smears in women with inflammatory bowel disease. *Am J Gastroenterol* 2008; **103**: 631-636 [PMID: 17941962 DOI: 10.1111/j.1572-0241.2007.01582.x]
36 Digestive Disease Week and the 107th Annual Meeting of the American Gastroenterological Association Institute, May 20-25, 2006, Los Angeles, California, USA. *Gastroenterology* 2006; **130** (4

Suppl 2): A1-A911
37 **Bhatia J**, Bratcher J, Korelitz B, Vakher K, Mannor S, Shevchuk M, Panagopoulos G, Ofer A, Tamas E, Kotsali P, Vele O. Abnormalities of uterine cervix in women with inflammatory bowel disease. *World J Gastroenterol* 2006; **12**: 6167-6171 [PMID: 17036389]
38 **Lees CW**, Critchley J, Chee N, Beez T, Gailer RE, Williams AR, Shand AG, Arnott ID, Satsangi J. Lack of association between cervical dysplasia and IBD: a large case-control study. *Inflamm Bowel Dis* 2009; **15**: 1621-1629 [PMID: 19618462 DOI: 10.1002/ibd.20959]
39 **Singh H**, Demers AA, Nugent Z, Mahmud SM, Kliewer EV, Bernstein CN. Risk of cervical abnormalities in women with inflammatory bowel disease: a population-based nested case-control study. *Gastroenterology* 2009; **136**: 451-458 [PMID: 18996382 DOI: 10.1053/j.gastro.2008.10.021]
40 **Centers for Disease Control and Prevention (CDC)**. Advisory Committee on Immunization Practices (ACIP) recommended immunization schedules for persons aged 0 through 18 years and adults aged 19 years and older--United States, 2013. *MMWR Surveill Summ* 2013; **62** Suppl 1: 1 [PMID: 23364301]
41 **Akinsanya-Beysolow I**, Jenkins R, Meissner HC. Advisory Committee on Immunization Practices (ACIP) recommended immunization schedule for persons aged 0 through 18 years--United States, 2013. *MMWR Surveill Summ* 2013; **62** Suppl 1: 2-8 [PMID: 23364302]
42 **Centers for Disease Control and Prevention (CDC)**. FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010; **59**: 630-632 [PMID: 20508594]
43 **Centers for Disease Control and Prevention (CDC)**. FDA licensure of bivalent human papillomavirus vaccine (HPV2, Cervarix) for use in females and updated HPV vaccination recommendations from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010; **59**: 626-629 [PMID: 20508593]
44 **Markowitz LE**, Dunne EF, Saraiya M, Lawson HW, Chesson H, Unger ER. Quadrivalent Human Papillomavirus Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007; **56**: 1-24 [PMID: 17380109]
45 **Jacobson DL**, Bousvaros A, Ashworth L, Carey R, Shrier LA, Burchett SK, Renna H, Lu Y. Immunogenicity and tolerability to human papillomavirus-like particle vaccine in girls and young women with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 1441-1449 [PMID: 23567780 DOI: 10.1097/MIB.0b013e318281341b]
46 Prevention and Control of Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2013; **62**: 1-22
47 **Stein KE**. Thymus-independent and thymus-dependent responses to polysaccharide antigens. *J Infect Dis* 1992; **165** Suppl 1: S49-S52 [PMID: 1588177]
48 **Gisbert JP**, Villagrasa JR, Rodríguez-Nogueiras A, Chaparro M. Efficacy of hepatitis B vaccination and revaccination and factors impacting on response in patients with inflammatory bowel disease. *Am J Gastroenterol* 2012; **107**: 1460-1466 [PMID: 23034605 DOI: 10.1038/ajg.2012.79]
49 **Loras C**, Saro C, Gonzalez-Huix F, Mínguez M, Merino O, Gisbert JP, Barrio J, Bernal A, Gutiérrez A, Piqueras M, Calvet X, Andreu M, Abad A, Ginard D, Bujanda L, Panés J, Torres M, Fernández-Bañares F, Viver JM, Esteve M. Prevalence and factors related to hepatitis B and C in inflammatory bowel disease patients in Spain: a nationwide, multicenter study. *Am J Gastroenterol* 2009; **104**: 57-63 [PMID: 19098850 DOI: 10.1038/ajg.2008.4]
50 **Tolentino YF**, Fogaca HS, Zaltman C, Ximenes LL, Coelho HS. Hepatitis B virus prevalence and transmission risk factors in inflammatory bowel disease patients at Clementino Fraga Filho university hospital. *World J Gastroenterol* 2008; **14**: 3201-3206 [PMID: 18506926]
51 **Gisbert JP**, Chaparro M, Esteve M. Review article: prevention and management of hepatitis B and C infection in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; **33**: 619-633 [PMID: 21416659]
52 **Shale MJ**, Seow CH, Coffin CS, Kaplan GG, Panaccione R, Ghosh S. Review article: chronic viral infection in the anti-tumour necrosis factor therapy era in inflammatory bowel disease. *Aliment Pharmacol Ther* 2010; **31**: 20-34 [PMID: 19681818]
53 **Mast EE**, Weinbaum CM, Fiore AE, Alter MJ, Bell BP, Finelli L, Rodewald LE, Douglas JM, Janssen RS, Ward JW. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: immunization of adults. *MMWR Recomm Rep* 2006; **55**: 1-33; quiz CE1-4 [PMID: 17159833]
54 **Vida PL**, Gómez CF, García SV, Iglesias FE, Castillo ML, Cerezo RA, Casáis JL, De Dios VJ. [Adequate rate of response to hepatitis B virus vaccination in patients with inflammatory bowel disease]. *Med Clin* 2009; **132**: 331
55 **Altunöz ME**, Senateş E, Yeşil A, Calhan T, Ovünç AO. Patients with inflammatory bowel disease have a lower response rate to HBV vaccination compared to controls. *Dig Dis Sci* 2012; **57**: 1039-1044 [PMID: 22147248 DOI: 10.1007/s10620-011-1980-8]
56 **Nyström J**, Cardell K, Björnsdottir TB, Fryden A, Hultgren C, Sällberg M. Improved cell mediated immune responses after successful re-vaccination of non-responders to the hepatitis B virus surface antigen (HBsAg) vaccine using the combined hepatitis A and B vaccine. *Vaccine* 2008; **26**: 5967-5972 [PMID: 18804140 DOI: 10.1016/j.vaccine.2008.08.054]
57 **Radzikowski A**, Banaszkiewicz A, Łazowska-Przeorek I, Grzybowska-Chlebowczyk U, Woś H, Pytrus T, Iwańczak B, Kowalska-Duplaga K, Fyderek K, Gawrońska A, Karolewska-Bochenek K, Kotowska M, Albrecht P. Immunogenecity of hepatitis A vaccine in pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 1117-1124 [PMID: 20818674 DOI: 10.1002/ibd.21465]
58 **Moses J**, Alkhouri N, Shannon A, Feldstein A, Carter-Kent C. Response to hepatitis A vaccine in children with inflammatory bowel disease receiving infliximab. *Inflamm Bowel Dis* 2011; **17**: E160 [PMID: 21953938 DOI: 10.1002/ibd.21892]
59 **Park SH**, Yang SK, Park SK, Kim JW, Yang DH, Jung KW, Kim KJ, Ye BD, Byeon JS, Myung SJ, Kim JH. Efficacy of hepatitis A vaccination and factors impacting on seroconversion in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2014; **20**: 69-74 [PMID: 24284413 DOI: 10.1097/01.MIB.0000437736.91712.a1]
60 **Marin M**, Güris D, Chaves SS, Schmid S, Seward JF. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007; **56**: 1-40 [PMID: 17585291]
61 **Ansari F**, Baker RD, Patel R, Baker SS. Varicella immunity in inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2011; **53**: 386-388 [PMID: 21505365]
62 **Cullen G**, Baden RP, Cheifetz AS. Varicella zoster virus infection in inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 2392-2403 [PMID: 22434654 DOI: 10.1002/ibd.22950]
63 **Rahier JF**, Moutschen M, Van Gompel A, Van Ranst M, Louis E, Segaert S, Masson P, De Keyser F. Vaccinations in patients with immune-mediated inflammatory diseases. *Rheumatology* (Oxford) 2010; **49**: 1815-1827 [PMID: 20591834 DOI: 10.1093/rheumatology/keq183]
64 **Levin MJ**. Varicella vaccination of immunocompromised children. *J Infect Dis* 2008; **197** Suppl 2: S200-S206 [PMID: 18419398 DOI: 10.1086/522133]
65 **Harpaz R**, Ortega-Sanchez I, Seward J. Advisory Committee on Immunization Practices (ACIP) Centers for Disease Control and Prevention (CDC). Prevention of herpes zoster: Recommendations of the advisory committee on immunization practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2008; **57**: 30
66 **Gupta G**, Lautenbach E, Lewis JD. Incidence and risk factors for

herpes zoster among patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2006; **4**: 1483-1490 [PMID: 17162240 DOI: 10.1016/j.cgh.2006.09.019]

67 **Long MD**, Martin C, Sandler RS, Kappelman MD. Increased risk of herpes zoster among 108 604 patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2013; **37**: 420-429 [PMID: 23240738 DOI: 10.1111/apt.12182]

68 **Moscandrew M**, Mahadevan U, Kane S. General health maintenance in IBD. *Inflamm Bowel Dis* 2009; **15**: 1399-1409 [PMID: 19591135 DOI: 10.1002/ibd.20944]

69 **Zhang J**, Xie F, Delzell E, Chen L, Winthrop KL, Lewis JD, Saag KG, Baddley JW, Curtis JR. Association between vaccination for herpes zoster and risk of herpes zoster infection among older patients with selected immune-mediated diseases. *JAMA* 2012; **308**: 43-49 [PMID: 22760290 DOI: 10.1001/jama.2012.7304]

70 **Bernstein CN**, Rawsthorne P, Blanchard JF. Population-based case-control study of measles, mumps, and rubella and inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 759-762 [PMID: 17230540 DOI: 10.1002/ibd.20089]

71 **McLean HQ**, Fiebelkorn AP, Temte JL, Wallace GS. Prevention of measles, rubella, congenital rubella syndrome, and mumps, 2013: summary recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2013; **62**: 1-34 [PMID: 23760231]

72 **Naganuma M**, Nagahori M, Fujii T, Morio J, Saito E, Watanabe M. Poor recall of prior exposure to varicella zoster, rubella, measles, or mumps in patients with IBD. *Inflamm Bowel Dis* 2013; **19**: 418-422 [PMID: 22605673 DOI: 10.1002/ibd.23027]

73 **Wasan SK**, Baker SE, Skolnik PR, Farraye FA. A practical guide to vaccinating the inflammatory bowel disease patient. *Am J Gastroenterol* 2010; **105**: 1231-1238 [PMID: 20104218 DOI: 10.1038/ajg.2009.733]

74 **Watson JC**, Hadler SC, Dykewicz CA, Reef S, Phillips L. Measles, mumps, and rubella--vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1998; **47**: 1-57 [PMID: 9639369]

75 **Thompson NP**, Fleming DM, Pounder RE, Wakefield AJ. Crohn's disease, measles, and measles vaccination: a case-control failure. *Lancet* 1996; **347**: 263 [PMID: 8551906]

76 **Davis RL**, Kramarz P, Bohlke K, Benson P, Thompson RS, Mullooly J, Black S, Shinefield H, Lewis E, Ward J, Marcy SM, Eriksen E, Destefano F, Chen R. Measles-mumps-rubella and other measles-containing vaccines do not increase the risk for inflammatory bowel disease: a case-control study from the Vaccine Safety Datalink project. *Arch Pediatr Adolesc Med* 2001; **155**: 354-359 [PMID: 11231801 DOI: 10.1001/archpedi.155.3.354]

77 **Feeney M**, Ciegg A, Winwood P, Snook J. A case-control study of measles vaccination and inflammatory bowel disease. The East Dorset Gastroenterology Group. *Lancet* 1997; **350**: 764-766 [PMID: 9297995]

P- Reviewer: Raman M, Stocco G, Triantafyllou K **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3197

World J Gastroenterol 2015 March 21; 21(11): 3197-3205
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Hyponatremia in cirrhosis: Pathophysiology and management

Savio John, Paul J Thuluvath

Savio John, Division of Gastroenterology, Department of Medicine, SUNY Upstate Medical University, Syracuse, NY 13210, United States
Paul J Thuluvath, University of Maryland School of Medicine and Mercy Medical Center, Baltimore, MD 21202, United States
Author contributions: John S reviewed the literature, drafted the manuscript and approved the final version of the article to be published; Thuluvath JP reviewed the literature, made critical revisions related to the content of the article and approved the final version of the article to be published.
Conflict-of-interest: None conflicting interest related to the manuscript submitted for publication.

Correspondence to: Paul J Thuluvath, MD, FAASLD, FRCP, Professor of Medicine and Surgery, University of Maryland School of Medicine and Mercy Medical Center, 301 Saint Paul Place, Baltimore, MD 21202, United States. thuluvath@gmail.com
Telephone: +1-410-3329356
Fax: +1-410-7835884
Received: October 20, 2014
Peer-review started: October 21, 2014
First decision: November 14, 2014
Revised: December 2, 2014
Accepted: January 30, 2015
Article in press: January 30, 2015
Published online: March 21, 2015

Abstract

Hyponatremia is frequently seen in patients with ascites secondary to advanced cirrhosis and portal hypertension. The development of ascites in patients with cirrhosis is multi-factorial. Portal hypertension and the associated systemic vasodilation lead to activation of the sodium-retaining neurohumoral mechanisms which include the renin-angiotensin-aldosterone system, sympathetic nervous system and antidiuretic hormone (ADH). The net effect is the avid retention of sodium and water to compensate for the low effective circulatory volume resulting in the development of ascites. Although not apparent in the early stages of cirrhosis, the progression of cirrhosis and ascites leads to impairment of the kidneys to eliminate solute-free water. This leads to additional compensatory mechanisms including non-osmotic secretion of ADH, also known as arginine vasopressin, further worsening excess water retention and thereby hyponatremia. Hyponatremia is associated with increased morbidity and mortality in patients with cirrhosis, and is an important prognostic marker both before and after liver transplant. The management of hyponatremia in this setting is a challenge as conventional therapy for hyponatremia including fluid restriction and loop diuretics are frequently inefficacious. In this review, we discuss the pathophysiology and various treatment modalities, including selective vasopressin receptor antagonists, for the management of hyponatremia in patients with cirrhosis.

Key words: Hyponatremia in cirrhosis; Dilutional hyponatremia; Hypervolemic hyponatremia; Vasopressin receptor antagonists; Vaptans

Core tip: Hyponatremia is the most common electrolyte abnormality observed in hospitalized patients and is a common finding in patients with advanced cirrhosis. The management of hyponatremia in cirrhosis is challenging as conventional therapy for hyponatremia including fluid restriction and loop diuretics are frequently inefficacious. Vaptans, drugs that selectively antagonizes the effects of arginine vasopressin on the V_2 receptors in the kidney tubules, represent a logical step in the treatment of hyponatremia. The currently

available vaptans, however, are not approved for use in patients with cirrhosis due to the increased risk for hepatic failure and mortality.

John S, Thuluvath PJ. Hyponatremia in cirrhosis: Pathophysiology and management. *World J Gastroenterol* 2015; 21(11): 3197-3205 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3197.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3197

INTRODUCTION

Hyponatremia is the most common electrolyte abnormality observed in hospitalized patients[1]. Hyponatremia in cirrhosis is currently defined as a serum sodium level of less than 130 meq/L[2]. It has been suggested that the prevalence of a serum sodium concentration less than 135, 130 and 120 meq/L in patients with cirrhosis and ascites is 49.4%, 21.6% and 1.2%, respectively[3]. Patients with cirrhosis may develop hyponatremia due to either hypovolemia (example: loss of extracellular fluid due to diuretics) or hypervolemia (expanded extracellular fluid volume due to the inability of the kidneys to excrete solute-free water proportionate to the amount of free water ingested). In this review, we will discuss the pathogenesis, prognostic value and management of dilutional hyponatremia (hypervolemic hyponatremia) in patients with cirrhosis and portal hypertension.

PATHOGENESIS

Systemic vasodilation

Systemic vasodilation and arterial underfilling play a major role in development of hyponatremia in patients with cirrhosis and portal hypertension (Figure 1). A hyperdynamic circulation, characterized by an increased cardiac output, markedly reduced systemic vascular resistance and reduced mean arterial pressure, is a common cardiovascular physiological manifestation of patients with cirrhosis and advanced portal hypertension[4,5]. The marked reduction in vascular resistance predominantly involves the splanchnic arterial circulation[6]. The opening of portasystemic collaterals[5] and the increased synthesis of circulating vasodilators, including nitric oxide (NO), glucagon, vasoactive intestinal peptide, substance P, platelet activating factor, prostaglandins and prostacyclins play a crucial role in the pathogenesis of splanchnic vasodilation[7] (Figure 2). The accumulating circumstantial evidence favors a key role for NO in the pathogenesis of splanchnic vasodilation in patients with advanced cirrhosis and portal hypertension[8-10]. The activation of nitric oxide synthase in the endothelial cells is multi-factorial, which include mechanical stimuli due to 'shear stress', vascular endothelial growth factors, tumor necrosis factor alpha, and more importantly endotoxins or bacterial DNA[11,12] that are less efficiently cleared from the gastrointestinal tract due to portal systemic shunting and defective reticuloendothelial cell function in cirrhosis.

It has been suggested that endotoxemia may be a causative factor for increased systemic prostacyclin synthesis, and could be reversed to some extent by antibacterial agents[13]. It is possible that when one of the vasoactive mediators, such as NO or prostacyclin, is inhibited, other vasoactive pathways such as the angiotensin-Ⅱ, norepinephrine, vasopressin and the augmented sympathetic tone, are up-regulated thereby preventing the correction of the splanchnic vasodilation. The complex relationship among these vasoactive systems implies that no one factor is likely to be solely responsible for the splanchnic vasodilation seen in patients with portal hypertension. This may explain the difficulty in developing pharmacological agents to counteract splanchnic vasodilation.

Water balance and role of antidiuretic hormone (also known arginine vasopressin)

The total body water and osmolality are maintained within normal limits in such a way that an increase in water intake (normally 1.5-3 L/d; may vary from 0.5-20 L/d under extreme conditions) is followed by an increase in renal solute-free water excretion and a decrease in water intake is ensued by a decrease in free water excretion. The serum osmolality (and hence serum sodium) is tightly regulated primarily at the level of hypothalamus *via* the release of antidiuretic hormone (ADH). A rise or fall in serum osmolality is accompanied by a corresponding increase or decrease of ADH secretion. Under normal physiologic conditions, the kidneys are in a state of antidiuresis with a 24-h urine osmolality higher than plasma osmolality[14].

The collecting duct has minimal water permeability under normal conditions, but permeability increases when ADH is released in response to hyperosmolality and hypovolemia. The enhanced binding of vasopressin to the V_2 receptors on the basolateral membrane of the cells lining the renal collecting ducts leads to production of cyclic AMP and subsequent activation of protein kinase A. This in turn phosphorylates microtubular subunits that aggregate to form specific water channel, aquaporin-2 (AQP-2), that are translocated from the cytoplasmic vesicles to the apical plasma membrane. This process allows the reabsorption of large volumes of water from the collecting duct, which leads to an increase in body water content and hypervolemic hyponatremia[15-20] (Figure 3). Under physiologic conditions, when serum osmolality increases, ADH secretion increases, aquaporin channels in the renal collecting duct are activated, resulting in water reabsorption. A fall in serum osmolality leads to inactivation of the renal aquaporin channels and excretion of dilute urine to maintain the volume status and serum osmolality. The rapid adaptation of the free water excretion depends on the presence of intact osmoreceptors in the anterior hypothalamus, the release

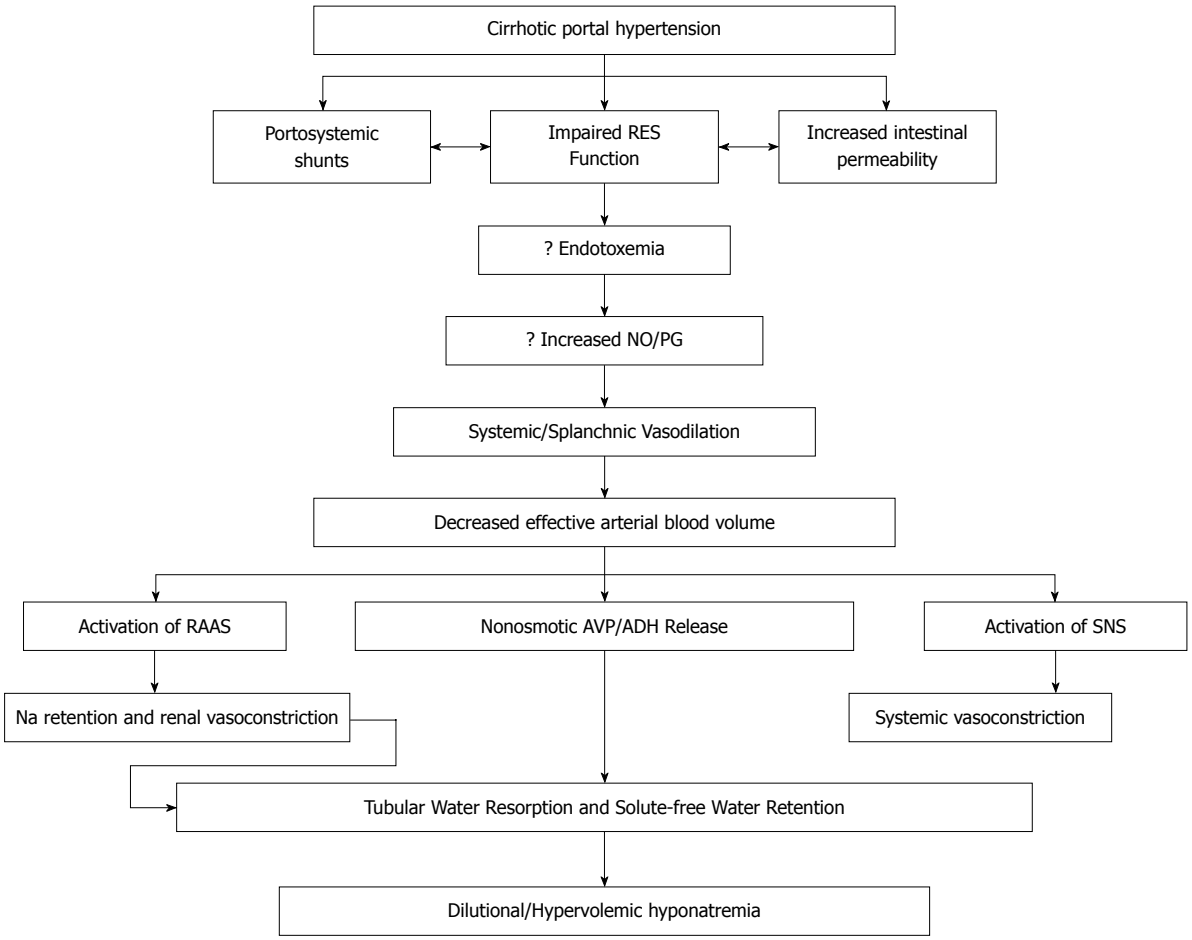


Figure 1 Proposed mechanisms for the development of hyponatremia. SNS: Sympathetic nervous system; RAAS: Renin-angiotensin-aldosterone system; NO: Nitric oxide; RES: Reticuloendothelial system; PG: Prostaglandin; AVP: Arginine vasopressin; ADH: Antidiuretic hormone.

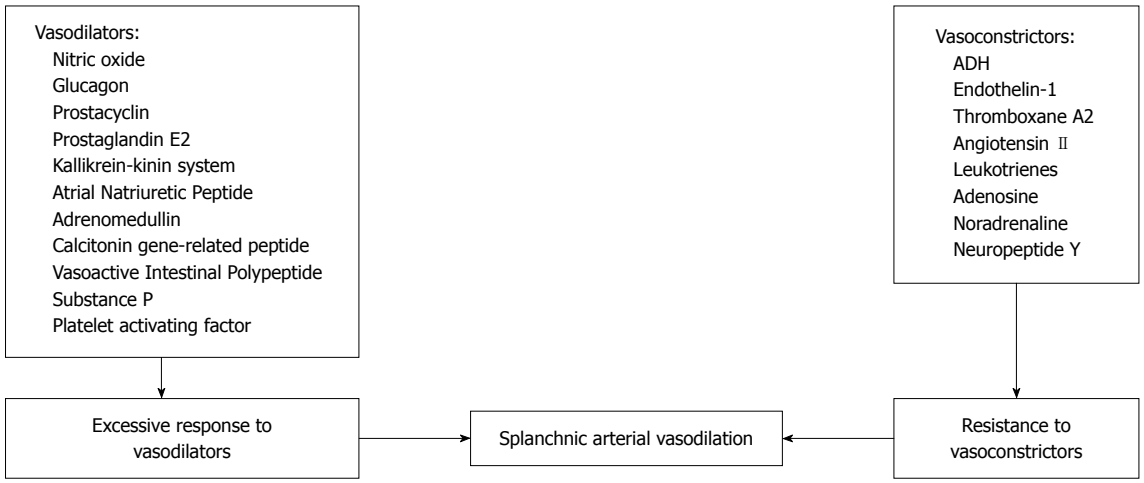


Figure 2 Mechanisms involved in the splanchnic vasodilation in cirrhosis. ADH: Antidiuretic hormone.

of ADH and the appropriate interaction between the ADH and AQP-2.

ADH is a polypeptide hormone that is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and stored in the posterior pituitary gland. Increased plasma osmolality and hypovolemia are the principal physiological stimuli for vasopressin secretion. Thus both osmotic and non-osmotic stimulations regulate ADH release. The osmotic pathway is mediated *via* osmoreceptors located in the anterior hypothalamus close to the supraoptic nuclei. These receptors sense the intracellular water content in the neurons (by their swelling and shrinking) and respond linearly to the changes in plasma osmolality[21]. The major non-osmotic

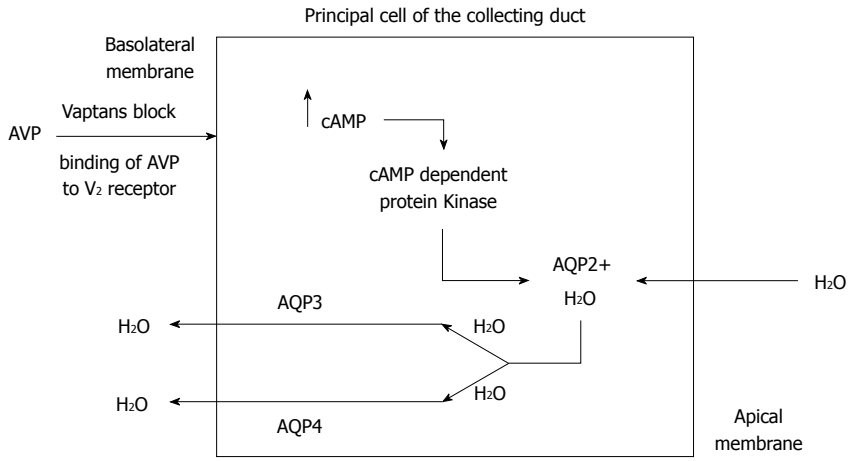


Figure 3 Mechanism of action of vaptans. AVP: Arginine vasopressin; AQP2: Aquaporin-2; AQP3: Aquaporin-3; AQP4: Aquaporin-4.

Table 1 Vasopressin receptors

Receptor	Location	Function
V_{1a}	Vascular smooth muscle	Vasoconstriction and myocardial hypertrophy
	Platelets	Platelet aggregation
	Hepatocytes	Glycogenolysis
	Myometrium	Uterine contractions
V_{1b}	Anterior pituitary	ACTH release
V_2	Basolateral membrane in collecting tubule	Water reabsorption
	Vascular endothelium	Release of vonWillebrand factor and factor Ⅷ
	Vascular smooth muscle	Vasodilation

pathway for ADH release involves the autonomic nervous system which is mediated via the baroreceptors located in the atria, ventricle, aortic arch, and carotid sinus. These baroreceptors communicate to the hypothalamus via parasympathetic pathways and cause a release of ADH in response to hypovolemia. Please refer to Table 1 for the details of vasopressin receptor subtypes.

Non-osmotic stimulation of renin-angiotensin-aldosterone system, sympathetic nervous system and ADH

The systemic/splanchnic vasodilation and arterial underfilling in patients with cirrhosis and portal hypertension lead to a decrease in the effective circulatory volume and a reduction in stretch at the carotid and renal baroreceptors. In order to restore the effective circulatory volume, the sodium-retaining neurohumoral mechanisms, such as the renin-angiotensin-aldosterone system, sympathetic nervous system and ADH, are activated leading to maximal retention of sodium and water.

Water and sodium retention secondary to impaired renal elimination of solute-free water clearly has been shown to occur in decompensated cirrhotic patients with ascites and edema. This impairment could be subclinical and only detected by a water loading test in compensated cirrhosis[22-26]. The role that ADH plays in mediating this abnormal water excretion was studied by Bichet *et al*[27] who measured plasma ADH concentrations before and after the water load test in cirrhotic patients with and without ascites. There was a significant difference in the inability to suppress ADH after the water load test in decompensated cirrhotic patients with ascites as compared to compensated cirrhotic patients, despite the presence of a low serum osmolality in decompensated cirrhotic patients.

The relative role of osmotic and non-osmotic pathways for the hypersecretion of ADH in patients with cirrhosis has been debated. Most cirrhotic patients have low serum osmolality and sodium levels, and one would expect to see suppression of ADH release if the stimulation was primarily from the osmoreceptors[2]. The ADH, norepinephrine, and aldosterone levels as well as renin activity were significantly higher in cirrhotic patients with ascites after the water load test implying that there was activation of the sodium-retaining neurohumoral mechanisms. It appears that the decrease in systemic vascular resistance leads to effective arterial underfilling, which causes baroreceptor mediated nonosmotic stimulation of ADH and other vasoconstricting systems leading to the activation of sodium retaining neurohumoral mechanisms in order to restore perfusion pressure[6]. These findings may suggest that the hypo-osmotic stimuli to suppress ADH release are overridden by the nonosmotic stimuli secondary to arterial under filling[28]. Thus in order to prevent impending vascular collapse from effective circulatory volume depletion, the body sacrifices the osmolar homeostasis and releases ADH in response to the non-osmotic stimulus of the endogenous vasoconstrictor agents. The net result is enhanced sodium and water retention to correct the depletion of circulatory volume and this occurs despite the presence of increased total body extracellular sodium, plasma volume, and cardiac output. As suppression of ADH release is required to excrete a water load, the inability of kidneys to excrete water in the presence of the non-osmotically triggered

ADH release leads to the development of a dilutional or hypervolemic hyponatremia. Thus the hyponatremia in this patient population is purely dilutional and does not reflect a sodium deficient state.

Many other factors including elevated atrial natriuretic peptide[29], decreased renal production of PGE-2[30-34] and decreased metabolism of ADH have been implicated in the development of hyponatremia in cirrhosis[35,36].

Prognostic value of hyponatremia in cirrhosis

In patients without cirrhosis, hyponatremia depending on its severity may lead to a range of symptoms including mild cognitive dysfunction, falls, seizures, coma and very rarely death[37]. Hyponatremia in cirrhosis is a chronic process and this allows the brain to adapt to the hypo-osmolality of the extracellular fluid. The most important factor in determining the severity of neurologic symptoms in patients with hyponatremia is the acuity of fall of serum sodium rather than the absolute reduction of serum sodium. Hence patients with cirrhosis and hyponatremia are less likely to have severe neurologic symptoms[38]. However, hyponatremia may pose a second osmotic hit to cerebral edema and astrocyte swelling, in addition to the astrocyte dysfunction caused by increased intracellular glutamine concentration from ammonia metabolism, thereby precipitating hepatic encephalopathy[38].

The quality of life is poor in patients with cirrhosis and hyponatremia due to the requirement for strict fluid restriction. Hyponatremia has been found to be an independent predictive factor of the impaired health related quality life[39] as well as hepatic encephalopathy[40]. Numerous studies have shown that the severity of hyponatremia and ascites is a major determinant of disease severity and prognosis in cirrhosis[26,41-48]. In one study, the serum sodium level before the onset of spontaneous bacterial peritonitis (SBP) was an independent predictor of renal failure triggered by SBP[49]. It has also been suggested that serum sodium is an earlier and more sensitive test than serum creatinine to detect circulatory dysfunction resulting in renal failure and/or death[47]. Although patients with hyponatremia are at a very high risk for developing hepatorenal syndrome, low serum sodium in hepatorenal syndrome is not only due to high ADH levels but also due to decreased GFR and proximal sodium reabsorption[38].

Patients with hyponatremia were found to have a higher risk of early death before transplantation independent of the severity of cirrhosis as assessed by the MELD scores[48]. Hence, some investigators have advocated an expedited liver transplantation under a 'sickest first' model in cirrhotic patients with MELD scores below 21, persistent ascites and hyponatremia[48]. It has been suggested that serum sodium could be incorporated into the MELD score[48], and this may provide a more accurate survival prediction than MELD alone[50]. Other studies have also identified hyponatremia to be a risk factor for increased morbidity and mortality after liver transplantation[51,52].

TREATMENT

Management of hyponatremia in the presence moderate to severe ascites is challenging for both physicians and patients. Hypovolemic hyponatremia should be treated with fluid resuscitation to restore the circulatory volume and withdrawal of the precipitating factor (usually diuretic therapy). On the other hand, hypervolemic/dilutional hyponatremia in cirrhosis is ideally managed with fluid restriction and measures to enhance the renal solute-free water excretion. The majority of patients find it difficult to adhere to fluid restriction, and discontinuation of diuretics may further worsen ascites and hydrothorax requiring repeated paracentesis or thoracocentesis.

Rapid correction (> 9 mEq/L in 24 h) of serum sodium may lead to serious neurological complications such as central pontine myelinolysis or seizures. Hyponatremia could pose significant risk to patients if they were to undergo liver transplantation where it is not always possible to maintain fluctuations in serum sodium levels to less than 10 mEq/L over 24 h. However, treatment for hyponatremia is indicated when the serum sodium is less than 120 meq/L or the patient has neurologic symptoms that might be due to hyponatremia. The general principles of the treatment of hyponatremia are broadly outlined below.

Water restriction

The mainstay of therapy of hyponatremia in patients with cirrhosis is fluid restriction (1-1.5 L/d) to a level sufficient to induce a negative water balance. Fluid restriction should be considered if the patient has neurologic symptoms that might be due to hyponatremia or when the serum sodium is less than 120 mEq/L, which occurs in about 1% of patients with cirrhosis[3]. There is no role for routine free water restriction in patients with mild, asymptomatic hyponatremia. To be effective, fluid intake should be less than urine output to account for the endogenous production of water by the body. In the authors' experience, compliance with fluid restriction is poor in these patients even in the hospital setting and often difficult to achieve. Patients on strict fluid restriction may be encouraged to suck on ice chips or lollipops to quench the thirst. A good indicator of adequate water restriction is the change in plasma sodium concentration within the first 24-48 h. If there is no increase in the plasma sodium levels within the first 48-72 h, either the patient is not following the water restriction or a stricter water restriction is needed. Sodium restriction (2 g/d) should be continued in addition to fluid restriction as these patients also have ascites.

Hypertonic saline

Hypertonic saline is indicated only in symptomatic patients who are intolerant or unresponsive to free water restriction, those with profound hyponatremia (<

110 mEq/L), or within hours of liver transplantation to prevent the likelihood for an emergent rapid correction in the operating room when the serum sodium levels are somewhat higher (between 120-130 mEq/L). Extreme care should be exercised not to overcorrect the serum sodium levels above 9 mEq/L per 24 h to avoid the risks of central pontine myelinolysis, quadriplegia, coma or death. As hypertonic sodium chloride infusion leads to increasing ascites and edema, it is usually not recommended for the treatment of hypervolemic hyponatremia, except in cases of profound hyponatremia as discussed above.

Correction of hypokalemia

Correction of hypokalemia also appears to be important in patients with cirrhosis and hyponatremia for two reasons: hypokalemia promotes the development of hepatic encephalopathy; correction of hypokalemia tends to raise serum sodium concentration. Hypokalemia predisposes to hepatic encephalopathy by at least two mechanisms: hypokalemia increases renal ammonia synthesis; the concomitant alkalemia increases the fraction of unionized ammonia in the plasma. As potassium is as osmotically active as sodium, supplementation of potassium can raise serum sodium and osmolality in patients with hyponatremia[53].

Albumin infusion

Intravenous albumin infusion might be useful in the short term, although long term use has not been studied and this approach is expensive and impractical[54].

Pharmacological therapy

The objectives of pharmacological therapy are to increase solute-free water excretion. There have been many attempts to achieve this goal with varying success rates, and this is a field in evolution. The target of pharmacological therapy has focused on the release or action of ADH [arginine vasopressin (AVP)]. The potential options include use of κ-opioid agonists to block the central release of ADH, blockade of the V_2 receptor of ADH with specific antagonists; and finally alteration of the effect of ADH at the level of the collecting duct in the kidney. Democycline could block the action of ADH in the collecting ducts, but could cause renal failure and hence is not recommended. Therefore only κ-opioid agonists and V_2 receptor antagonists have been studied in both animals and humans.

κ-opioid agonists

κ-opioid agonists inhibit ADH release from the neurohypophysis, and have been shown to exert an aquaretic effect in animal models and in patients with cirrhosis. In the only human study, niravoline (0.5-2 mg, iv) was given to 18 patients with cirrhosis[55,56]. There was a marked aquaretic effect between 1 and 2 h after administration with a return to basal values at 24 h[57]. The aquaretic effect was not sustained, and moreover, it was associated with major neurological side effects including personality disorders and mild confusion. Hence, no large scale studies have been performed with κ-opioid agonists.

Vasopressin receptor antagonists or vaptans

The biologic effects of AVP (ADH) are mediated *via* specific receptors called V_{1a}, V_{1b}, and V_2 receptors as discussed earlier in this review. Anti-diuretic properties of ADH are mediated primarily through the V_2 receptors, which are found exclusively in the renal collecting ducts. Activation of V_2 receptors is responsible for water reabsorption. The development of V_2 receptor antagonists was therefore a logical step in the management of fluid overload and hyponatremia as effective V_2 receptor antagonists could theoretically produce pure aquaresis (Figure 3).

The initial studies on vaptans in cirrhosis were done on patients with cirrhosis without hyponatremia[13,58]. These studies demonstrated the efficacy of oral vaptans in increasing the urine volume and solute-free water excretion resulting in a negative fluid balance. The subsequent studies with vaptans have consistently demonstrated their efficacy in improving serum sodium levels in the short term[59-64], but with increased risk for mortality in patients with cirrhosis, as explained below.

Tolvaptan, satavaptan and lixivaptan are all oral agents which selectively block the V_2 receptor. The intravenous agent, conivaptan, which blocks both V_2 and V_1 receptors may lead to further reduction in blood pressure, increase the risk of variceal bleeding via the V_{1a} receptor blockade[65]. Tolvaptan is a selective non-peptide V_2 receptor antagonist and when this drug was added to standard diuretic therapy for periods ranging from 25 to 60 d in patients with heart failure[61,63], treated patients had significantly lower weight and improvement in edema as well as serum sodium levels compared to those who received placebo. This drug was initially approved by the United States Food and Drug Administration (FDA) for use in hyponatremia based on a randomized controlled trial involving a predominant patient population comprising of those with congestive heart failure. Sixty three patients with cirrhosis with a CTP score less than 10 and serum sodium less than 120 were also included in the above study[61]. However, based on the findings of multicenter trials evaluating the effect of tolvaptan on the progression of disease in polycystic kidney disease[66,67], the FDA determined that tolvaptan should not be used in patients with liver disease or cirrhosis due to the risks for liver failure and death. Similarly, a one year follow-up study on patients on satavaptan showed increased mortality compared to placebo, resulting in withdrawal of the drug by the pharmaceutical company[68].

Domeclocycline, another ADH antagonist, which increased free water excretion and thus corrects

hyponatremia, should not be used in cirrhosis due to its nephrotoxic potential[69]. Since most patients with hyponatremia have advanced cirrhosis, the side-effect profile of single dose-finding studies must be interpreted with caution, as in the case of the preliminary studies on vaptans, because adverse events are more likely to occur after long term administration in an unselected population with greater co-morbidities.

Terlipressin, by virtue of its strong effect on vasopressin V_1 receptor, has therapeutic potential in portal hypertensive bleeding as well as hepatorenal syndrome. Terlipressin is also a partial agonist of renal vasopressin V_2 receptors and acute reduction in serum sodium level has been documented in patients who are initiated on terlipressin[70]. The resultant hyponatremia, although severe in some patients, is usually reversible after withdrawal of terlipressin therapy. Hence serum sodium levels should be monitored while patients are on therapy with terlipressin.

CONCLUSION

Hyponatremia is very common in patients with cirrhosis and the routine correction of asymptomatic hyponatremia is not recommended. The main indications for correction of hyponatremia are presence of neurologic symptoms that might be due to hyponatremia and serum sodium less than 120 mEq/L. The only exception is in patients who are likely to receive liver transplantation within hours when their serum sodium concentration is less than 130 mEq/L to avoid rapid correction in the operating room as it may be associated with serious neurological complications. Correction of hypokalemia and fluid restriction are the mainstays of treatment. Administration of hypertonic saline may be considered in a monitored setting to correct profound hyponatremia (serum sodium < 110 mEq/L) and in the immediate pre-liver transplant period to prevent the risk of osmotic demyelination syndrome. No vasopressin receptor antagonist is currently approved by the FDA for treatment of hyponatremia in patients with liver disease or cirrhosis. The availability of selective and efficacious oral V_2 receptor antagonists, without major side effects, will be a major development for the management of hyponatremia.

REFERENCES

1 **Baran D**, Hutchinson TA. The outcome of hyponatremia in a general hospital population. *Clin Nephrol* 1984; **22**: 72-76 [PMID: 6478674]

2 **Ginés P**, Berl T, Bernardi M, Bichet DG, Hamon G, Jiménez W, Liard JF, Martin PY, Schrier RW. Hyponatremia in cirrhosis: from pathogenesis to treatment. *Hepatology* 1998; **28**: 851-864 [PMID: 9731583 DOI: 10.1002/hep.510280337]

3 **Angeli P**, Wong F, Watson H, Ginès P. Hyponatremia in cirrhosis: Results of a patient population survey. *Hepatology* 2006; **44**: 1535-1542 [PMID: 17133458 DOI: 10.1002/hep.21412]

4 **Abelmann WH**. Hyperdynamic circulation in cirrhosis: a historical perspective. *Hepatology* 1994; **20**: 1356-1358 [PMID: 7927272 DOI: 10.1002/hep.1840200537]

5 **Groszmann RJ**. Hyperdynamic circulation of liver disease 40 years later: pathophysiology and clinical consequences. *Hepatology* 1994; **20**: 1359-1363 [PMID: 7927273 DOI: 10.1002/hep.1840200538]

6 **Schrier RW**, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodés J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988; **8**: 1151-1157 [PMID: 2971015 DOI: 10.1002/hep.1840080532]

7 **Ginès P**, Fernández-Esparrach G, Arroyo V, Rodés J. Pathogenesis of ascites in cirrhosis. *Semin Liver Dis* 1997; **17**: 175-189 [PMID: 9308123 DOI: 10.1055/s-2007-1007196]

8 **Vallance P**, Moncada S. Hyperdynamic circulation in cirrhosis: a role for nitric oxide? *Lancet* 1991; **337**: 776-778 [PMID: 1706450 DOI: 10.1016/0140-6736(91)91384-7]

9 **Iwakiri Y**, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. *Hepatology* 2006; **43**: S121-S131 [PMID: 16447289 DOI: 10.1002/hep.20993]

10 **Battista S**, Bar F, Mengozzi G, Zanon E, Grosso M, Molino G. Hyperdynamic circulation in patients with cirrhosis: direct measurement of nitric oxide levels in hepatic and portal veins. *J Hepatol* 1997; **26**: 75-80 [PMID: 9148026]

11 **Such J**, Francés R, Muñoz C, Zapater P, Casellas JA, Cifuentes A, Rodríguez-Valera F, Pascual S, Sola-Vera J, Carnicer F, Uceda F, Palazón JM, Pérez-Mateo M. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, nonneutrocytic ascites. *Hepatology* 2002; **36**: 135-141 [PMID: 12085357 DOI: 10.1053/jhep.2002.33715]

12 **Francés R**, Benlloch S, Zapater P, González JM, Lozano B, Muñoz C, Pascual S, Casellas JA, Uceda F, Palazón JM, Carnicer F, Pérez-Mateo M, Such J. A sequential study of serum bacterial DNA in patients with advanced cirrhosis and ascites. *Hepatology* 2004; **39**: 484-491 [PMID: 14768002 DOI: 10.1002/hep.20055]

13 **Guarner C**, Soriano G, Such J, Teixidó M, Ramis I, Bulbena O, Roselló J, Guarner F, Gelpi E, Balanzó J. Systemic prostacyclin in cirrhotic patients. Relationship with portal hypertension and changes after intestinal decontamination. *Gastroenterology* 1992; **102**: 303-309 [PMID: 1727763]

14 **Arroyo V**, Jiménez W. Complications of cirrhosis. II. Renal and circulatory dysfunction. Lights and shadows in an important clinical problem. *J Hepatol* 2000; **32**: 157-170 [PMID: 10728802 DOI: 10.1016/S0168-8278(00)80423-7]

15 **Knepper MA**, Wade JB, Terris J, Ecelbarger CA, Marples D, Mandon B, Chou CL, Kishore BK, Nielsen S. Renal aquaporins. *Kidney Int* 1996; **49**: 1712-1717 [PMID: 8743483 DOI: 10.1038/ki.1996.253]

16 **Nielsen S**, Marples D, Frøkiaer J, Knepper M, Agre P. The aquaporin family of water channels in kidney: an update on physiology and pathophysiology of aquaporin-2. *Kidney Int* 1996; **49**: 1718-1723 [PMID: 8743484 DOI: 10.1038/ki.1996.254]

17 **King LS**, Agre P. Pathophysiology of the aquaporin water channels. *Annu Rev Physiol* 1996; **58**: 619-648 [PMID: 8815812 DOI: 10.1146/annurev.ph.58.030196.003155]

18 **Knepper MA**. Molecular physiology of urinary concentrating mechanism: regulation of aquaporin water channels by vasopressin. *Am J Physiol* 1997; **272**: F3-12 [PMID: 9039043]

19 **Fushimi K**, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S. Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* 1993; **361**: 549-552 [PMID: 8429910 DOI: 10.1038/361549a0]

20 **Agre P**, Nielsen S. The aquaporin family of water channels in kidney. *Nephrologie* 1996; **17**: 409-415 [PMID: 8987045]

21 **Oliet SH**, Bourque CW. Mechanosensitive channels transduce osmosensitivity in supraoptic neurons. *Nature* 1993; **364**: 341-343 [PMID: 7687327 DOI: 10.1038/364341a0]

22 **Epstein M**. Derangements of renal water handling in liver disease. *Gastroenterology* 1985; **89**: 1415-1425 [PMID: 3902555]

23 **Arroyo V**, Clària J, Saló J, Jiménez W. Antidiuretic hormone and the pathogenesis of water retention in cirrhosis with ascites. *Semin Liver Dis* 1994; **14**: 44-58 [PMID: 8016662 DOI: 10.1055/s-2007-1007297]

24 **Ginès P**, Abraham WT, Schrier RW. Vasopressin in pathophysiological states. *Semin Nephrol* 1994; **14**: 384-397 [PMID: 7938953]

25 Vaamonde CA. Renal water handling in liver disease, Epstein M, ed. The Kidney in Liver Disease, 4th ed. Philadelphia: Hanley & Belfus, Inc., 1996: 33-74

26 **Arroyo V**, Rodés J, Gutiérrez-Lizárraga MA, Revert L. Prognostic value of spontaneous hyponatremia in cirrhosis with ascites. *Am J Dig Dis* 1976; **21**: 249-256 [PMID: 1266841 DOI: 10.1007/BF01095898]

27 **Bichet D**, Szatalowicz V, Chaimovitz C, Schrier RW. Role of vasopressin in abnormal water excretion in cirrhotic patients. *Ann Intern Med* 1982; **96**: 413-417 [PMID: 7065556 DOI: 10.7326/0003-4819-96-4-413]

28 **Schrier RW**. Water and sodium retention in edematous disorders: role of vasopressin and aldosterone. *Am J Med* 2006; **119**: S47-S53 [PMID: 16843085 DOI: 10.1016/j.amjmed.2006.05.007]

29 **Warner L**, Skorecki K, Blendis LM, Epstein M. Atrial natriuretic factor and liver disease. *Hepatology* 1993; **17**: 500-513 [PMID: 8444424]

30 **Hébert RL**, Jacobson HR, Breyer MD. PGE2 inhibits AVP-induced water flow in cortical collecting ducts by protein kinase C activation. *Am J Physiol* 1990; **259**: F318-F325 [PMID: 2167017]

31 **Orloff J**, Handler JS, Bergstrom S. Effect of prostaglandin (pge-1) on the permeability response of toad bladder to vasopressin, theophylline and adenosine 3',5'-monophosphate. *Nature* 1965; **205**: 397-398 [PMID: 14243428 DOI: 10.1038/205397a0]

32 **Anderson RJ**, Berl T, McDonald KD, Schrier RW. Evidence for an in vivo antagonism between vasopressin and prostaglandin in the mammalian kidney. *J Clin Invest* 1975; **56**: 420-426 [PMID: 1150880 DOI: 10.1172/JCI108108]

33 **Walker LA**, Frölich JC. Dose-dependent stimulation of renal prostaglandin synthesis by deamino-8-D-arginine vasopressin in rats with hereditary diabetes insipidus. *J Pharmacol Exp Ther* 1981; **217**: 87-91 [PMID: 7205662]

34 **Pérez-Ayuso RM**, Arroyo V, Camps J, Rimola A, Gaya J, Costa J, Rivera F, Rodés J. Evidence that renal prostaglandins are involved in renal water metabolism in cirrhosis. *Kidney Int* 1984; **26**: 72-80 [PMID: 6434791 DOI: 10.1038/ki.1984.136]

35 **Solis-Herruzo JA**, Gonzalez-Gamarra A, Castellano G, Muñoz-Yagüe MT. Metabolic clearance rate of arginine vasopressin in patients with cirrhosis. *Hepatology* 1992; **16**: 974-979 [PMID: 1398505 DOI: 10.1002/hep.1840160420]

36 **Kim JK**, Summer SN, Howard RL, Schrier RW. Vasopressin gene expression in rats with experimental cirrhosis. *Hepatology* 1993; **17**: 143-147 [PMID: 8423035]

37 **Adrogué HJ**, Madias NE. Hyponatremia. *N Engl J Med* 2000; **342**: 1581-1589 [PMID: 10824078 DOI: 10.1056/NEJM200005253422107]

38 **Ginès P**, Guevara M. Hyponatremia in cirrhosis: pathogenesis, clinical significance, and management. *Hepatology* 2008; **48**: 1002-1010 [PMID: 18671303 DOI: 10.1002/hep.22418]

39 **Solà E**, Watson H, Graupera I, Turón F, Barreto R, Rodríguez E, Pavesi M, Arroyo V, Guevara M, Ginès P. Factors related to quality of life in patients with cirrhosis and ascites: relevance of serum sodium concentration and leg edema. *J Hepatol* 2012; **57**: 1199-1206 [PMID: 22824819 DOI: 10.1016/j.jhep.2012.07.020]

40 **Guevara M**, Baccaro ME, Torre A, Gómez-Ansón B, Ríos J, Torres F, Rami L, Monté-Rubio GC, Martín-Llahí M, Arroyo V, Ginès P. Hyponatremia is a risk factor of hepatic encephalopathy in patients with cirrhosis: a prospective study with time-dependent analysis. *Am J Gastroenterol* 2009; **104**: 1382-1389 [PMID: 19455124 DOI: 10.1038/ajg.2009.293]

41 **Llach J**, Ginès P, Arroyo V, Rimola A, Titó L, Badalamenti S, Jiménez W, Gaya J, Rivera F, Rodés J. Prognostic value of arterial pressure, endogenous vasoactive systems, and renal function in cirrhotic patients admitted to the hospital for the treatment of ascites. *Gastroenterology* 1988; **94**: 482-487 [PMID: 3335320]

42 **Ginés P**, Quintero E, Arroyo V, Terés J, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128 [PMID: 3804191]

43 **Cosby RL**, Yee B, Schrier RW. New classification with prognostic value in cirrhotic patients. *Miner Electrolyte Metab* 1989; **15**: 261-266 [PMID: 2682175]

44 **Fernández-Esparrach G**, Sánchez-Fueyo A, Ginès P, Uriz J, Quintó L, Ventura PJ, Cárdenas A, Guevara M, Sort P, Jiménez W, Bataller R, Arroyo V, Rodés J. A prognostic model for predicting survival in cirrhosis with ascites. *J Hepatol* 2001; **34**: 46-52 [PMID: 11211907]

45 **Shear L**, Kleinerman J, Gabuzda GJ. Renal failure in patients with cirrhosis of the liver. i. clinical and pathologic characteristics. *Am J Med* 1965; **39**: 184-198 [PMID: 14320684 DOI: 10.1016/0002-9343(65)90041-0]

46 **Arroyo V**, Bosch J, Gaya-Beltrán J, Kravetz D, Estrada L, Rivera F, Rodés J. Plasma renin activity and urinary sodium excretion as prognostic indicators in nonazotemic cirrhosis with ascites. *Ann Intern Med* 1981; **94**: 198-201 [PMID: 7008667 DOI: 10.7326/0003-4819-94-2-198]

47 **Ruf AE**, Kremers WK, Chavez LL, Descalzi VI, Podesta LG, Villamil FG. Addition of serum sodium into the MELD score predicts waiting list mortality better than MELD alone. *Liver Transpl* 2005; **11**: 336-343 [PMID: 15719386 DOI: 10.1002/lt.20329]

48 **Heuman DM**, Abou-Assi SG, Habib A, Williams LM, Stravitz RT, Sanyal AJ, Fisher RA, Mihas AA. Persistent ascites and low serum sodium identify patients with cirrhosis and low MELD scores who are at high risk for early death. *Hepatology* 2004; **40**: 802-810 [PMID: 15382176 DOI: 10.1002/hep.20405]

49 **Follo A**, Llovet JM, Navasa M, Planas R, Forns X, Francitorra A, Rimola A, Gassull MA, Arroyo V, Rodés J. Renal impairment after spontaneous bacterial peritonitis in cirrhosis: incidence, clinical course, predictive factors and prognosis. *Hepatology* 1994; **20**: 1495-1501 [PMID: 7982650 DOI: 10.1002/hep.1840200619]

50 **Biggins SW**, Kim WR, Terrault NA, Saab S, Balan V, Schiano T, Benson J, Therneau T, Kremers W, Wiesner R, Kamath P, Klintmalm G. Evidence-based incorporation of serum sodium concentration into MELD. *Gastroenterology* 2006; **130**: 1652-1660 [PMID: 16697729 DOI: 10.1053/j.gastro.2006.02.010]

51 **Londoño MC**, Guevara M, Rimola A, Navasa M, Taurà P, Mas A, García-Valdecasas JC, Arroyo V, Ginès P. Hyponatremia impairs early posttransplantation outcome in patients with cirrhosis undergoing liver transplantation. *Gastroenterology* 2006; **130**: 1135-1143 [PMID: 16618408 DOI: 10.1053/j.gastro.2006.02.017]

52 **Dawwas MF**, Lewsey JD, Neuberger JM, Gimson AE. The impact of serum sodium concentration on mortality after liver transplantation: a cohort multicenter study. *Liver Transpl* 2007; **13**: 1115-1124 [PMID: 17663412 DOI: 10.1002/lt.21154]

53 **Rose BD**. New approach to disturbances in the plasma sodium concentration. *Am J Med* 1986; **81**: 1033-1040 [PMID: 3799631 DOI: 10.1016/0002-9343(86)90401-8]

54 **McCormick PA**, Mistry P, Kaye G, Burroughs AK, McIntyre N. Intravenous albumin infusion is an effective therapy for hyponatraemia in cirrhotic patients with ascites. *Gut* 1990; **31**: 204-207 [PMID: 2311979 DOI: 10.1136/gut.31.2.204]

55 **Bosch-Marcé M**, Jiménez W, Angeli P, Leivas A, Clària J, Graziotto A, Arroyo V, Rivera F, Rodés J. Aquaretic effect of the kappa-opioid agonist RU 51599 in cirrhotic rats with ascites and water retention. *Gastroenterology* 1995; **109**: 217-223 [PMID: 7797019 DOI: 10.1016/0016-5085(95)90287-2]

56 **Moreau R**, Cailmail S, Hamon G, Lebrec D. Renal and haemodynamic responses to a novel kappa opioid receptor agonist, niravoline (RU 51,599), in rats with cirrhosis. *J Gastroenterol Hepatol* 1996; **11**: 857-863 [PMID: 8889966 DOI: 10.1111/j.1440-1746.1996.tb00093.x]

57 **Gadano A**, Moreau R, Pessione F, Trombino C, Giuily N, Sinnassamy P, Valla D, Lebrec D. Aquaretic effects of niravoline, a kappa-opioid agonist, in patients with cirrhosis. *J Hepatol* 2000; **32**: 38-42 [PMID: 10673065 DOI: 10.1016/S0168-8278(00)80187-7]

58 **Inoue T**, Ohnishi A, Matsuo A, Kawai B, Kunihiro N, Tada Y, Koizumi F, Chau T, Okada K, Yamamura Y, Tanaka T. Therapeutic and diagnostic potential of a vasopressin-2 antagonist for impaired

water handling in cirrhosis. *Clin Pharmacol Ther* 1998; **63**: 561-570 [PMID: 9630829 DOI: 10.1016/S0009-9236(98)90107-2]

59 **Gerbes AL**, Gülberg V, Ginès P, Decaux G, Gross P, Gandjini H, Djian J. Therapy of hyponatremia in cirrhosis with a vasopressin receptor antagonist: a randomized double-blind multicenter trial. *Gastroenterology* 2003; **124**: 933-939 [PMID: 12671890 DOI: 10.1053/gast.2003.50143]

60 **Wong F**, Blei AT, Blendis LM, Thuluvath PJ. A vasopressin receptor antagonist (VPA-985) improves serum sodium concentration in patients with hyponatremia: a multicenter, randomized, placebo-controlled trial. *Hepatology* 2003; **37**: 182-191 [PMID: 12500203 DOI: 10.1053/jhep.2003.50021]

61 **Schrier RW**, Gross P, Gheorghiade M, Berl T, Verbalis JG, Czerwiec FS, Orlandi C. Tolvaptan, a selective oral vasopressin V2-receptor antagonist, for hyponatremia. *N Engl J Med* 2006; **355**: 2099-2112 [PMID: 17105757 DOI: 10.1056/NEJMoa065181]

62 **Ginès P**, Wong F, Watson H, Milutinovic S, del Arbol LR, Olteanu D. Effects of satavaptan, a selective vasopressin V(2) receptor antagonist, on ascites and serum sodium in cirrhosis with hyponatremia: a randomized trial. *Hepatology* 2008; **48**: 204-213 [PMID: 18508290 DOI: 10.1002/hep.22293]

63 **Thuluvath PJ**, Maheshwari A, Wong F, Yoo HW, Schrier RW, Parikh C, Steare S, Korula J. Oral V2 receptor antagonist (RWJ-351647) in patients with cirrhosis and ascites: a randomized, double-blind, placebo-controlled, single ascending dose study. *Aliment Pharmacol Ther* 2006; **24**: 973-982 [PMID: 16948809 DOI: 10.1111/j.1365-2036.2006.03088.x]

64 **Berl T**, Quittnat-Pelletier F, Verbalis JG, Schrier RW, Bichet DG, Ouyang J, Czerwiec FS. Oral tolvaptan is safe and effective in chronic hyponatremia. *J Am Soc Nephrol* 2010; **21**: 705-712 [PMID: 20185637 DOI: 10.1681/ASN.2009080857]

65 **Greenberg A**, Verbalis JG. Vasopressin receptor antagonists. *Kidney Int* 2006; **69**: 2124-2130 [PMID: 16672911 DOI: 10.1038/sj.ki.5000432]

66 **Higashihara E**, Torres VE, Chapman AB, Grantham JJ, Bae K, Watnick TJ, Horie S, Nutahara K, Ouyang J, Krasa HB, Czerwiec FS. Tolvaptan in autosomal dominant polycystic kidney disease: three years' experience. *Clin J Am Soc Nephrol* 2011; **6**: 2499-2507 [PMID: 21903984 DOI: 10.2215/CJN.03530411]

67 **Torres VE**, Chapman AB, Devuyst O, Gansevoort RT, Grantham JJ, Higashihara E, Perrone RD, Krasa HB, Ouyang J, Czerwiec FS. Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N Engl J Med* 2012; **367**: 2407-2418 [PMID: 23121377 DOI: 10.1056/NEJMoa1205511]

68 **Wong F**, Watson H, Gerbes A, Vilstrup H, Badalamenti S, Bernardi M, Ginès P. Satavaptan for the management of ascites in cirrhosis: efficacy and safety across the spectrum of ascites severity. *Gut* 2012; **61**: 108-116 [PMID: 21836029 DOI: 10.1136/gutjnl-2011-300157]

69 **Miller PD**, Linas SL, Schrier RW. Plasma demeclocycline levels and nephrotoxicity. Correlation in hyponatremic cirrhotic patients. *JAMA* 1980; **243**: 2513-2515 [PMID: 6770106]

70 **Solà E**, Lens S, Guevara M, Martín-Llahí M, Fagundes C, Pereira G, Pavesi M, Fernández J, González-Abraldes J, Escorsell A, Mas A, Bosch J, Arroyo V, Ginès P. Hyponatremia in patients treated with terlipressin for severe gastrointestinal bleeding due to portal hypertension. *Hepatology* 2010; **52**: 1783-1790 [PMID: 20931555 DOI: 10.1002/hep.23893]

P- Reviewer: Barreto R, Ruiz-Margain A, Stanciu C
S- Editor: Yu J **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3206

World J Gastroenterol 2015 March 21; 21(11): 3206-3213
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Novel CD9-targeted therapies in gastric cancer

Yoko Murayama, Kenji Oritani, Shusaku Tsutsui

Yoko Murayama, Shusaku Tsutsui, Department of Gastroenterology and Hepatology, Itami City Hospital, Itami 664-8540, Japan
Kenji Oritani, Department of Hematology/Oncology, Graduate School of Medicine, Osaka University, Suita 565-0871, Japan
Author contributions: Murayama Y was responsible for the literature review, and preparation of the manuscript; Oritani K prepared the final version of the manuscript; Tsutsui S provided intellectual support.

Correspondence to: Yoko Murayama, MD, PhD, Department of Gastroenterology and Hepatology, Itami City Hospital, 1-100 Koyaike, Itami 664-8540, Japan. murayama@hosp.itami.hyogo.jp
Telephone: +81-72-7773773
Fax: +81-72-7819888
Received: July 23, 2014
Peer-review started: July 24, 2014
First decision: August 15, 2014
Revised: November 13, 2014
Accepted: December 16, 2014
Article in press: December 16, 2014
Published online: March 21, 2015

Abstract

There are 33 human tetraspanin proteins, emerging as key players in malignancy, the immune system, fertilization, cellular signaling, adhesion, morphology, motility, proliferation, and tumor invasion. CD9, a member of the tetraspanin family, associates with and influences a variety of cell-surface molecules. Through these interactions, CD9 modifies multiple cellular events, including adhesion, migration, proliferation, and survival. CD9 is therefore considered to play a role in several stages during cancer development. Reduced CD9 expression is generally related to venous vessel invasion and metastasis as well as poor prognosis. We found that treatment of mice bearing human gastric cancer cells with anti-CD9 antibody successfully inhibited tumor progression *via* antiproliferative, proapoptotic, and antiangiogenic effects, strongly indicating that CD9 is a possible therapeutic target in patients with gastric cancer. Here, we describe the possibility of CD9 manipulation as a novel therapeutic strategy in gastric cancer, which still shows poor prognosis.

Key words: CD9; Tetraspanin; Gastric cancer; Tumorigenicity; Therapeutic target

Core tip: Tetraspanin CD9 is a cell-surface protein with four transmembrane domains and is found in several organs. Although CD9 was primarily identified as a tumor suppressor, it exhibits diverse functions through its association with various partner proteins. CD9 relates to tumor proliferation, apoptosis, migration, adhesion, and angiogenesis, therefore involving several steps of tumor formation: communication with the environment, dissemination, and metastasis. In this review, we describe the possibility of CD9 manipulation as a novel therapeutic strategy to improve clinical outcome in gastric cancer.

Murayama Y, Oritani K, Tsutsui S. Novel CD9-targeted therapies in gastric cancer. *World J Gastroenterol* 2015; 21(11): 3206-3213 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3206.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3206

INTRODUCTION

Gastric cancer is one of the most common malignancies, remaining a major public health issue as the fourth most common cancer and the second leading cause of cancer death worldwide[1], with a particularly high

incidence in Japan, China, South Korea, Chile and Costa Rica. The large regional incidence variations possibly reflect different prevalences of *Helicobacter pylori* infection, which is responsible for > 60% of gastric cancer globally. Advanced gastric cancer is an aggressive disease, and the prognosis remains poor. The 5-year survival rate for locoregional disease is 25%-35%[2-4] and the median survival ranges from 10 to 14 mo in advanced disease[5,6]. Although various treatment modalities have been developed and the mortality rate of gastric cancer has gradually decreased over recent decades[7], many of them have failed to eliminate gastric cancer cells curatively[8]. Therefore, a novel therapeutic strategy is clinically desired.

CD9, a member of the tetraspanin family, has been reported to relate to growth and invasion of tumor cells. There are many reports of the relationship between CD9 expression and disease prognosis. In addition, molecular mechanisms of CD9 functions have been gradually clarified. In this field, we also reported apoptotic signals after CD9 ligation in gastric cancer cells, as well as the treatment of gastric-cancer-bearing mice with anti-CD9 antibody.

We review the characteristics of CD9 and discuss the possibility of CD9 as a novel therapeutic target in gastric cancer.

CD9 FUNCTIONS

Tetraspanins, which have four putative membrane-spanning domains, are integral membrane proteins including at least 33 distinct family members, such as CD9,CD37, CD53, CD63, CD81, CD82, and CD151[9-11]. Members of this family are involved in many physiological and pathological processes, such as fertilization, cellular adhesion, motility, and tumor invasion[9-12]. To date, tetraspanins are believed to act as molecular facilitators or adaptors, which form a network of interaction among the cell-surface molecules, known as the "tetraspanin web" or tetraspan-enriched microdomains[12,13]. Notably, some tetraspanin proteins have key roles in tumor initiation, promotion, metastasis, and angiogenesis.

CD9, which was identified as a suppressor of cancer spread[14], belongs to the tetraspanin family. Like other tetraspanins, CD9 has four putative transmembrane domains, which provide the short N- and C-terminal cytoplasmic domains, a small intracellular loop, and two extracellular loops[11,12] (Figure 1). CD9 is widely expressed on the surface of several types of cells, including many malignant tumor cells as well as normal hematopoietic, endothelial and epithelial cells[11,12].

CD9 interacts with a number of transmembrane proteins, including integrins, immunoglobulin superfamily member EWI proteins (EWI-2 and EWI-F) and other tetraspanins (*e.g.*, CD81 and CD151)[10-13], Claudin-1[15], epidermal growth factor receptor (EGFR)[16], and membrane-bound ligands for EGFR[17-19] (Table 1). These interactions form functional complexes, which facilitate cell adhesion, motility, and signaling[10,20-24]. For examples, antibody (Ab) ligation of CD9 induces homotypic aggregation of pre-B cells and augments their adhesion to bone marrow fibroblasts through the modification of integrins[10]. Treatment with anti-CD9 Ab can induce strong adhesion between stromal and hematopoietic cells[25,26] as well as inhibit the migration of malignant cells[27]. In addition, CD9 acts as a co-receptor for diphtheria toxin. CD9 does not bind directly to the toxin, but interacts with the diphtheria toxin receptor (transmembrane precursor of heparin-binding epidermal-growth-factor-like growth factor; HB-EGF), leading to the elevation of juxtacrine activity of HB-EGF[28,29]. Also, CD9 functionally associates with Fcγ receptors, and co-cross-linking of CD9-Fcγ receptors modifies signals for phagocytosis and inflammatory responses on macrophages[30].

CD9 affects physical processes, such as cell proliferation, apoptosis and tumor metastasis[31-33]. Treatment of cells with anti-CD9 Ab has revealed antiproliferative effects[16,18] *via* the suppression of extracellular signal-regulated kinase (ERK) 1/2 activity[31]. In addition, CD9 ligation concurrently induces apoptosis *via* the selective activation of the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 mitogen-activated protein kinase (MAPK) pathway, as well as caspase-3 and the p46 Shc isoform[31]. Moreover, CD9 can associate with conventional protein kinase C (PKC) isoforms including PKCα and PKCβ[34], as well as type Ⅱ phosphatidylinositol 4-kinase[35], which could contribute to tumor-suppressor functions. In addition, CD9 may affect the Wnt signaling pathway

Table 1 CD9 associated with partner proteins

Partner protein	Function	Ref.
EWI-2	Modulates integrin-dependent cell motility, morphology and/or spreading	[5-6,8,44,45,50]
EWI-F	Functions unknown	[5-6,8,46,47]
Integrin β1	CD9 modulates integrin-dependent cell morphology, cell migration, signaling and adhesion strengthening	[5,11]
Other tetraspanins (*e.g.*, CD81, CD151)	Form TEMs	[7,8]
Claudin-1	CD9 stabilizes expression of non-junctional Claudin-1	[10]
EGFR	CD9 enhances the internalization of EGFR and reduces EGF-EGFR-induced signals	[11]
HB-EGF	CD9 upregulates both diphtheria toxin binding and mitogenic functions of HB-EGF	[23,24]
PKC isoforms	Contribute to signaling and tumor-suppressor functions	[29]
Type Ⅱ PI4K	Contribute to signaling and tumor-suppressor functions	[30]

EGFR: Epidermal growth factor receptor; HB-EGF: Heparin-binding epidermal-growth-factor-like growth factor; PKC: Protein kinase C; TEMS: Tetraspan-enriched microdomains.

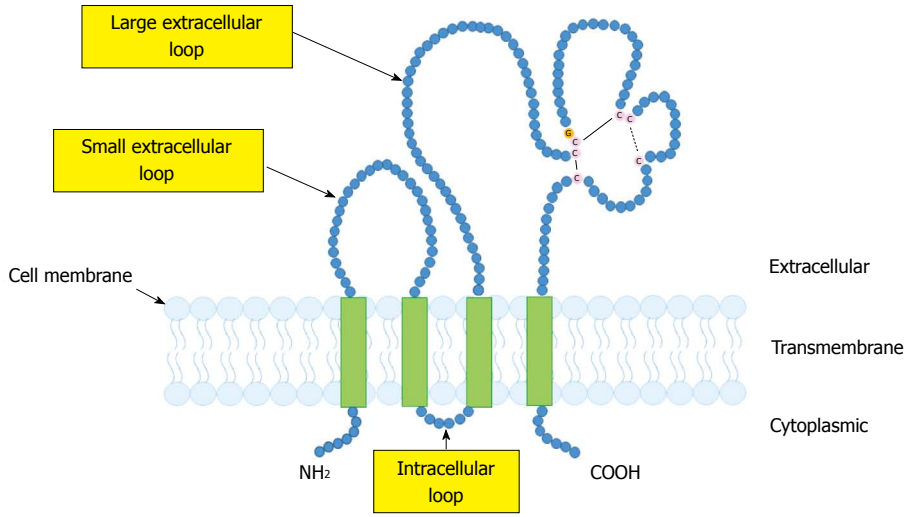


Figure 1 Structural features of CD9. CD9 has four putative transmembrane domains, which provide the short N- and C-terminal cytoplasmic domains, a small intracellular loop, and two extracellular loops. C: Cysteine; G: Glycine.

by downregulating Wnt genes[36]. Expression of CD9 also acts to protect transforming growth factor α from cleavage, thereby regulating cell proliferation and migration[19]. Therefore, CD9 expression has an ability to regulate a variety of intracellular signals.

CD9 AND CANCER

From experiments manipulating CD9 in tumor cell lines, CD9 has been demonstrated to be primarily a suppressor of metastasis[27,37-40]. Several clinical studies have also shown an important prognostic value of CD9. The reduced CD9 expression is associated with poor prognosis in melanoma[41], non-small-cell lung cancer[28], and breast[37,42], colon[43], pancreatic[44], ovarian[45] and prostate[46] cancer. Expression of CD9 is also related to metastasis of the gastrointestinal carcinoma[43,44,47,48]. For example, reduced CD9 expression is significantly associated with more venous vessel invasion and liver metastasis in patients with colon cancer[27,43]. Although diverse physiological functions (clinical data) of CD9 have been suggested[49,50], we and others have found that the amount of CD9 is inversely correlated with lymph node status in gastric cancer[48] and in esophageal squamous cell carcinoma[47]. Moreover, expression of CD9 protein in gastric cancer tissues was significantly stronger in patients without regional lymph node or distant metastasis than in those with metastasis[51]. Furthermore, the reduction of CD9 protein was associated with distant metastasis of gastric cancer. Thus, decreased levels of CD9 are strongly associated with an increased risk of recurrence, especially in patients with N0 nodal status and M0 metastatic status. Low levels of CD9 expression are related to poor prognosis. These findings are consistent with previous reports. Therefore, reduced CD9 expression is generally related to more venous vessel invasion and metastasis as well as poor prognosis in most common types of cancer.

As mentioned above, many investigators believe that CD9 is a suppressor of tumor development.

POSSIBILITY OF CD9-TARGETED THERAPY IN GASTRIC CANCER

Anti-CD9 monoclonal Abs (mAbs), ALB6 and PAINS-13 are ligand-mimic Abs, therefore, Ab ligation of CD9 with these antibodies enhances, but does not inhibit, CD9 functions (Figure 2). We first introduce some interesting data concerning mechanisms of CD9 functions obtained by using these Abs. We previously reported that treatment with anti-CD9 mAb (ALB6), which enhances CD9 functions, inhibited cell growth in CD9-positive tumor cell lines (MKN-28, MKN-45, SW480, HT-29, CaCO2, MIA-PaCa-2 and A459)[31]. In a gastric cancer line MKN-28, CD9 ligation induced apoptosis. ALB6 treatment activated JNK/SAPK and p38 MAPK as well as caspase-3[31]. Notably, ALB6 treatment selectively induced tyrosine phosphorylation of the p46 Shc isoform, and overexpression of its dominant-negative form completely cancelled the ALB6-induced activation of JNK/SAPK, p38 MAPK and caspase-3, leading to loss of apoptosis. Therefore, Ab ligation of CD9 induced apoptotic signals *via* restricted activation of the p46 Shc isoform. We also reported that CD9 ligation enhanced the internalization of EGFR[16]. ALB6 treatment induced a dotted or patch-like aggregation composed of CD9-EGFR and CD9-β1 integrin on the surface of MKN-28 cells. Furthermore, expression of CD9 specifically attenuated EGFR signaling in CD9-overexpressing CHO cells *via* the downregulation of surface expression of EGFR[16]. Therefore, CD9 expression negatively regulates cell surface EGFR expression levels. Finally, we examined *in vivo* effects of ALB6 Ab to treat patients with gastric cancer. MKN-28 cells were inoculated subcutaneously

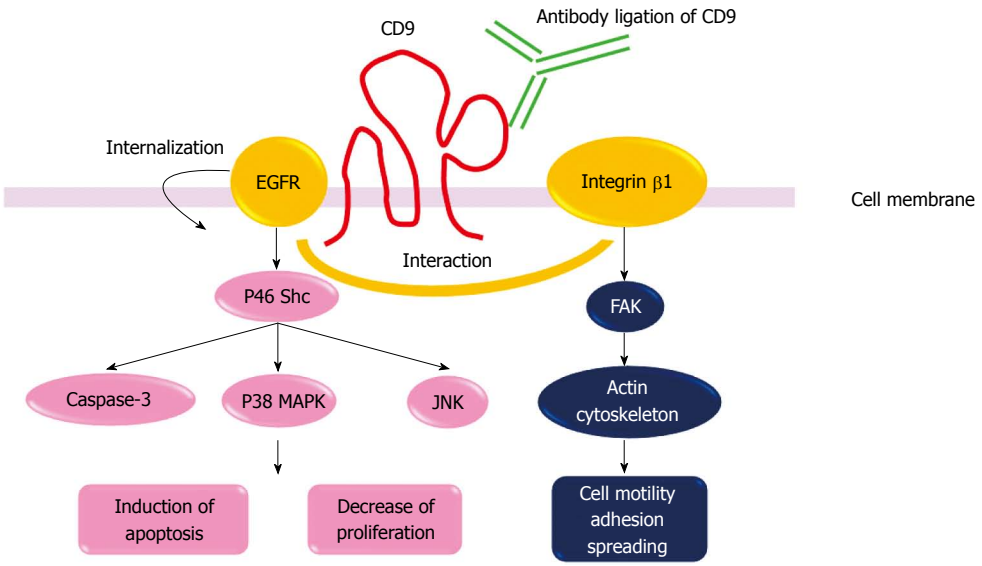


Figure 2 CD9 signaling. CD9-EGFR and CD9-β1 integrin co-localize on the cell surface. CD9 enhances the internalization of EGFR and reduces EGF-EGFR-induced signals[11]. CD9 ligation induced apoptosis *via* the selective activation of JNK and p38 MAPK pathway as well as caspase-3 and the p46 Shc isoform[26]. CD9 modulates integrin-dependent cell motility, cell migration, adhesion strengthening, and spreading[5,11]. EGFR: Epidermal growth factor receptor; p38 MAPK: p38 mitogen-activated-protein kinase; JNK: c-Jun NH_2-terminal kinase; FAK: Focal adhesion kinase.

into SCID mice. After a tumor was visualized, the MKN-28-bearing mice were injected with ALB6 or control Ab three times per week. In the ALB6 treatment group, tumor volume was significantly suppressed, and the apoptotic indexes were increased. Therefore, administration of mice bearing human gastric cancer cells with anti-CD9 Ab successfully inhibited tumor progression[52]. Similar to our results, it has been reported that anti-CD9 mAb PAINS 13 inhibited *in vivo* tumor growth of colon cancer cells[53]. The inhibition of cell proliferation in colon carcinoma cells caused by anti-CD9 mAbs PAINS-13 was related to the enhanced integrin-dependent adhesion and the increased expression of membrane tumor necrosis factor (TNF)-α.

Therefore, TNF-α partly mediates the antiproliferative effects of CD9 in this case.

Overexpression of vascular endothelial growth factor (VEGF)-A is associated with tumor angiogenesis, nodal metastasis, and poor prognosis in cancer patients[54,55]. A report that CD9 gene transduction could downregulate VEGF-A expression is now available[36]. In this situation, CD9 is also likely to regulate tumor development negatively.

With regard to interactions between CD9 and integrins, CD9 seems to positively and/or negatively involve tumor development through functional modification of integrins. Indeed, the enhancement of integrin-mediated cell adhesion by CD9 inhibits metastasis and invasion of tumor cells and contributes to cell-adhesion-mediated drug resistance[56].

PRESENT TREATMENT FOR PATIENTS WITH GASTRIC CANCER

Improving molecular characterization has translated into better survival in select patients with advanced gastric and esophageal cancer. Trastuzumab, an antibody targeting the anti-human epidermal growth factor receptor 2 (HER2) extracellular domain, induces antibody-dependent cellular cytotoxicity and inhibits the HER2 downstream signals. In the ToGA study, standard chemotherapy regimens (capecitabine plus cisplatin or fluorouracil plus cisplatin) combined with trastuzumab resulted in a longer survival time than standard regimens without trastuzumab in patients with HER2-positive gastric cancer[57,58]. In addition, ramucirumab, an mAb targeting vascular endothelial growth factor receptor (VEGFR)-2, is the first biological treatment that showed survival benefits as a single-agent therapy for the second-line chemotherapy (REGARD trial) in patients with advanced gastric cancer who progressed after first-line chemotherapy[59]. An early report of the phase Ⅲ RAINBOW trial, testing ramucirumab in combination with paclitaxel for the second-line therapy after platinum-fluoropyrimidine failure, also demonstrated an overall survival benefit of 9.6 mo *vs* 7.4 mo as compared with paclitaxel alone[60]. With recent success of ramucirumab, investigations with several other antiangiogenic agents have begun. These include the VEGFR-2 inhibitor, apatinib, and the multi-

targeted tyrosine kinase receptor inhibitors, axitinib and pazopanib[60]. In addition to the HER family and VEGFRs, the phosphatidylinositol 3-kinase-AKT-mammalian target of rapamycin (mTOR) and the c-MET signaling pathways are promising candidates, and some molecular targeting agents are now in clinical investigation[61].

FUTURE PROSPECTS

A number of recent reports have suggested that tetraspanin targeting by Abs, soluble large-loop proteins, RNAi technology, or adenoviral transduction methods could be therapeutically beneficial[62]. In the case of CD9, we and others have proposed that CD9 ligation is likely to be useful to treat malignancies. Ectopic expression of CD9 in small-cell lung carcinoma cells inhibited their proliferation[63], and adenoviral transduction of CD9 inhibited lymph node metastasis in an orthotopic lung cancer model[40]. With cDNA expression microarray experiments, CD9 was reported to be one of the genes upregulated in gastric cancer[64]. Thus, CD9 expression in non-cancerous tissues is lower than that in gastric cancer tissues, indicating that adverse effects of anti-CD9 treatment on normal gastrointestinal tissues might be tolerable.

Tumor growth is dependent on angiogenesis, which forms new blood vessels[65]. Targeting tumor vessels provides several advantages over traditional anti-tumor approaches. CD9 enhancement contributes to tumor angiogenesis, presumably by affecting endothelial cell function, although their contributions to angiogenesis have not been shown using *de novo* tumor models. It was previously reported that CD9 gene transduction could downregulate VEGF-A expression, which is essential for angiogenesis[36]. Therefore, enhancement of CD9 functions may also be worthwhile in particular circumstances.

With regard to tumor metastasis, CD9 is involved in cell adhesion *via* enhancing integrin functions. In addition, associations of CD9 with EWI-2[10,11,13,66,67], EWI-F[68,69], EPCAM[70], Claudin-1[10] or HB-EGF[23,24] could have different effects on tumor cell invasion and metastasis. Indeed, the CD9 partners EWI-F[71] and EWI-2 can markedly affect cell migration[72], and EWI-2 influences the association of CD9 with membrane-type 1 matrix metalloproteinase (MT1-MMP; also known as MMP14) and MMP2[73], which could alter proteolysis during invasion. Thus, CD9 acts on multiple steps of tumorigenesis, and because CD9 function is dependent on its associating proteins, efficacy of the CD9-targeting therapy may be determined by expression of these associating molecules as well as CD9 itself.

CONCLUSION

Molecular mechanisms for CD9 functions have been understood through identification of CD9-associating proteins. Ab ligation of CD9 is a powerful tool to change CD9 functions, and we showed apoptotic signals after CD9 ligation in gastric cancer cells as well as successful treatment of gastric-cancer-bearing mice with anti-CD9 Ab. CD9 influences intracellular signals, cell adhesion, and cell proliferation, and is involved in several events during development of gastric cancer. Taken together with evidence from clinical data, the manipulation of CD9 is likely to have the potential to improve clinical results of therapy for gastric cancer. When implementing CD9-targeted therapy in gastric cancer, we should come up with various ideas to enhance CD9 functions.

A new therapy to target HER2, VEGFR-2, is responsible for a significant increase in survival of patients with advanced gastric cancer. Unfortunately, advanced gastric cancer continues to have a poor prognosis. In the future, new strategies to target CD9 will hopefully be developed and implemented for gastric cancer treatment.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Cunningham D**, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11-20 [PMID: 16822992 DOI: 10.1056/NEJMoa055531]

3 **Smalley SR**, Benedetti JK, Haller DG, Hundahl SA, Estes NC, Ajani JA, Gunderson LL, Goldman B, Martenson JA, Jessup JM, Stemmermann GN, Blanke CD, Macdonald JS. Updated analysis of SWOG-directed intergroup study 0116: a phase III trial of adjuvant radiochemotherapy versus observation after curative gastric cancer resection. *J Clin Oncol* 2012; **30**: 2327-2333 [PMID: 22585691 DOI: 10.1200/JCO.2011.36.7136]

4 **van Hagen P**, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, Richel DJ, Nieuwenhuijzen GA, Hospers GA, Bonenkamp JJ, Cuesta MA, Blaisse RJ, Busch OR, ten Kate FJ, Creemers GJ, Punt CJ, Plukker JT, Verheul HM, Spillenaar Bilgen EJ, van Dekken H, van der Sangen MJ, Rozema T, Biermann K, Beukema JC, Piet AH, van Rij CM, Reinders JG, Tilanus HW, van der Gaast A. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med* 2012; **366**: 2074-2084 [PMID: 22646630 DOI: 10.1056/NEJMoa1112088]

5 **Cunningham D**, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008; **358**: 36-46 [PMID: 18172173 DOI: 10.1056/NEJMoa073149]

6 **Waddell T**, Chau I, Cunningham D, Gonzalez D, Okines AF, Okines C, Wotherspoon A, Saffery C, Middleton G, Wadsley J, Ferry D, Mansoor W, Crosby T, Coxon F, Smith D, Waters J, Iveson T, Falk S, Slater S, Peckitt C, Barbachano Y. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 481-489 [PMID: 23594787 DOI: 10.1016/S1470-2045(13)70096-2]

7 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]

8 **Gallo A**, Cha C. Updates on esophageal and gastric cancers. *World J Gastroenterol* 2006; **12**: 3237-3242 [PMID: 16718845]

9 **Maecker HT**, Todd SC, Levy S. The tetraspanin superfamily: molecular facilitators. *FASEB J* 1997; **11**: 428-442 [PMID: 9194523]

10 **Berditchevski F**. Complexes of tetraspanins with integrins: more than meets the eye. *J Cell Sci* 2001; **114**: 4143-4151 [PMID: 11739647]

11 **Hemler ME**. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu Rev Cell Dev Biol* 2003; **19**: 397-422 [PMID: 14570575 DOI: 10.1146/annurev.cellbio.19.111301.153609]

12 **Boucheix C**, Rubinstein E. Tetraspanins. *Cell Mol Life Sci* 2001; **58**: 1189-1205 [PMID: 11577978 DOI: 10.1007/PL00000933]

13 **Hemler ME**. Tetraspanin functions and associated microdomains. *Nat Rev Mol Cell Biol* 2005; **6**: 801-811 [PMID: 16314869 DOI: 10.1038/nrm1736]

14 **Miyake M**, Koyama M, Seno M, Ikeyama S. Identification of the motility-related protein (MRP-1), recognized by monoclonal antibody M31-15, which inhibits cell motility. *J Exp Med* 1991; **174**: 1347-1354 [PMID: 1720807 DOI: 10.1084/jem.174.6.1347]

15 **Kovalenko OV**, Yang XH, Hemler ME. A novel cysteine cross-linking method reveals a direct association between claudin-1 and tetraspanin CD9. *Mol Cell Proteomics* 2007; **6**: 1855-1867 [PMID: 17644758 DOI: 10.1074/mcp.M700183-MCP200]

16 **Murayama Y**, Shinomura Y, Oritani K, Miyagawa J, Yoshida H, Nishida M, Katsube F, Shiraga M, Miyazaki T, Nakamoto T, Tsutsui S, Tamura S, Higashiyama S, Shimomura I, Hayashi N. The tetraspanin CD9 modulates epidermal growth factor receptor signaling in cancer cells. *J Cell Physiol* 2008; **216**: 135-143 [PMID: 18247373 DOI: 10.1002/jcp.21384]

17 **Higashiyama S**, Iwamoto R, Goishi K, Raab G, Taniguchi N, Klagsbrun M, Mekada E. The membrane protein CD9/DRAP 27 potentiates the juxtacrine growth factor activity of the membrane-anchored heparin-binding EGF-like growth factor. *J Cell Biol* 1995; **128**: 929-938 [PMID: 7876316 DOI: 10.1083/jcb.128.5.929]

18 **Inui S**, Higashiyama S, Hashimoto K, Higashiyama M, Yoshikawa K, Taniguchi N. Possible role of coexpression of CD9 with membrane-anchored heparin-binding EGF-like growth factor and amphiregulin in cultured human keratinocyte growth. *J Cell Physiol* 1997; **171**: 291-298 [PMID: 9180898]

19 **Shi W**, Fan H, Shum L, Derynck R. The tetraspanin CD9 associates with transmembrane TGF-alpha and regulates TGF-alpha-induced EGF receptor activation and cell proliferation. *J Cell Biol* 2000; **148**: 591-602 [PMID: 10662783 DOI: 10.1083/jcb.148.3.591]

20 **Jones PH**, Bishop LA, Watt FM. Functional significance of CD9 association with beta 1 integrins in human epidermal keratinocytes. *Cell Adhes Commun* 1996; **4**: 297-305 [PMID: 9117348 DOI: 10.3109/15419069609010773]

21 **Baudoux B**, Castanares-Zapatero D, Leclercq-Smekens M, Berna N, Poumay Y. The tetraspanin CD9 associates with the integrin alpha6beta4 in cultured human epidermal keratinocytes and is involved in cell motility. *Eur J Cell Biol* 2000; **79**: 41-51 [PMID: 10711425 DOI: 10.1078/S0171-9335(04)70006-0]

22 **Yáñez-Mó M**, Alfranca A, Cabañas C, Marazuela M, Tejedor R, Ursa MA, Ashman LK, de Landázuri MO, Sánchez-Madrid F. Regulation of endothelial cell motility by complexes of tetraspan molecules CD81/TAPA-1 and CD151/PETA-3 with alpha3 beta1 integrin localized at endothelial lateral junctions. *J Cell Biol* 1998; **141**: 791-804 [PMID: 9566977]

23 **Berditchevski F**, Odintsova E. Characterization of integrin-tetraspanin adhesion complexes: role of tetraspanins in integrin signaling. *J Cell Biol* 1999; **146**: 477-492 [PMID: 10427099 DOI: 10.1083/jcb.146.2.477]

24 **Shaw AR**, Domanska A, Mak A, Gilchrist A, Dobler K, Visser L, Poppema S, Fliegel L, Letarte M, Willett BJ. Ectopic expression of human and feline CD9 in a human B cell line confers beta 1 integrin-dependent motility on fibronectin and laminin substrates and enhanced tyrosine phosphorylation. *J Biol Chem* 1995; **270**: 24092-24099 [PMID: 7592610 DOI: 10.1074/jbc.270.41.24092]

25 **Oritani K**, Wu X, Medina K, Hudson J, Miyake K, Gimble JM, Burstein SA, Kincade PW. Antibody ligation of CD9 modifies production of myeloid cells in long-term cultures. *Blood* 1996; **87**: 2252-2261 [PMID: 8630385]

26 **Aoyama K**, Oritani K, Yokota T, Ishikawa J, Nishiura T, Miyake K, Kanakura Y, Tomiyama Y, Kincade PW, Matsuzawa Y. Stromal cell CD9 regulates differentiation of hematopoietic stem/progenitor cells. *Blood* 1999; **93**: 2586-2594 [PMID: 10194438]

27 **Cajot JF**, Sordat I, Silvestre T, Sordat B. Differential display cloning identifies motility-related protein (MRP1/CD9) as highly expressed in primary compared to metastatic human colon carcinoma cells. *Cancer Res* 1997; **57**: 2593-2597 [PMID: 9205061]

28 **Higashiyama M**, Taki T, Ieki Y, Adachi M, Huang CL, Koh T, Kodama K, Doi O, Miyake M. Reduced motility related protein-1 (MRP-1/CD9) gene expression as a factor of poor prognosis in non-small cell lung cancer. *Cancer Res* 1995; **55**: 6040-6044 [PMID: 8521390]

29 **Iwamoto R**, Higashiyama S, Mitamura T, Taniguchi N, Klagsbrun M, Mekada E. Heparin-binding EGF-like growth factor, which acts as the diphtheria toxin receptor, forms a complex with membrane protein DRAP27/CD9, which up-regulates functional receptors and diphtheria toxin sensitivity. *EMBO J* 1994; **13**: 2322-2330 [PMID: 8194524]

30 **Kaji K**, Takeshita S, Miyake K, Takai T, Kudo A. Functional association of CD9 with the Fc gamma receptors in macrophages. *J Immunol* 2001; **166**: 3256-3265 [PMID: 11207280 DOI: 10.4049/jimmunol.166.5.3256]

31 **Murayama Y**, Miyagawa J, Oritani K, Yoshida H, Yamamoto K, Kishida O, Miyazaki T, Tsutsui S, Kiyohara T, Miyazaki Y, Higashiyama S, Matsuzawa Y, Shinomura Y. CD9-mediated activation of the p46 Shc isoform leads to apoptosis in cancer cells. *J Cell Sci* 2004; **117**: 3379-3388 [PMID: 15226408 DOI: 10.1242/jcs.01201]

32 **Tachibana I**, Hemler ME. Role of transmembrane 4 superfamily (TM4SF) proteins CD9 and CD81 in muscle cell fusion and myotube maintenance. *J Cell Biol* 1999; **146**: 893-904 [PMID: 10459022 DOI: 10.1083/jcb.146.4.893]

33 **Ono M**, Handa K, Withers DA, Hakomori S. Motility inhibition and apoptosis are induced by metastasis-suppressing gene product CD82 and its analogue CD9, with concurrent glycosylation. *Cancer Res* 1999; **59**: 2335-2339 [PMID: 10344740]

34 **Zhang XA**, Bontrager AL, Hemler ME. Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific beta(1) integrins. *J Biol Chem* 2001; **276**: 25005-25013 [PMID: 11325968 DOI: 10.1074/jbc.M102156200]

35 **Yauch RL**, Hemler ME. Specific interactions among transmembrane 4 superfamily (TM4SF) proteins and phosphoinositide 4-kinase. *Biochem J* 2000; **351** Pt 3: 629-637 [PMID: 11042117]

36 **Huang CL**, Liu D, Masuya D, Kameyama K, Nakashima T, Yokomise H, Ueno M, Miyake M. MRP-1/CD9 gene transduction downregulates Wnt signal pathways. *Oncogene* 2004; **23**: 7475-7483 [PMID: 15334057 DOI: 10.1038/sj.onc.1208063]

37 **Adachi M**, Taki T, Ieki Y, Huang CL, Higashiyama M, Miyake M. Correlation of KAI1/CD82 gene expression with good prognosis in patients with non-small cell lung cancer. *Cancer Res* 1996; **56**: 1751-1755 [PMID: 8620488]

38 **Miyake M**, Nakano K, Ieki Y, Adachi M, Huang CL, Itoi S, Koh T, Taki T. Motility related protein 1 (MRP-1/CD9) expression: inverse correlation with metastases in breast cancer. *Cancer Res* 1995; **55**: 4127-4131 [PMID: 7664290]

39 **Kusukawa J**, Ryu F, Kameyama T, Mekada E. Reduced expression of CD9 in oral squamous cell carcinoma: CD9 expression inversely related to high prevalence of lymph node metastasis. *J Oral Pathol Med* 2001; **30**: 73-79 [PMID: 11168850 DOI: 10.1034/j.1600-0714.2001.300202.x]

40 **Takeda T**, Hattori N, Tokuhara T, Nishimura Y, Yokoyama M, Miyake M. Adenoviral transduction of MRP-1/CD9 and KAI1/CD82 inhibits lymph node metastasis in orthotopic lung cancer model. *Cancer Res* 2007; **67**: 1744-1749 [PMID: 17308116 DOI: 10.1158/0008-5472.CAN-06-3090]

41 **Si Z**, Hersey P. Expression of the neuroglandular antigen and analogues in melanoma. CD9 expression appears inversely related to metastatic potential of melanoma. *Int J Cancer* 1993; **54**: 37-43 [PMID: 8478146 DOI: 10.1002/ijc.2910540107]

42 **Huang CI**, Kohno N, Ogawa E, Adachi M, Taki T, Miyake M. Correlation of reduction in MRP-1/CD9 and KAI1/CD82 expression with recurrences in breast cancer patients. *Am J Pathol* 1998; **153**: 973-983 [PMID: 9736046 DOI: 10.1016/S0002-9440(10)65639-8]

43 **Mori M**, Mimori K, Shiraishi T, Haraguchi M, Ueo H, Barnard GF, Akiyoshi T. Motility related protein 1 (MRP1/CD9) expression in colon cancer. *Clin Cancer Res* 1998; **4**: 1507-1510 [PMID: 9626469]

44 **Sho M**, Adachi M, Taki T, Hashida H, Konishi T, Huang CL, Ikeda N, Nakajima Y, Kanehiro H, Hisanaga M, Nakano H, Miyake M. Transmembrane 4 superfamily as a prognostic factor in pancreatic cancer. *Int J Cancer* 1998; **79**: 509-516 [PMID: 9761121]

45 **Houle CD**, Ding XY, Foley JF, Afshari CA, Barrett JC, Davis BJ. Loss of expression and altered localization of KAI1 and CD9 protein are associated with epithelial ovarian cancer progression. *Gynecol Oncol* 2002; **86**: 69-78 [PMID: 12079303 DOI: 10.1006/gyno.2002.6729]

46 **Wang JC**, Bégin LR, Bérubé NG, Chevalier S, Aprikian AG, Gourdeau H, Chevrette M. Down-regulation of CD9 expression during prostate carcinoma progression is associated with CD9 mRNA modifications. *Clin Cancer Res* 2007; **13**: 2354-2361 [PMID: 17406028 DOI: 10.1158/1078-0432.CCR-06-1692]

47 **Uchida S**, Shimada Y, Watanabe G, Li ZG, Hong T, Miyake M, Imamura M. Motility-related protein (MRP-1/CD9) and KAI1/CD82 expression inversely correlate with lymph node metastasis in oesophageal squamous cell carcinoma. *Br J Cancer* 1999; **79**: 1168-1173 [PMID: 10098753 DOI: 10.1038/sj.bjc.6690186]

48 **Murayama Y**, Miyagawa J, Shinomura Y, Kanayama S, Isozaki K, Yamamori K, Mizuno H, Ishiguro S, Kiyohara T, Miyazaki Y, Taniguchi N, Higashiyama S, Matsuzawa Y. Significance of the association between heparin-binding epidermal growth factor-like growth factor and CD9 in human gastric cancer. *Int J Cancer* 2002; **98**: 505-513 [PMID: 11920609 DOI: 10.1002/ijc.10198]

49 **Fricker G**, Drewe J, Vonderscher J, Kissel T, Beglinger C. Enteral absorption of octreotide. *Br J Pharmacol* 1992; **105**: 783-786 [PMID: 1504712 DOI: 10.1016/j.jss.2004.01.014]

50 **Soyuer S**, Soyuer I, Unal D, Ucar K, Yildiz OG, Orhan O. Prognostic significance of CD9 expression in locally advanced gastric cancer treated with surgery and adjuvant chemoradiotherapy. *Pathol Res Pract* 2010; **206**: 607-610 [PMID: 20547009 DOI: 10.1016/j.prp.2010.04.004]

51 **Chen Z**, Gu S, Trojanowicz B, Liu N, Zhu G, Dralle H, Hoang-Vu C. Down-regulation of TM4SF is associated with the metastatic potential of gastric carcinoma TM4SF members in gastric carcinoma. *World J Surg Oncol* 2011; **9**: 43 [PMID: 21521534 DOI: 10.1186/1477-7819-9-43]

52 **Nakamoto T**, Murayama Y, Oritani K, Boucheix C, Rubinstein E, Nishida M, Katsube F, Watabe K, Kiso S, Tsutsui S, Tamura S, Shinomura Y, Hayashi N. A novel therapeutic strategy with anti-CD9 antibody in gastric cancers. *J Gastroenterol* 2009; **44**: 889-896 [PMID: 19468669 DOI: 10.1007/s00535-009-0081-3]

53 **Ovalle S**, Gutiérrez-López MD, Olmo N, Turnay J, Lizarbe MA, Majano P, Molina-Jiménez F, López-Cabrera M, Yáñez-Mó M, Sánchez-Madrid F, Cabañas C. The tetraspanin CD9 inhibits the proliferation and tumorigenicity of human colon carcinoma cells. *Int J Cancer* 2007; **121**: 2140-2152 [PMID: 17582603 DOI: 10.1002/ijc.22902]

54 **Dvorak HF**, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 1995; **146**: 1029-1039 [PMID: 7538264]

55 **Masuya D**, Huang C, Liu D, Kameyama K, Hayashi E, Yamauchi A, Kobayashi S, Haba R, Yokomise H. The intratumoral expression of vascular endothelial growth factor and interleukin-8 associated with angiogenesis in nonsmall cell lung carcinoma patients. *Cancer* 2001; **92**: 2628-2638 [PMID: 11745198]

56 **Carloni V**, Mazzocca A, Mello T, Galli A, Capaccioli S. Cell fusion promotes chemoresistance in metastatic colon carcinoma. *Oncogene* 2013; **32**: 2649-2660 [PMID: 22751128 DOI: 10.1038/onc.2012.268]

57 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]

58 **de Mello RA**, Marques AM, Araújo A. HER2 therapies and gastric cancer: a step forward. *World J Gastroenterol* 2013; **19**: 6165-6169 [PMID: 24115812 DOI: 10.3748/wjg.v19.i37.6165]

59 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768 DOI: 10.1016/S0140-6736(13)61719-5]

60 **Wilke H**, Cutsem EV, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Ohtsu A, Rougier P, Emig M, Carlesi R, Chandrawansa K, Muro K. RAINBOW: A global, phage III, randomized, double-blind study of ramucirumab plus paclitaxel vs placebo plus paclitaxel in the treatment of metastatic gastroesophageal junction (GEJ) and gastric adenocarcinoma following disease progression on first-line platinum- and fluoropyrimidine-containing combination therapy rainbow IMCL CP12-0922(I4T-IE-JVBE). *J Clin Oncol* 2014; **32** Suppl 3: (abstr LBA7)

61 **Yang W**, Raufi A, Klempner SJ. Targeted therapy for gastric cancer: molecular pathways and ongoing investigations. *Biochim Biophys Acta* 2014; **1846**: 232-237 [PMID: 24858418 DOI: 10.1016/j.bbcan]

62 **Hemler ME**. Targeting of tetraspanin proteins--potential benefits and strategies. *Nat Rev Drug Discov* 2008; **7**: 747-758 [PMID: 18758472 DOI: 10.1038/nrd2659]

63 **Zheng R**, Yano S, Zhang H, Nakataki E, Tachibana I, Kawase I, Hayashi S, Sone S. CD9 overexpression suppressed the liver metastasis and malignant ascites via inhibition of proliferation and motility of small-cell lung cancer cells in NK cell-depleted SCID mice. *Oncol Res* 2005; **15**: 365-372 [PMID: 16491954]

64 **Liu LX**, Liu ZH, Jiang HC, Qu X, Zhang WH, Wu LF, Zhu AL, Wang XQ, Wu M. Profiling of differentially expressed genes in human gastric carcinoma by cDNA expression array. *World J Gastroenterol* 2002; **8**: 580-585 [PMID: 12174360]

65 **Folkman J**. Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg* 1972; **175**: 409-416 [PMID: 5077799 DOI: 10.1097/00000658-197203000-00014]

66 **Stipp CS**, Kolesnikova TV, Hemler ME. EWI-2 is a major CD9 and CD81 partner and member of a novel Ig protein subfamily. *J Biol Chem* 2001; **276**: 40545-40554 [PMID: 11504738 DOI: 10.1074/jbc.M107338200]

67 **Charrin S**, Le Naour F, Labas V, Billard M, Le Caer JP, Emile JF, Petit MA, Boucheix C, Rubinstein E. EWI-2 is a new component of the tetraspanin web in hepatocytes and lymphoid cells. *Biochem J* 2003; **373**: 409-421 [PMID: 12708969 DOI: 10.1042/BJ20030343]

68 **Charrin S**, Le Naour F, Oualid M, Billard M, Faure G, Hanash SM, Boucheix C, Rubinstein E. The major CD9 and CD81 molecular partner. Identification and characterization of the complexes. *J Biol Chem* 2001; **276**: 14329-14337 [PMID: 11278880]

69 **Stipp CS**, Orlicky D, Hemler ME. FPRP, a major, highly stoichiometric, highly specific CD81- and CD9-associated protein. *J Biol Chem* 2001; **276**: 4853-4862 [PMID: 11087758 DOI: 10.1074/jbc.M009859200]

70 **Le Naour F**, André M, Greco C, Billard M, Sordat B, Emile JF, Lanza F, Boucheix C, Rubinstein E. Profiling of the tetraspanin web of human colon cancer cells. *Mol Cell Proteomics* 2006; **5**: 845-857 [PMID: 16467180 DOI: 10.1074/mcp.M500330-MCP200]

71 **Chambrion C**, Le Naour F. The tetraspanins CD9 and CD81 regulate CD9P1-induced effects on cell migration. *PLoS One* 2010; **5**: e11219 [PMID: 20574531 DOI: 10.1371/journal.pone.0011219]

72 **Stipp CS**, Kolesnikova TV, Hemler ME. EWI-2 regulates alpha3beta1 integrin-dependent cell functions on laminin-5. *J Cell Biol* 2003; **163**: 1167-1177 [PMID: 14662754 DOI: 10.1083/jcb.200309113]

73 **Kolesnikova TV**, Kazarov AR, Lemieux ME, Lafleur MA, Kesari S, Kung AL, Hemler ME. Glioblastoma inhibition by cell surface immunoglobulin protein EWI-2, in vitro and in vivo. *Neoplasia* 2009; **11**: 77-86, 4p following 86 [PMID: 19107234]

P- Reviewer: Luo QF, Qi F **S- Editor**: Yu J
L- Editor: Kerr C **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3214

World J Gastroenterol 2015 March 21; 21(11): 3214-3222
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Ghrelin-ghrelin *O*-acyltransferase system in the pathogenesis of nonalcoholic fatty liver disease

Shao-Ren Zhang, Xiao-Ming Fan

Shao-Ren Zhang, Xiao-Ming Fan, Department of Gastroenterology and Hepatology, Jinshan Hospital of Fudan University, Shanghai 201508, China

Author contributions: Fan XM conceived of the review and revised the paper; Zhang SR reviewed the literature and wrote the paper.

Supported by a research grant for Key Clinical Discipline Construction of Shanghai Municipality, China, No. ZK2012B20.

Conflict-of-interest: None of the authors have any conflicts-of-interest to disclose.

Correspondence to: Xiao-Ming Fan, MD, PhD, Professor, Department of Gastroenterology and Hepatology, Jinshan Hospital of Fudan University, No. 1508 Longhang Road, Shanghai 201508, China. xiaomingfan57@sina.com
Telephone: +86-21-34189990
Fax: +86-21-57943141

Received: October 19, 2014
Peer-review started: October 20, 2014
First decision: December 11, 2014
Revised: December 29, 2014
Accepted: January 30, 2015
Article in press: January 30, 2015
Published online: March 21, 2015

Abstract

Nonalcoholic fatty liver disease (NAFLD) is currently considered as the most common liver disease in Western countries, and is rapidly becoming a serious threat to public health worldwide. However, the underlying mechanisms leading to the development of NAFLD are still not fully understood. The ghrelin-ghrelin O-acyltransferase (GOAT) system has recently been found to play a crucial role in both the development of steatosis and its progression to nonalcoholic steatohepatitis. Ghrelin, the natural ligand of the growth hormone secretagogue receptor, is a 28-amino acid peptide possessing a unique acylation on the serine in position 3 catalyzed by GOAT. The ghrelin-GOAT system is involved in insulin resistance, lipid metabolism dysfunction, and inflammation, all of which play important roles in the pathogenesis of NAFLD. A better understanding of ghrelin-GOAT system biology led to the identification of its potential roles in NAFLD. Molecular targets modulating ghrelin-GOAT levels and the biologic effects are being studied, which provide a new insight into the pathogenesis of NAFLD. This review probes into the possible relationship between the ghrelin-GOAT system and NAFLD, and considers the potential mechanisms by which the ghrelin-GOAT system brings about insulin resistance and other aspects concerning NAFLD.

Key words: Energy homeostasis; Ghrelin-ghrelin *O*-acyltransferase system; Insulin resistance; Lipid metabolism; Nonalcoholic fatty liver disease

Core tip: Nonalcoholic fatty liver disease (NAFLD) is a progressive disorder that can lead to impaired liver function and, ultimately, liver failure. The ghrelin-ghrelin *O*-acyltransferase (GOAT) system has recently been found to play a crucial role in both the development of steatosis and its progression to nonalcoholic steatohepatitis. This review probes into the possible relationship between ghrelin-GOAT system and NAFLD, and considers the potential mechanisms by which the ghrelin-GOAT system brings about insulin resistance and other aspects concerning NAFLD.

Zhang SR, Fan XM. Ghrelin-ghrelin *O*-acyltransferase system in the pathogenesis of nonalcoholic fatty liver disease. *World J*

Gastroenterol 2015; 21(11): 3214-3222 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3214.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3214

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a metabolic disorder syndrome that is not due to the abuse of alcohol. The term NAFLD encompasses a spectrum of histologically defined liver disorders. The disease can progress from macrovesicular lipid accumulation in the hepatocytes (termed steatosis) to nonalcoholic steatohepatitis (NASH) to outright fibrosis, cirrhosis, and even hepatocellular carcinoma[1-3] (Figure 1). The occurrence of NAFLD is strongly linked to obesity, insulin resistance (IR) and other aspects of the metabolic syndrome.

The reported prevalence of NAFLD in the United States and other Western countries ranges from 30% to 46%[4-6]. This disease has also become prevalent in Eastern countries where it has become a significant public health concern[7,8]. However, patients with NAFLD often have normal liver aminotransferases and the potential presence of NAFLD may be neglected by clinicians[9-12]. Patients with NAFLD are always at high risk for cardiometabolic complications, such as type 2 diabetes (T2DM) and cardiovascular disease[13-16].

The exact pathogenesis of NAFLD remains unknown. A number of environmental and genetic factors are involved in the NAFLD development and progression (Figure 1). The "two-hits hypothesis" is currently the most recognized theory to explain NAFLD development and progression[17]. Fat accumulation in hepatocytes is considered as the primary insult, while the following events, including mitochondrial dysfunction, lipid peroxidation, IR, and oxidative stress, result in liver cell inflammation and apoptosis, which eventually progress from simple steatosis to NASH[18-20]. Although the "two-hits hypothesis" of NAFLD pathogenesis is currently the most recognized theory, the "multi-hits hypothesis" that involves lipotoxicity, oxidative stress, mitochondrial dysfunction, a chronic inflammatory state, and endoplasmic reticulum stress, is getting more and more attention. The "multi-hits hypothesis" summarizes the complex factors and interactions between cytokines, free fatty acids (FFAs) metabolism, inflammation, and IR in NAFLD[21,22].

As oxidative stress and inflammation are key events in the progression from simple steatosis to NASH, retardation of these processes may reverse the development of NAFLD[18,20]. Thus, use of low side-effect agents that ameliorate those key events of NAFLD may provide important therapeutic evidence for the development of NAFLD. In recent years, a number of chemical agents have been found to have protective efficacy against NAFLD-induced liver injury, oxidative stress, and inflammation[23-26].

Recently, several studies have shown some advances in the pathogenesis of NAFLD. Advances in the understanding of autophagy have provided insights into the relationship between autophagy and NAFLD. Autophagy might stimulate lipid metabolism and have therapeutic potential in NAFLD[27]. *Helicobacter pylori* infection is involved in the pathogenesis of IR, which is closely linked with NAFLD[28]. The role of *H. pylori* infection in the development of NAFLD is gaining attention because its eradication is easy and much less expensive than long-term treatment of the other risk factors. Besides, overexpression of miR-185, an endogenous non-protein coding small RNA molecule, improved insulin sensitivity and reduced liver steatosis in an NAFLD animal model, and thus may be a therapeutic target[29]. In recent years, several adipocytokines and proinflammatory cytokines, which decrease or enhance IR, were also found to be involved in the pathogenesis of NAFLD[30-32]. Nevertheless, the complicated mechanisms of NAFLD are not entirely clear at present. Future better-designed research will provide more insights into the pathogenesis and therapeutic strategies for NAFLD.

The ghrelin-ghrelin *O*-acyltransferase (GOAT) system has recently been reported to play a crucial role in both the development of steatosis and progression to NASH. The ghrelin-GOAT system is involved in IR, lipid metabolism dysfunction, and inflammation, all of which play important roles in the pathogenesis of NAFLD[33-36]. Therefore, there is an urgent need to better understand the mechanisms of ghrelin-GOAT system involvement in NAFLD. This review will illuminate the relationship between the ghrelin-GOAT system and pathogenesis of NAFLD.

OVERVIEW OF THE GHRELIN-GOAT SYSTEM

Ghrelin

Ghrelin is a small peptide and hormone comprised of 28 amino acids[37] that is mainly produced by the stomach and the pancreas, which stimulates appetite and is a potent stimulator of growth hormone through the action of its receptor, the growth horomone secretagogue receptor[38,39]. A number of studies have shown that exogenous administration of ghrelin produces multiple physiologic effects, including the ability to increase food intake and decrease energy expenditure[38,40]. Ghrelin undergoes a post-translational modification, in which the third serine residue is covalently linked to a medium-chain fatty acid, typically octanoate[38]. The *O*-n-octanoylation of ghrelin is unique[41], and only the octanoylated form, which represents 10%-15% of circulating ghrelin, is able to stimulate body weight gain and food intake[42,43].

There are two forms of ghrelin: acylated and des-acyl ghrelin (DAG). Without food intake, both forms rise gradually in the plasma. Although some effects of DAG are still controversial and its receptor has not been identified, the biologic activities of DAG have

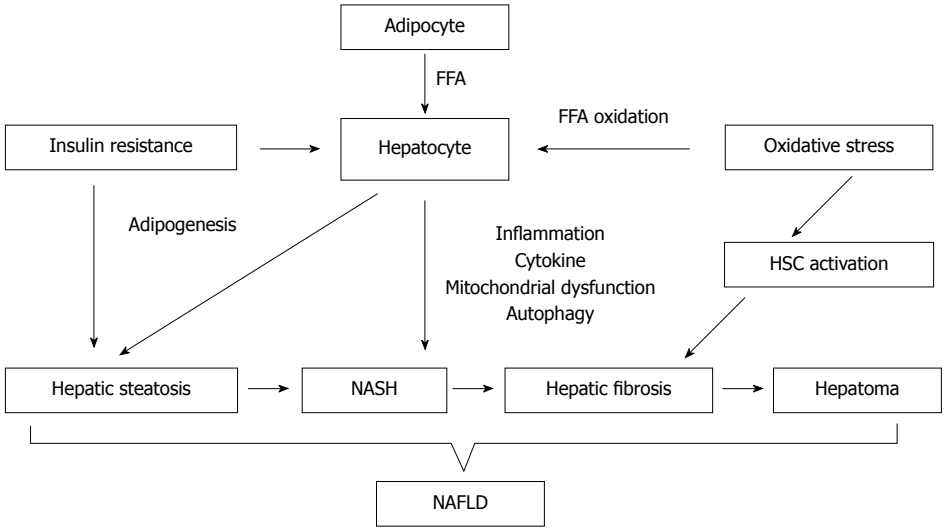


Figure 1 Pathogenesis and natural history of nonalcoholic fatty liver disease. FFA: Free fatty acid; HSC: Hepatic stellate cell; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

been reported, including gastric motility[44,45], adiposity, and glucose metabolism[46]. Further evidence for metabolic function of ghrelin has been provided by phenotypic analysis of rodents with genetic deletions of either ghrelin[47] or its receptor, GHS-R1a[48].

Ghrelin O-acyltransferase

Ghrelin is acylated on the serine in position 3. Both forms of ghrelin may result from the processing of preproghrelin[49]. The acylation is catalyzed by GOAT during the processing of the peptide[29,50]. Ingestion of either medium-chain fatty acids or medium-chain triglycerides can enhance acylation of ghrelin[51].

Lim *et al*[52] found that GOAT is expressed in all human tissues studied (stomach, adrenal cortex, breast, and right and left colon). The widespread expression of GOAT corresponds to the widespread distribution of ghrelin expression. GOAT expression was high in the stomach and gut, the major ghrelin-secreting tissues, and in the pituitary, ghrelin showed autocrine and paracrine effects. In addition to the important endocrine effects of acylated ghrelin, the paracrine effects of locally synthesized and acylated ghrelin may also be important. The concept was supported by the identification of GOAT expression in various tissues. It will be helpful to search for GOAT inhibitors as an alternative approach to reduce the actions of ghrelin, such as feeding and adiposity. As the action of GOAT and its inhibition are very specific to ghrelin, this may be a promising therapeutic target.

The activity of GOAT is modulated by fasting and satiation[53-55]. Although feeding suppresses both acylated ghrelin and DAG, long-term fasting inhibits ghrelin acylation but not total ghrelin secretion[56]. The exact effect of fasting and feeding on *GOAT* mRNA expression remains vague[57,58]. GOAT has been confirmed as a leptin-regulated gene[57], and González *et al*[58] found that exogenous leptin administration markedly increased *GOAT* mRNA levels in the gastric mucosa of fasted rats. It has been indicated that fasting low-leptin levels prevent an increase in *GOAT* mRNA levels, and therefore, GOAT can be added to the list of leptin-regulated genes under this specific condition. Leptin is the primary signal through which the hypothalamus senses the nutritional state and modulates food intake and energy balance. Leptin plays an opposite functional role of ghrelin in food intake and it also regulates ghrelin receptor GHS-R1a[59,60]. Increased *GOAT* mRNA levels relevant to chronic malnutrition may elucidate the potential mechanism responsible for increased acylated ghrelin levels in anorexia nervosa[61].

Alimentary lipids are important for the activation of GOAT. In fact, *GOAT* knockout mice subjected to a diet containing 10% medium-chain triglycerides exhibited lower body weights, possibly due to lower fat mass, compared to wild-type mice[57]. In addition, large amounts of acyl ghrelin were produced by *GOAT* transgenic mice[57]. An important function of ghrelin is the maintenance of viability during famine. The study of wild-type and *GOAT* knockout mice subjected to a 60% calorie-restricted diet showed 30% and 75% body weight loss, respectively, which could explain this hypothesis[62].

GHRELIN-GOAT SYSTEM AND NAFLD

The ghrelin-GOAT system is linked to energy and lipid metabolism, IR, inflammation, and apoptotic cell death, which are common to both obesity and NAFLD. Therefore, the role of the ghrelin-GOAT system in NAFLD has become a subject of considerable interest in recent years.

The relation of the ghrelin gene products and their involvement in metabolic and inflammatory pathways linked with the development of NAFLD were recently

reported[34]. It was found that patients with NASH had a twofold higher concentration of DAG than patients with non-NASH. Ghrelin concentration positively correlated with fibrosis stage. Apparently, products of the ghrelin gene may be important for the pathogenesis of NASH and fibrosis. The report by Li *et al*[33] showed that both administration of ghrelin during the induction of NAFLD and after the establishment of NAFLD could improve liver injury *via* attenuating alanine aminotransferase/aspartate transaminase, oxidative stress, inflammation, apoptosis, and restoring hepatic lipid metabolism. Such effects might partly act through targeting the PI3K/Akt and LKB1/AMPK pathways. Therefore, ghrelin can be a critical therapeutic agent against NAFLD. However, other research yielded different results. One study reported that the plasma levels of ghrelin in obese individuals were lower than those in normal-weight people, indicating that ghrelin may not be related to the progression of obesity[63].

The role of ghrelin in appetite regulation and energy metabolism is well established and it is now recognized as a very promising target for the treatment of NAFLD[64]. GHS-R antagonists and ghrelin antibodies are being studied in these systems[65]. An anti-obesity vaccine that prevents ghrelin from reaching the central nervous system has been developed[66]. A glucagon-like peptide-1 receptor agonist, exendin-4, has shown the effect of inhibiting ghrelin secretion[67].

Besides ghrelin and GHS-R, GOAT has also been implicated as a potential target for anti-NAFLD treatment[35,36]. GOAT inhibition will lead to decreased ghrelin acylation and increased levels of DAG, which is suggested to be beneficial for glucose homeostasis[68,69]. At the moment, there are no anti-NAFLD drugs on the market that target the ghrelin system. This is mainly due to the variation or lack of efficacy, potency, non-selectivity, poor bioavailability, sustained weight loss, and/or adverse side effects. However, specific antagonists are being developed and their relevance to clinical practice is being studied.

Role of the ghrelin-GOAT system in IR

IR is a disorder in insulin signaling in many organs, including the liver, fat, and muscle, and is a major characteristic of obesity, T2DM, and NAFLD. IR is an essential requirement for NAFLD and is believed to influence "the first hit" in NAFLD. Some research has illuminated that IR is a typical character of NAFLD[70-73]. NAFLD is highly prevalent among patients with T2DM[74]. By addressing NAFLD both as a consequence and as a cause of IR through lessons learned from the liver of patients with T2DM, Takamura *et al*[75] presented the remarkable changes in the liver in NAFLD. The development of NAFLD appears to be associated with food intake, as diet is an important contributor to its pathogenesis[76].

In recent years, ghrelin has been found to play a direct role in glucose homeostasis. A number of reports have demonstrated ghrelin expression in pancreatic islets[77-80] and the ability of ghrelin to regulate insulin secretion and promote β-cell proliferation and survival[81,82]. However, the role of ghrelin in the secretion and action of insulin remains controversial. Some studies show that ghrelin increases insulin secretion[79,81,83-85], whereas other reports show that it inhibits it[86-89].

Mice genetically deficient in the GOAT enzyme lack acylated ghrelin and exhibit a modest decrease in body weight and fat mass when fed a diet rich in medium-chain triglycerides[57]. When these mice were subjected to a period of severe caloric restriction, they were unable to maintain normal blood glucose levels, resulting in eventual death, unless either ghrelin or growth hormone was provided[62]. A recent report showed that a GOAT-specific acyltransferase inhibitor could improve glucose tolerance and reduce weight gain, indicating the effect of the ghrelin-GOAT system on glucose homeostasis[90].

IR patients with NAFLD show decreased insulin sensitivity not only in muscle, but also in liver and adipose tissue[73,91]. The adipose tissue becomes resistant to the anti-lipolytic effect of insulin, and the release of fatty acids is increased in IR[92]. An important source of FFAs is the increased spillover from chylomicrons under postprandial conditions[93]. It was supposed that ectopic fat may be a defense mechanism against lipotoxicity[94,95], and that patients with NAFLD develop NASH and cirrhosis only after a second hit. Therefore, it is not surprising that gut hormones that are known to control the uptake of nutrients by organs are now increasingly investigated in NAFLD.

Role of the ghrelin-GOAT system in lipid and energy metabolism

The first step in the development of NAFLD is hepatic steatosis, which is characterized by macrovesicular accumulation of triglycerides in the cytoplasm of hepatocytes. Sources of increased hepatic lipids in NAFLD include excess dietary chylomicron remnants, increased new lipogenesis, or excess FFAs released from the lipolysis of adipose tissue[96-98]. Saturated fat seems to stimulate hepatic lipid accumulation and progression into NASH, whereas unsaturated fat, choline, antioxidants, and high-protein diets rich in isoflavones seem to have a more preventive effect. Li *et al*[99] recently found that ghrelin activated hepatocyte lipogenesis *via* the mTOR-PPARγ signaling pathway; ghrelin-induced lipogenesis was mediated by mTOR, and the effect was significantly attenuated by PPARγ antagonism in cultured hepatocytes and in *PPAR*γ-deficient mice.

Lipid accumulation within the liver represents an equilibrium between synthesis and utilization. The indiscriminate lipid metabolism and increased lipid flux through the liver result in intracellular stress, apoptosis, and consequent liver damage[100]. IR in adipose tissue

with uncontrolled lipolysis resulting in enhanced FFA delivery to the liver has been postulated to be a critical factor in development of NAFLD. IR in adipose tissue has been shown to correlate with severity of liver biopsy findings in NASH[101]. Adipose tissue tumor necrosis factor (TNF)-α and circulating interleukin 6 are associated with IR and circulating FFA levels, and both are increased in patients with NAFLD[102-104]. It is entirely plausible that endocrine abnormalities with hormonal excess and deficiencies may be implicated in the pathogenesis of NAFLD.

Ghrelin stimulates food intake and decreases energy expenditure in rats[48,105-108]. Ghrelin also increases appetite and stimulates food intake in humans[105]. A recent study evaluated ghrelin levels and their relationship with NAFLD and IR in obese adolescents, and found that ghrelin was negatively correlated with weight[109]. Ghrelin concentrations decrease with weight gain resulting from overfeeding, pregnancy, or olanzapine treatment[110-113]. Indeed, ghrelin stimulates the gene expression of lipogenic enzymes, such as acetyl CoA carboxylase, stearoyl CoA desaturase, and fatty acid synthase in white adipose tissue[114].

In order to determine *GOAT* expression and functional regulation, measurement of its protein levels and activity will be critical. Recently, genetic variation in *GOAT* was found in association with anorexia nervosa[115], though whether it may also be involved in NAFLD remains unknown. Inhibition of GOAT by a peptide-based bisubstrate analog (GO-CoA-Tat) reduced weight gain and improved glucose tolerance in wild-type mice[84]. In fact, GOAT is the only enzyme responsible for ghrelin acylation and its alteration will only affect the physiologic process of ghrelin acylation. In the future, special medicine targeting GOAT may be designed as a novel therapeutic approach for NAFLD.

Role of the ghrelin-GOAT system in inflammation

A small portion of patients with NAFLD will develop inflammation and fibrosis, termed NASH, which is a more progressive, inflammatory disease phenotype of NAFLD[18]. In recent years, the roles of ghrelin in immunity regulation under inflammatory conditions and liver protection have been being clarified. In the gastrointestinal tract, administration of exogenous ghrelin ameliorates the release of proinflammatory cytokines, promotes cell proliferation, and reduces apoptosis after TNF-α- or lipopolysaccharide-induced inflammation[116]. Administration of ghrelin has therapeutic effects for several inflammatory diseases in rodent models, including sepsis[117], intestinal ischemia and reperfusion injury[118], pancreatic disease[119], cardiovascular disease[120], and gastrointestinal disease[121]. In addition, a recent study demonstrated that pretreatment with ghrelin prior to carbon tetrachloride intoxication attenuated liver injury and oxidative stress[122]. Thus, inflammation represents an important mechanism for the development of NAFLD to NASH. Now that recent research has suggested the anti-inflammatory role of ghrelin in many organs, it is possible that future study will identify its pharmacologic role in the development of NAFLD.

CONCLUSION

NAFLD is becoming a serious threat to public health worldwide. However, the underlying mechanisms leading to the development of NAFLD are not fully understood. The involvement of the ghrelin-GOAT system in NAFLD and a better understanding of its biology have led to the identification of pharmacologic targets and the development of pharmacologic compounds for the treatment of NAFLD and related diseases. Thus, the ghrelin-GOAT system represents a promising target for the treatment of NAFLD.

REFERENCES

1 **Dowman JK**, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2011; **33**: 525-540 [PMID: 21198708 DOI: 10.1111/j.1365-2036.2010.04556.x]

2 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419 [PMID: 10348825 DOI: 10.1016/S0016-5085(99)70506-8]

3 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140 [PMID: 12105842 DOI: 10.1053/gast.2002.34168]

4 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

5 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

6 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]

7 **Fan JG**. Epidemiology of alcoholic and nonalcoholic fatty liver disease in China. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 11-17 [PMID: 23855290 DOI: 10.1111/jgh.12036]

8 **Farrell GC**, Wong VW, Chitturi S. NAFLD in Asia--as common and important as in the West. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 307-318 [PMID: 23458891]

9 **Fracanzani AL**, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; **48**: 792-798 [PMID: 18752331 DOI: 10.1002/hep.22429]

10 **Kotronen A**, Juurinen L, Hakkarainen A, Westerbacka J,

Cornér A, Bergholm R, Yki-Järvinen H. Liver fat is increased in type 2 diabetic patients and underestimated by serum alanine aminotransferase compared with equally obese nondiabetic subjects. *Diabetes Care* 2008; **31**: 165-169 [PMID: 17934148 DOI: 10.2337/dc07-1463]

11 **Gastaldelli A**, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, Buzzigoli E, Sironi AM, Cersosimo E, Ferrannini E, Defronzo RA. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 2007; **133**: 496-506 [PMID: 17681171 DOI: 10.1053/j.gastro.2007.04.068]

12 **Bellentani S**, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**: 112-117 [PMID: 10644271 DOI: 10.7326/0003-4819-132-2-200001180-00004]

13 **Bhatia LS**, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? *Eur Heart J* 2012; **33**: 1190-1200 [PMID: 22408036 DOI: 10.1093/eurheartj/ehr453]

14 **Targher G**, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010; **363**: 1341-1350 [PMID: 20879883]

15 **Targher G**, Byrne CD. Clinical Review: Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *J Clin Endocrinol Metab* 2013; **98**: 483-495 [PMID: 23293330 DOI: 10.1210/jc.2012-3093]

16 **Fabbrini E**, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010; **51**: 679-689 [PMID: 20041406 DOI: 10.1002/hep.23280]

17 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]

18 **Harmon RC**, Tiniakos DG, Argo CK. Inflammation in nonalcoholic steatohepatitis. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 189-200 [PMID: 21476914 DOI: 10.1586/egh.11.21]

19 **Alkhouri N**, Carter-Kent C, Feldstein AE. Apoptosis in nonalcoholic fatty liver disease: diagnostic and therapeutic implications. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 201-212 [PMID: 21476915 DOI: 10.1586/egh.11.6]

20 **Koek GH**, Liedorp PR, Bast A. The role of oxidative stress in non-alcoholic steatohepatitis. *Clin Chim Acta* 2011; **412**: 1297-1305 [PMID: 21514287 DOI: 10.1016/j.cca.2011.04.013]

21 **Polyzos SA**, Kountouras J, Zavos C. Nonalcoholic fatty liver disease: the pathogenetic roles of insulin resistance and adipocytokines. *Curr Mol Med* 2009; **9**: 299-314 [PMID: 19355912 DOI: 10.2174/156652409787847191]

22 **Liu J**, Xu Y, Hu Y, Wang G. The role of fibroblast growth factor 21 in the pathogenesis of non-alcoholic fatty liver disease and implications for therapy. *Metabolism* 2015; **64**: 380-390 [PMID: 25516477 DOI: 10.1016/j.metabol.2014.11.009]

23 **Pan M**, Song YL, Xu JM, Gan HZ. Melatonin ameliorates nonalcoholic fatty liver induced by high-fat diet in rats. *J Pineal Res* 2006; **41**: 79-84 [PMID: 16842545 DOI: 10.1111/j.1600-079X.2006.00346.x]

24 **Panchal SK**, Poudyal H, Arumugam TV, Brown L. Rutin attenuates metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats. *J Nutr* 2011; **141**: 1062-1069 [PMID: 21508207 DOI: 10.3945/jn.111.137877]

25 **Song Z**, Deaciuc I, Zhou Z, Song M, Chen T, Hill D, McClain CJ. Involvement of AMP-activated protein kinase in beneficial effects of betaine on high-sucrose diet-induced hepatic steatosis. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G894-G902 [PMID: 17702954 DOI: 10.1152/ajpgi.00133.2007]

26 **Park HJ**, DiNatale DA, Chung MY, Park YK, Lee JY, Koo SI, O'Connor M, Manautou JE, Bruno RS. Green tea extract attenuates hepatic steatosis by decreasing adipose lipogenesis and enhancing hepatic antioxidant defenses in ob/ob mice. *J Nutr Biochem* 2011; **22**: 393-400 [PMID: 20655714 DOI: 10.1016/j.jnutbio.2010.03.009]

27 **Sinha RA**, Farah BL, Singh BK, Siddique MM, Li Y, Wu Y, Ilkayeva OR, Gooding J, Ching J, Zhou J, Martinez L, Xie S, Bay BH, Summers SA, Newgard CB, Yen PM. Caffeine stimulates hepatic lipid metabolism by the autophagy-lysosomal pathway in mice. *Hepatology* 2014; **59**: 1366-1380 [PMID: 23929677 DOI: 10.1002/hep.26667]

28 **Li M**, Shen Z, Li YM. Potential role of Helicobacter pylori infection in nonalcoholic fatty liver disease. *World J Gastroenterol* 2013; **19**: 7024-7031 [PMID: 24222944 DOI: 10.3748/wjg.v19.i41.7024]

29 **Wang XC**, Zhan XR, Li XY, Yu JJ, Liu XM. MicroRNA-185 regulates expression of lipid metabolism genes and improves insulin sensitivity in mice with non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 17914-17923 [PMID: 25548489 DOI: 10.3748/wjg.v20.i47.17914]

30 **El-Wakkad A**, Hassan Nel-M, Sibaii H, El-Zayat SR. Proinflammatory, anti-inflammatory cytokines and adiponkines in students with central obesity. *Cytokine* 2013; **61**: 682-687 [PMID: 23306429 DOI: 10.1016/j.cyto.2012.11.010]

31 **Zuo H**, Shi Z, Yuan B, Dai Y, Wu G, Hussain A. Association between serum leptin concentrations and insulin resistance: a population-based study from China. *PLoS One* 2013; **8**: e54615 [PMID: 23349940 DOI: 10.1371/journal.pone.0054615]

32 **Ozcelik F**, Yuksel C, Arslan E, Genc S, Omer B, Serdar MA. Relationship between visceral adipose tissue and adiponectin, inflammatory markers and thyroid hormones in obese males with hepatosteatosis and insulin resistance. *Arch Med Res* 2013; **44**: 273-280 [PMID: 23602473 DOI: 10.1016/j.arcmed.2013.04.001]

33 **Li Y**, Hai J, Li L, Chen X, Peng H, Cao M, Zhang Q. Administration of ghrelin improves inflammation, oxidative stress, and apoptosis during and after non-alcoholic fatty liver disease development. *Endocrine* 2013; **43**: 376-386 [PMID: 22843123 DOI: 10.1007/s12020-012-9761-5]

34 **Estep M**, Abawi M, Jarrar M, Wang L, Stepanova M, Elariny H, Moazez A, Goodman Z, Chandhoke V, Baranova A, Younossi ZM. Association of obestatin, ghrelin, and inflammatory cytokines in obese patients with non-alcoholic fatty liver disease. *Obes Surg* 2011; **21**: 1750-1757 [PMID: 21744131 DOI: 10.1007/s11695-011-0475-1]

35 **Yang J**, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 2008; **132**: 387-396 [PMID: 18267071 DOI: 10.1016/j.cell.2008.01.017]

36 **Gualillo O**, Lago F, Dieguez C. Introducing GOAT: a target for obesity and anti-diabetic drugs? *Trends Pharmacol Sci* 2008; **29**: 398-401 [PMID: 18606462 DOI: 10.1016/j.tips.2008.06.003]

37 **Inui A**, Asakawa A, Bowers CY, Mantovani G, Laviano A, Meguid MM, Fujimiya M. Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ. *FASEB J* 2004; **18**: 439-456 [PMID: 15003990 DOI: 10.1096/fj.03-0641rev]

38 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660 [PMID: 10604470 DOI: 10.1038/45230]

39 **Castañeda TR**, Tong J, Datta R, Culler M, Tschöp MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol* 2010; **31**: 44-60 [PMID: 19896496 DOI: 10.1016/j.yfrne.2009.10.008]

40 **Wren AM**, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, Bloom SR. Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 2001; **50**: 2540-2547 [PMID: 11679432 DOI: 10.2337/diabetes.50.11.2540]

41 **Banks WA**, Tschöp M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther* 2002; **302**: 822-827 [PMID: 12130749 DOI: 10.1124/jpet.102.034827]

42 **Tschöp M**, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; **407**: 908-913 [PMID: 11057670 DOI:

10.1038/35038090]
43 **Akamizu T**, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, Tada H, Miura K, Shimizu A, Fukushima M, Yokode M, Tanaka K, Kangawa K. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. *Eur J Endocrinol* 2004; **150**: 447-455 [PMID: 15080773 DOI: 10.1530/eje.0.1500447]
44 **Chen CY**, Inui A, Asakawa A, Fujino K, Kato I, Chen CC, Ueno N, Fujimiya M. Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats. *Gastroenterology* 2005; **129**: 8-25 [PMID: 16012930 DOI: 10.1053/j.gastro.2005.04.015]
45 **Toshinai K**, Yamaguchi H, Sun Y, Smith RG, Yamanaka A, Sakurai T, Date Y, Mondal MS, Shimbara T, Kawagoe T, Murakami N, Miyazato M, Kangawa K, Nakazato M. Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. *Endocrinology* 2006; **147**: 2306-2314 [PMID: 16484324 DOI: 10.1210/en.2005-1357]
46 **Zhang W**, Chai B, Li JY, Wang H, Mulholland MW. Effect of des-acyl ghrelin on adiposity and glucose metabolism. *Endocrinology* 2008; **149**: 4710-4716 [PMID: 18535105 DOI: 10.1210/en.2008-0263]
47 **Wortley KE**, del Rincon JP, Murray JD, Garcia K, Iida K, Thorner MO, Sleeman MW. Absence of ghrelin protects against early-onset obesity. *J Clin Invest* 2005; **115**: 3573-3578 [PMID: 16322795 DOI: 10.1172/JCI26003]
48 **Zigman JM**, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD, Williams TD, Lachey JL, Seeley RJ, Lowell BB, Elmquist JK. Mice lacking ghrelin receptors resist the development of diet-induced obesity. *J Clin Invest* 2005; **115**: 3564-3572 [PMID: 16322794 DOI: 10.1172/JCI26002]
49 **Hosoda H**, Kojima M, Mizushima T, Shimizu S, Kangawa K. Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing. *J Biol Chem* 2003; **278**: 64-70 [PMID: 12414809 DOI: 10.1074/jbc.M205366200]
50 **Gutierrez JA**, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, Witcher DR, Luo S, Onyia JE, Hale JE. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci USA* 2008; **105**: 6320-6325 [PMID: 18443287 DOI: 10.1073/pnas.0800708105]
51 **Nishi Y**, Hiejima H, Hosoda H, Kaiya H, Mori K, Fukue Y, Yanase T, Nawata H, Kangawa K, Kojima M. Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin. *Endocrinology* 2005; **146**: 2255-2264 [PMID: 15677766 DOI: 10.1210/en.2004-0695]
52 **Lim CT**, Kola B, Grossman A, Korbonits M. The expression of ghrelin O-acyltransferase (GOAT) in human tissues. *Endocr J* 2011; **58**: 707-710 [PMID: 21646729 DOI: 10.1507/endocrj.K11E-117]
53 **Cummings DE**, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714-1719 [PMID: 11473029 DOI: 10.2337/diabetes.50.8.1714]
54 **Cummings DE**. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol Behav* 2006; **89**: 71-84 [PMID: 16859720 DOI: 10.1016/j.physbeh.2006.05.022]
55 **Tschöp M**, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, Folwaczny C. Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest* 2001; **24**: RC19-RC21 [PMID: 11434675 DOI: 10.1007/BF03351037]
56 **Liu J**, Prudom CE, Nass R, Pezzoli SS, Oliveri MC, Johnson ML, Veldhuis P, Gordon DA, Howard AD, Witcher DR, Geysen HM, Gaylinn BD, Thorner MO. Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. *J Clin Endocrinol Metab* 2008; **93**: 1980-1987 [PMID: 18349056 DOI: 10.1210/jc.2007-2235]
57 **Kirchner H**, Gutierrez JA, Solenberg PJ, Pfluger PT, Czyzyk TA, Willency JA, Schürmann A, Joost HG, Jandacek RJ, Hale JE, Heiman ML, Tschöp MH. GOAT links dietary lipids with the endocrine control of energy balance. *Nat Med* 2009; **15**: 741-745 [PMID: 19503064 DOI: 10.1038/nm.1997]
58 **González CR**, Vázquez MJ, López M, Diéguez C. Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa. *J Mol Endocrinol* 2008; **41**: 415-421 [PMID: 18835978 DOI: 10.1677/JME-08-0102]
59 **Nogueiras R**, Tovar S, Mitchell SE, Rayner DV, Archer ZA, Dieguez C, Williams LM. Regulation of growth hormone secretagogue receptor gene expression in the arcuate nuclei of the rat by leptin and ghrelin. *Diabetes* 2004; **53**: 2552-2558 [PMID: 15448083 DOI: 10.2337/diabetes.53.10.2552]
60 **López M**, Tovar S, Vázquez MJ, Williams LM, Diéguez C. Peripheral tissue-brain interactions in the regulation of food intake. *Proc Nutr Soc* 2007; **66**: 131-155 [PMID: 17343779 DOI: 10.1017/S0029665107005368]
61 **Soriano-Guillén L**, Barrios V, Campos-Barros A, Argente J. Ghrelin levels in obesity and anorexia nervosa: effect of weight reduction or recuperation. *J Pediatr* 2004; **144**: 36-42 [PMID: 14722516 DOI: 10.1016/j.jpeds.2003.10.036]
62 **Zhao TJ**, Liang G, Li RL, Xie X, Sleeman MW, Murphy AJ, Valenzuela DM, Yancopoulos GD, Goldstein JL, Brown MS. Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc Natl Acad Sci USA* 2010; **107**: 7467-7472 [PMID: 20231469 DOI: 10.1073/pnas.1002271107]
63 **Cummings DE**, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 2002; **346**: 1623-1630 [PMID: 12023994 DOI: 10.1056/NEJMoa012908]
64 **Soares JB**, Roncon-Albuquerque R, Leite-Moreira A. Ghrelin and ghrelin receptor inhibitors: agents in the treatment of obesity. *Expert Opin Ther Targets* 2008; **12**: 1177-1189 [PMID: 18694382 DOI: 10.1517/14728222.12.9.1177]
65 **Schellekens H**, Dinan TG, Cryan JF. Lean mean fat reducing "ghrelin" machine: hypothalamic ghrelin and ghrelin receptors as therapeutic targets in obesity. *Neuropharmacology* 2010; **58**: 2-16 [PMID: 19573543 DOI: 10.1016/j.neuropharm.2009.06.024]
66 **Zorrilla EP**, Iwasaki S, Moss JA, Chang J, Otsuji J, Inoue K, Meijler MM, Janda KD. Vaccination against weight gain. *Proc Natl Acad Sci USA* 2006; **103**: 13226-13231 [PMID: 16891413 DOI: 10.1073/pnas.0605376103]
67 **Pérez-Tilve D**, González-Matías L, Alvarez-Crespo M, Leiras R, Tovar S, Diéguez C, Mallo F. Exendin-4 potently decreases ghrelin levels in fasting rats. *Diabetes* 2007; **56**: 143-151 [PMID: 17192476 DOI: 10.2337/db05-0996]
68 **Ariyasu H**, Takaya K, Iwakura H, Hosoda H, Akamizu T, Arai Y, Kangawa K, Nakao K. Transgenic mice overexpressing des-acyl ghrelin show small phenotype. *Endocrinology* 2005; **146**: 355-364 [PMID: 15471959 DOI: 10.1210/en.2004-0629]
69 **Iwakura H**, Hosoda K, Son C, Fujikura J, Tomita T, Noguchi M, Ariyasu H, Takaya K, Masuzaki H, Ogawa Y, Hayashi T, Inoue G, Akamizu T, Hosoda H, Kojima M, Itoh H, Toyokuni S, Kangawa K, Nakao K. Analysis of rat insulin II promoter-ghrelin transgenic mice and rat glucagon promoter-ghrelin transgenic mice. *J Biol Chem* 2005; **280**: 15247-15256 [PMID: 15701644 DOI: 10.1074/jbc.M411358200]
70 **Sanyal AJ**, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**: 1183-1192 [PMID: 11266382 DOI: 10.1053/gast.2001.23256]
71 **Yki-Järvinen H**. Liver fat in the pathogenesis of insulin resistance and type 2 diabetes. *Dig Dis* 2010; **28**: 203-209 [PMID: 20460912 DOI: 10.1159/000282087]
72 **Fabbrini E**, Magkos F, Mohammed BS, Pietka T, Abumrad NA, Patterson BW, Okunade A, Klein S. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA* 2009; **106**: 15430-15435 [PMID: 19706383 DOI: 10.1073/pnas.0904944106]
73 **Bugianesi E**, Gastaldelli A, Vanni E, Gambino R, Cassader M,

Baldi S, Ponti V, Pagano G, Ferrannini E, Rizzetto M. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 2005; **48**: 634-642 [PMID: 15747110 DOI: 10.1007/s00125-005-1682-x]

74 **Targher G**, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, Day C, Arcaro G. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007; **30**: 1212-1218 [PMID: 17277038 DOI: 10.2337/dc06-2247]

75 **Takamura T**, Misu H, Ota T, Kaneko S. Fatty liver as a consequence and cause of insulin resistance: lessons from type 2 diabetic liver. *Endocr J* 2012; **59**: 745-763 [PMID: 22893453 DOI: 10.1507/endocrj.EJ12-0228]

76 **de Wit NJ**, Afman LA, Mensink M, Müller M. Phenotyping the effect of diet on non-alcoholic fatty liver disease. *J Hepatol* 2012; **57**: 1370-1373 [PMID: 22796155 DOI: 10.1016/j.jhep.2012.07.003]

77 **Date Y**, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes* 2002; **51**: 124-129 [PMID: 11756331 DOI: 10.2337/diabetes.51.1.124]

78 **Prado CL**, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L. Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proc Natl Acad Sci USA* 2004; **101**: 2924-2929 [PMID: 14970313 DOI: 10.1073/pnas.0308604100]

79 **Dezaki K**, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, Yada T. Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca2+ signaling in beta-cells: implication in the glycemic control in rodents. *Diabetes* 2004; **53**: 3142-3151 [PMID: 15561944 DOI: 10.2337/diabetes.53.12.3142]

80 **Wierup N**, Svensson H, Mulder H, Sundler F. The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regul Pept* 2002; **107**: 63-69 [PMID: 12137967 DOI: 10.1016/S0167-0115(02)00067-8]

81 **Irako T**, Akamizu T, Hosoda H, Iwakura H, Ariyasu H, Tojo K, Tajima N, Kangawa K. Ghrelin prevents development of diabetes at adult age in streptozotocin-treated newborn rats. *Diabetologia* 2006; **49**: 1264-1273 [PMID: 16570155 DOI: 10.1007/s00125-006-0226-3]

82 **Granata R**, Settanni F, Biancone L, Trovato L, Nano R, Bertuzzi F, Destefanis S, Annunziata M, Martinetti M, Catapano F, Ghè C, Isgaard J, Papotti M, Ghigo E, Muccioli G. Acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 3',5'-cyclic adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphatidyl inositol 3-Kinase/Akt signaling. *Endocrinology* 2007; **148**: 512-529 [PMID: 17068144 DOI: 10.1210/en.2006-0266]

83 **Adeghate E**, Ponery AS. Ghrelin stimulates insulin secretion from the pancreas of normal and diabetic rats. *J Neuroendocrinol* 2002; **14**: 555-560 [PMID: 12121492 DOI: 10.1046/j.1365-2826.2002.00811.x]

84 **Sun Y**, Asnicar M, Smith RG. Central and peripheral roles of ghrelin on glucose homeostasis. *Neuroendocrinology* 2007; **86**: 215-228 [PMID: 17898534 DOI: 10.1159/000109094]

85 **Lee HM**, Wang G, Englander EW, Kojima M, Greeley GH. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. *Endocrinology* 2002; **143**: 185-190 [PMID: 11751608 DOI: 10.1210/endo.143.1.8602]

86 **Dezaki K**, Sone H, Koizumi M, Nakata M, Kakei M, Nagai H, Hosoda H, Kangawa K, Yada T. Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes* 2006; **55**: 3486-3493 [PMID: 17130496 DOI: 10.2337/db06-0878]

87 **Doi A**, Shono T, Nishi M, Furuta H, Sasaki H, Nanjo K. IA-2beta, but not IA-2, is induced by ghrelin and inhibits glucose-stimulated insulin secretion. *Proc Natl Acad Sci USA* 2006; **103**: 885-890 [PMID: 16418280 DOI: 10.1073/pnas.0502470102]

88 **Colombo M**, Gregersen S, Xiao J, Hermansen K. Effects of ghrelin and other neuropeptides (CART, MCH, orexin A and B, and GLP-1) on the release of insulin from isolated rat islets. *Pancreas* 2003; **27**: 161-166 [PMID: 12883265 DOI: 10.1097/00006676-200308000-00009]

89 **Wierup N**, Yang S, McEvilly RJ, Mulder H, Sundler F. Ghrelin is expressed in a novel endocrine cell type in developing rat islets and inhibits insulin secretion from INS-1 (832/13) cells. *J Histochem Cytochem* 2004; **52**: 301-310 [PMID: 14966197 DOI: 10.1177/002215540405200301]

90 **Barnett BP**, Hwang Y, Taylor MS, Kirchner H, Pfluger PT, Bernard V, Lin YY, Bowers EM, Mukherjee C, Song WJ, Longo PA, Leahy DJ, Hussain MA, Tschöp MH, Boeke JD, Cole PA. Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor. *Science* 2010; **330**: 1689-1692 [PMID: 21097901 DOI: 10.1126/science.1196154]

91 **Lomonaco R**, Ortiz-Lopez C, Orsak B, Webb A, Hardies J, Darland C, Finch J, Gastaldelli A, Harrison S, Tio F, Cusi K. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 1389-1397 [PMID: 22183689 DOI: 10.1002/hep.25539]

92 **Arner P**. Insulin resistance in type 2 diabetes: role of fatty acids. *Diabetes Metab Res Rev* 2002; **18** Suppl 2: S5-S9 [PMID: 11921432 DOI: 10.1002/dmrr.254]

93 **Miles JM**, Nelson RH. Contribution of triglyceride-rich lipoproteins to plasma free fatty acids. *Horm Metab Res* 2007; **39**: 726-729 [PMID: 17952834 DOI: 10.1055/s-2007-990273]

94 **Choi SS**, Diehl AM. Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. *Curr Opin Lipidol* 2008; **19**: 295-300 [PMID: 18460922 DOI: 10.1097/MOL.0b013e3282ff5e55]

95 **Neuschwander-Tetri BA**. Nontriglyceride hepatic lipotoxicity: the new paradigm for the pathogenesis of NASH. *Curr Gastroenterol Rep* 2010; **12**: 49-56 [PMID: 20425484 DOI: 10.1007/s11894-009-0083-6]

96 **Cusi K**. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology* 2012; **142**: 711-725.e6 [PMID: 22326434 DOI: 10.1053/j.gastro.2012.02.003]

97 **Sozio MS**, Liangpunsakul S, Crabb D. The role of lipid metabolism in the pathogenesis of alcoholic and nonalcoholic hepatic steatosis. *Semin Liver Dis* 2010; **30**: 378-390 [PMID: 20960377 DOI: 10.1055/s-0030-1267538]

98 **Reddy JK**, Rao MS. Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G852-G858 [PMID: 16603729 DOI: 10.1152/ajpgi.00521.2005]

99 **Li Z**, Xu G, Qin Y, Zhang C, Tang H, Yin Y, Xiang X, Li Y, Zhao J, Mulholland M, Zhang W. Ghrelin promotes hepatic lipogenesis by activation of mTOR-PPARγ signaling pathway. *Proc Natl Acad Sci USA* 2014; **111**: 13163-13168 [PMID: 25157160 DOI: 10.1073/pnas.1411571111]

100 **Ibrahim SH**, Kohli R, Gores GJ. Mechanisms of lipotoxicity in NAFLD and clinical implications. *J Pediatr Gastroenterol Nutr* 2011; **53**: 131-140 [PMID: 21629127 DOI: 10.1097/MPG.0b013e31822578db]

101 **Musso G**, Cassader M, De Michieli F, Rosina F, Orlandi F, Gambino R. Nonalcoholic steatohepatitis versus steatosis: adipose tissue insulin resistance and dysfunctional response to fat ingestion predict liver injury and altered glucose and lipoprotein metabolism. *Hepatology* 2012; **56**: 933-942 [PMID: 22684858 DOI: 10.1002/hep.25739]

102 **Kern PA**, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 2001; **280**: E745-E751 [PMID: 11287357]

103 **Grigorescu M**, Crisan D, Radu C, Grigorescu MD, Sparchez Z, Serban A. A novel pathophysiological-based panel of biomarkers for the diagnosis of nonalcoholic steatohepatitis. *J Physiol Pharmacol* 2012; **63**: 347-353 [PMID: 23070083]

104 **Crespo J**, Cayón A, Fernández-Gil P, Hernández-Guerra M, Mayorga M, Domínguez-Díez A, Fernández-Escalante JC, Pons-Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 2001; **34**: 1158-1163 [PMID: 11732005 DOI:

10.1053/jhep.2001.29628]
105 **Wren AM**, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001; **86**: 5992 [PMID: 11739476 DOI: 10.1210/jcem.86.12.8111]
106 **Kamegai J**, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes* 2001; **50**: 2438-2443 [PMID: 11679419 DOI: 10.2337/diabetes.50.11.2438]
107 **Nakazato M**, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194-198 [PMID: 11196643 DOI: 10.1038/35051587]
108 **Shintani M**, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes* 2001; **50**: 227-232 [PMID: 11272130 DOI: 10.2337/diabetes.50.2.227]
109 **Arslan N**, Sayin O, Tokgoz Y. Evaluation of serum xenin and ghrelin levels and their relationship with nonalcoholic fatty liver disease and insulin resistance in obese adolescents. *J Endocrinol Invest* 2014; Epub ahead of print [PMID: 25200997]
110 **Williams DL**, Grill HJ, Cummings DE, Kaplan JM. Overfeeding-induced weight gain suppresses plasma ghrelin levels in rats. *J Endocrinol Invest* 2006; **29**: 863-868 [PMID: 17185893 DOI: 10.1007/BF03349188]
111 **Palik E**, Baranyi E, Melczer Z, Audikovszky M, Szöcs A, Winkler G, Cseh K. Elevated serum acylated (biologically active) ghrelin and resistin levels associate with pregnancy-induced weight gain and insulin resistance. *Diabetes Res Clin Pract* 2007; **76**: 351-357 [PMID: 17010469 DOI: 10.1016/j.diabres.2006.09.005]
112 **Hosojima H**, Togo T, Odawara T, Hasegawa K, Miura S, Kato Y, Kanai A, Kase A, Uchikado H, Hirayasu Y. Early effects of olanzapine on serum levels of ghrelin, adiponectin and leptin in patients with schizophrenia. *J Psychopharmacol* 2006; **20**: 75-79 [PMID: 16204328 DOI: 10.1177/0269881105056647]
113 **Otukonyong EE**, Dube MG, Torto R, Kalra PS, Kalra SP. High-fat diet-induced ultradian leptin and insulin hypersecretion are absent in obesity-resistant rats. *Obes Res* 2005; **13**: 991-999 [PMID: 15976141 DOI: 10.1038/oby.2005.116]
114 **Perez-Tilve D**, Heppner K, Kirchner H, Lockie SH, Woods SC, Smiley DL, Tschöp M, Pfluger P. Ghrelin-induced adiposity is independent of orexigenic effects. *FASEB J* 2011; **25**: 2814-2822 [PMID: 21543764 DOI: 10.1096/fj.11-183632]
115 **Müller TD**, Tschöp MH, Jarick I, Ehrlich S, Scherag S, Herpertz-Dahlmann B, Zipfel S, Herzog W, de Zwaan M, Burghardt R, Fleischhaker C, Klampfl K, Wewetzer C, Herpertz S, Zeeck A, Tagay S, Burgmer M, Pfluger PT, Scherag A, Hebebrand J, Hinney A. Genetic variation of the ghrelin activator gene ghrelin O-acyltransferase (GOAT) is associated with anorexia nervosa. *J Psychiatr Res* 2011; **45**: 706-711 [PMID: 21035823 DOI: 10.1016/j.jpsychires.2010.10.001]
116 **Waseem T**, Duxbury M, Ito H, Ashley SW, Robinson MK. Exogenous ghrelin modulates release of pro-inflammatory and anti-inflammatory cytokines in LPS-stimulated macrophages through distinct signaling pathways. *Surgery* 2008; **143**: 334-342 [PMID: 18291254 DOI: 10.1016/j.surg.2007.09.039]
117 **Chorny A**, Anderson P, Gonzalez-Rey E, Delgado M. Ghrelin protects against experimental sepsis by inhibiting high-mobility group box 1 release and by killing bacteria. *J Immunol* 2008; **180**: 8369-8377 [PMID: 18523304 DOI: 10.4049/jimmunol.180.12.8369]
118 **Wu R**, Dong W, Ji Y, Zhou M, Marini CP, Ravikumar TS, Wang P. Orexigenic hormone ghrelin attenuates local and remote organ injury after intestinal ischemia-reperfusion. *PLoS One* 2008; **3**: e2026 [PMID: 18431503 DOI: 10.1371/journal.pone.0002026]
119 **Kasımay O**, Işeri SO, Barlas A, Bangir D, Yeğen C, Arbak S, Yeğen BC. Ghrelin ameliorates pancreaticobiliary inflammation and associated remote organ injury in rats. *Hepatol Res* 2006; **36**: 11-19 [PMID: 16877038 DOI: 10.1016/j.hepres.2006.06.009]
120 **Huang CX**, Yuan MJ, Huang H, Wu G, Liu Y, Yu SB, Li HT, Wang T. Ghrelin inhibits post-infarct myocardial remodeling and improves cardiac function through anti-inflammation effect. *Peptides* 2009; **30**: 2286-2291 [PMID: 19747956 DOI: 10.1016/j.peptides.2009.09.004]
121 **Gonzalez-Rey E**, Chorny A, Delgado M. Therapeutic action of ghrelin in a mouse model of colitis. *Gastroenterology* 2006; **130**: 1707-1720 [PMID: 16697735 DOI: 10.1053/j.gastro.2006.01.041]
122 **Cetin E**, Kanbur M, Cetin N, Eraslan G, Atasever A. Hepatoprotective effect of ghrelin on carbon tetrachloride-induced acute liver injury in rats. *Regul Pept* 2011; **171**: 1-5 [PMID: 21640759 DOI: 10.1016/j.regpep.2011.05.010]

P- Reviewer: Gallego-Duran R, Liu HK, Mascitelli L **S- Editor**: Yu J
L- Editor: AmEditor **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3223

World J Gastroenterol 2015 March 21; 21(11): 3223-3231
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Biomarkers in nonalcoholic fatty liver disease-the emperor has no clothes?

Madhusudana Girija Sanal

Madhusudana Girija Sanal, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States

Author contributions: Sanal MG conceived the issues which formed the content of the manuscript and wrote the manuscript.
Supported by IIP fellowship (2013-2014), Albert Einstein College of Medicine, New York, through the generosity of the Gruss Lipper Family Foundation.
Conflict-of-interest: The author does not have any conflict of interest associated with this manuscript.

Correspondence to: Madhusudana Girija Sanal, MBBS, PhD, IIP Research Fellow, Albert Einstein College of Medicine, Room#323, Ullmann Building, 1300 Morris Park Avenue, Bronx, NY 10461, United States. sanalmg@gmail.com
Telephone: +1-347-3894440
Fax: +1-718-4303099
Received: November 22, 2014
Peer-review started: November 23, 2014
First decision: December 26, 2014
Revised: January 16, 2015
Accepted: February 11, 2015
Article in press: February 11, 2015
Published online: March 21, 2015

Abstract

Fatty liver is present in over ten percentage of the world population and it is a growing public health problem. Nonalcoholic fatty liver disease (NAFLD) is not a single disease, but encompasses a spectrum of diseases of different etiologies. It is difficult to find highly specific and sensitive diagnostic biomarkers when a disease is very complex. Therefore, we should aim to find relevant prognostic markers rather than accurate diagnostic markers which will help to minimize the frequency of liver biopsies to evaluate disease progression. There are several biomarker panels commercially available, however, there is no clear evidence that more sophisticated panels are better compared to simple criteria such as, presence of diabetes over five years, metabolic syndrome, obesity, obstructive sleep apnea, aspartate transaminase/alanine transaminase (ALT) ratio > 0.8 or ferritin levels > 1.5 times normal in patients with over six month history of raised ALT and/or ultrasonological evidence of fat in the liver. Currently the biomarker panels are not a replacement for a liver biopsy. However the need and benefit of liver biopsy in NAFLD is questionable because there is no convincing evidence that biopsy and detailed staging of NAFLD improves the management of NAFLD and benefits the patient. After all there is no evidence based treatment for NAFLD other than management of lifestyle and components of "metabolic syndrome".

Key words: Nonalcoholic fatty liver disease; Biomarkers; Fibrosis; Cirrhosis; Steatohepatitis; Liver biopsy

Core tip: Nonalcoholic fatty liver disease (NAFLD) is not a single disease, but encompasses a spectrum of diseases and this makes it very difficult to find highly specific and sensitive biomarkers. We should therefore aim to find relevant prognostic markers rather than accurate diagnostic markers which will help to minimize the frequency of liver biopsies to evaluate disease progression. There is no evidence that biopsy and detailed staging of NAFLD is important in the NAFLD management and benefits patients. Finally, there is no evidence based treatment for NAFLD other than management of 'metabolic syndrome' by pharmacological or non-pharmacological (lifestyle management/surgical) approaches.

Sanal MG. Biomarkers in nonalcoholic fatty liver disease-the emperor has no clothes? *World J Gastroenterol* 2015; 21(11): 3223-3231 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3223.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3223

INTRODUCTION

Fatty liver can be a sign of an underlying disorder but by itself it is not a disease. Nonalcoholic fatty liver disease (NAFLD) is not a single disease but encompasses a spectrum of diseases. No wonder that efforts to find a highly specific and sensitive biomarker for NAFLD have not become successful. About a quarter of fatty livers develop liver inflammation [nonalcoholic steatohepatitis (NASH)] and over a quarter of NASH patients develop severe fibrosis. We need biomarkers for the excess fat in liver, inflammation and fibrosis of liver. It is less likely that we could find liver specific proteins/molecules which can be used in commercial settings for identifying fat in liver. While there are several markers for inflammation, but it is difficult to find markers which are liver specific but superior to classic liver enzymes such as alanine transaminase (ALT). Similarly, it is difficult to find biomolecules which are specific for fibrosis of liver. We should therefore aim to find relevant prognostic markers rather than accurate diagnostic markers which will help to minimize the frequency of liver biopsies to evaluate disease progression. Despite several years of research, there is no clear evidence in the literature that any of the sophisticated algorithms or proprietary biomarker panels are good enough to avoid a liver biopsy compared to simple criteria such as, presence of diabetes over five years, metabolic syndrome, obesity, obstructive sleep apnea, aspartate transaminase (AST)/ALT ratio > 0.8 or ferritin levels > 1.5 times normal in patients with over six months history of raised ALT and/or ultrasonological evidence of fat in liver. Therefore, "more" is not necessarily 'the better' when it comes to the number of biomarkers, accuracy of diagnosis and staging of NAFLD. Moreover, the performance of biomarkers depends on the etiology of NAFLD and the stage of the disease and compromising their reliability. After all there is no evidence based treatment for NAFLD other than management of lifestyle and components of "metabolic syndrome". There is no convincing evidence that biopsy and detailed staging of NAFLD improves the management of NAFLD and benefits the patients. Appropriate combination of lifestyle adjustments, pharmacological and non-pharmacological (such as bariatric surgery) intervention to improve the underlying cause of NAFLD such as diabetes should be undertaken in all cases of NAFLD with diabetes over five years, metabolic syndrome, obesity, obstructive sleep apnea, AST/ALT ratio > 0.8 or ferritin levels > 1.5 times normal in patients with over six months history of raised ALT and/or ultrasonological evidence of fat in liver.

It is important to detect the development of inflammation in fatty liver because greater than a quarter of these patients develop fibrosis which is associated with a high mortality rate. Detection of inflammation requires microscopic examination of liver biopsy specimens. The diagnosis of nonalcoholic steatohepatitis (inflamed fatty liver) is therefore histological[1-3]. However, liver biopsy is an invasive procedure which involves some serious patient risk and suffers from sampling errors[3]. In association with liver biopsy, various studies have reported mortality as high as 2% in the literature[4]. Though liver biopsy is recommended for therapeutic decisions, clinical practice guidelines for NAFLD have been modified therefore to include noninvasive tests for diagnosis of NASH. The European Association for the Study of the Liver had a special topic conference in NAFLD which showed a renewed interest on noninvasive biomarkers[5]. The prospect of imaging techniques [such as real-time elastography, acoustic radiation force impulse elastography, magnetic resonance spectroscopy and certain magnetic resonance imaging (MRI) based techniques] are currently more promising when compared to the prospect of biomarkers in the evaluation of fibrosis. Many of the non-invasive diagnosis techniques now employed for NAFLD were actually developed for managing chronic hepatitis C. The most important criteria to be evaluated in hepatitis C virus (HCV) and NAFLD are inflammation and progression of fibrosis, the two most important turning points in the course of fatty liver disease progression.

While there are several markers for inflammation only liver enzymes are specific to liver and even few are sufficiently sensitive enough to be a serum biomarker for clinical use. For example cytokeratin-18 (CK-18) is a relatively useful marker to differentiate non-alcoholic steatohepatitis (NASH) from fatty liver without inflammation. However its plasma levels are altered in several inflammatory conditions involving apoptotic response such as chronic viral hepatitis, chronic lung and renal diseases. Therefore, CK-18 is not definitive enough for routine diagnostic use as a marker for staging NASH[6].

This review will focus on the limitations of biomarkers and diagnostic panels presently available in the diagnosis and management of NAFLD. Although tremendous advances are presently being made in non-invasive imaging methods and other non-biomarker based methods inclusive of ultrasound based methods such as transient ultrasound elastography, Doppler analysis, acoustic radiation force impulse (ARFI), real-time elastography, tissue strain imaging, supersonic shear imaging, magnetic resonance based techniques such as MRI, diffusion-weighted MRI, magnetic resonance spectroscopy, X-ray based imaging techniques such as computed tomography (CT) and radioisotope

based imaging techniques such as positron emission tomography and single photon emission computed tomography (SPECT), however they are beyond the scope of this review.

There exists a plethora of panels and scoring systems and plenty of redundancy exists among these tests. We will only consider some of these panels or scoring systems as detailed discussion about these all is also beyond the scope of this review. There are already many good reviews on biomarkers and diagnostic panels used in NAFLD, NASH and fibrosis[7-12].

MicroRNAs are implicated in pathogenesis of NAFLD, however more research is required to confirm and validate their usefulness as diagnostic or prognostic markers to qualify them for clinical use[13].

QUESTION OF BECOMING BETTER THAN THE GOLD STANDARD

An important fact to note is that when we decide the quality of a non-invasive test or biomarker, all non-invasive tests or biomarkers are compared against the "gold standard" and for NASH diagnosis it is liver biopsy. It is well documented that liver biopsy suffers from sample variability and inter-observer variability[3]. It is possible that in a proportion of samples where liver biopsy results were inaccurate but the biomarkers were correct, the comparative performance of bio-marker will be reported inferior despite the reality that they gave superior results.

MARKERS OF INFLAMMATION

Pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), are raised in plasma in NASH patients compared to patients who suffer from fatty liver without inflammation. There are several reports showing strong association between IL-6 and non-alcoholic steatohepatitis (NASH)[14]. However, IL-6 is raised in several inflammatory conditions including insulin resistance and triggers fibrosis in multiple organs[15] IL-6 is not only involved in inflammation and infection responses but also it has anti-inflammatory action, besides, it is also involved in the regulation of metabolic, regenerative, and neural processes[16]. TNF-α level is increased several fold in NASH, however it is also increased in several inflammatory diseases, cancer and infections. Obesity is characterized by increased plasma levels of TNF-α, IL-6 and acute phase reactant proteins like C-reactive protein (CRP). It may be noted that about 70% of adults age twenty years and over are overweight or obese according to Center for Disease Control and Prevention, United States[17]. Pentraxin-related protein (PTX3), also known as TNF-inducible gene 14 (TSG-14) protein is rapidly induced in many cell types, in particular by mononuclear phagocytes, fibroblasts and endothelial cells in response to inflammatory signals such as TNF-α[18]. To be useful, IL-6 and TNF-α, should be sufficiently specific and should be able to distinguish between a fatty liver without inflammation from one with inflammation. The same is true for markers such as CRP, adiponectin, resistin, leptin, visfatin or retinol-binding protein 4 and PTX3. Ferritin is an intracellular protein that binds to iron and releases it in a controlled fashion present in all cells. Ferritin level increases in response to infection and inflammation. Serum ferritin is an independent predictor of advanced hepatic fibrosis among patients with NAFLD[19]. Both inflammation and accumulated fat in liver creates oxidative stress. Partially oxidized fat causes cellular damage and is known to attract leukocytes resulting in inflammatory response. Measurement of oxidative stress therefore is an indirect predictor of inflammation. However, both obesity and diabetes are independently associated with oxidative stress and inflammation[20]. Accumulated fat in liver will undergo slow oxidation inside hepatocytes, generating free radicals which will initiate a cascade of free radical reactions. Several of the stable intermediates and final products of these reactions can be quantified. Products of free radical–mediated oxidation of linoleic acid (9- and 13-hydroxy octadecadienoic acid and 9-13-oxo-octadecadienoic acid) measured in plasma were significantly elevated in NASH patients with reference to patients with fatty liver without inflammation or patients with normal biopsies[21]. Several compounds such as oxidized low density lipoproteins, malonaldehyde, thiobarbituric acid reactive substances (TBARS) or compounds arising from oxidized tyrosine are useful markers of oxidative stress. However they are of limited use in clinical diagnosis or management of NASH[22,23].

The human body has an anti-free radical regimen to counteract oxidative/nitrosative stress which is depleted during chronic free radical stress conditions such as NASH. The degree of depletion of antioxidant components of mammalian systems, such as glutathione (which is considered the main regulator of redox balance) is a reasonable surrogate measure for oxidative stress[24]. However, oxidative/nitrosative stress is now recognized to be a common characteristic of many acute and chronic diseases in addition to the normal aging process[25].

MARKERS OF REPAIR AND REMODELING RESPONSE

Chronic inflammation results in cell death (apoptosis and necrosis) which in turn induces repair and remodeling responses. Liver has enormous regeneration potential[3,7,9] and during this process several biomolecules are released into the bloodstream mainly from damaged/dying cells, tissue matrix, infiltrated immune cells and possibly from regenerating cells. This includes, liver enzymes and other proteins such as aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), α2

macroglobulin (an inhibitor of fibrinolysis), haptoglobin (a protein which binds to free hemoglobin), apolipoprotein A1 (component of high density lipoprotein), tissue inhibitor of metalloproteinase 1 (TIMP1), Chitinase-3-like protein 1 (CHI3L1 also known as YKL-40, is a secreted glycoprotein) and constituents of extracellular matrix such as hyaluronic acid (HA) , laminin ,type Ⅳ collagen 7S domain, Pro-collagen Ⅲ (PⅢNP), procollagen Ⅰ carboxyl terminal peptide (PICP), procollagen Ⅳ C peptide, procollagen Ⅳ N peptide (7-S collagen), cytokeratin 18 (CK-18 or KRT18- a type Ⅰ cytokeratin present in glandular epithelia of the digestive, respiratory and urogenital tracts *etc.*)[7-12].

PRIMARY ETIOLOGICAL MARKERS OF NAFLD AND INDIRECT MARKERS ASSOCIATED WITH DECLINING LIVER FUNCTION AND HEALTH

Type 2 diabetes mellitus and adipose tissue dysfunction results in deposition of fat in liver[3]. Insulin resistance, dyslipidemia and obesity are therefore markers of fatty liver disease. Similarly, dysfunction of other organ systems may result in liver pathology. Liver is a key organ in maintaining good health and liver damage results in secondary damage to other organ systems. Liver damage is associated with changes in platelet values, renal and nervous system pathology. NAFLD is associated with cardiovascular risk and events associated with primary arterial hypertension[1-3].

MICRORNAS AS BIOMARKERS IN NAFLD

Recently, certain microRNAs were implicated in NAFLD, however, the available data is not sufficient to suggest their diagnostic use as markers of steatosis, inflammation or fibrosis. miR-122 and miR-34a levels were positively correlated with disease severity from simple steatosis to steatohepatitis. In both chronic hepatitis C (CHC) and NAFLD patients serum levels of miR-122 and miR-34a correlated with serum lipids, liver enzymes levels, and fibrosis stage and inflammation activity[26]. In a recent study, serum levels of circulating miRNAs, miR-21, miR-34a, miR-122 and miR-451 were found associated with nonalcoholic fatty liver disease and the serum level of miR-122 was correlated with the severity of liver steatosis[27]. Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation but corresponding data from human studies are not presently available or corroborative[28]. In another rat study, Venugopal *et al*[29], reported that liver fibrosis is associated with a down regulation of miRNA-150 and miRNA-194 in hepatic stellate cells and their overexpression causes decreased stellate cell activation. In a study by Alisi *et al*[30] in rats, the miRNAs analysis showed the significant down regulation of three miRNAs, (miR-122, miR-451 and miR-27) and the up regulation of three (miR-200a, miR-200b and miR-429) in high fat diet (standard diet with high fructose and high fat diet combined with high fructose).

NONALCOHOLIC STEATOHEPATITIS DIAGNOSTIC PANELS: THE MORE PARAMETERS THE BETTER?

Although, there exists a variety of scoring systems and panels for evaluating the progression of fatty liver to NASH and cirrhosis exists, none of these markers can be a replacement for liver biopsy. Some of these panels depend on a dozen or more variables to derive the scores while others depend only on three or four parameters (Table 1). Despite the difference in the number of factors and the complexity of the mathematics involved in the biomarker panel development, the difference in efficiency and accuracy in diagnosing and/or staging inflammation and fibrosis that is associated with fatty liver disease is not very much different between these tests (see below).

Brief review on the biomarkers/panels in NAFLD

In a paper published in 2001 Dixon *et al*[31] found that: (1) a raised index of Insulin; Resistance (OR = 9.3); (2) systemic hypertension (OR = 5.2); and (3) raised alanine aminotransferase (OR = 8.6) were independent predictors of NASH. A combination of any two or all three of these predictors showed a sensitivity of 0.8 and specificity of 0.89 for NASH. The accuracy of the test was found by receiver operating characteristic (ROC) analysis. They reported an area under the curve (AUC) equal to 0.90 for the combination of these three predictors[31].

A composite index for distinguishing steatosis from NASH was formulated by Palekar *et al*[32] which included the risk factors, age > 50 years, female gender, AST 45 IU/L, BMI 30 kg/m^2, AST/ALT ratio $\geqslant$ 0.80, and HA $\geqslant$ 55 mcg/L, and its accuracy was determined by ROC analysis to be 0.763. The presence of three or more risk factors had sensitivity and specificity of 73.7% and 65.7% respectively[32].

A commercial panel, the "NashTest" from BioPredictive a French company, combines α2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, fasting blood glucose (FBS), triglycerides (TG), cholesterol, ALT and AST, with parameters adjusted for patient's age, gender, weight and height[33]. According to Thierry Poynard, the inventor of this patented test, the accuracy of NashTest was determined by ROC analysis. The AUC of the "NashTest" for diagnosing NASH in the training and validation groups were, 0.79 and 0.79 (P = 0.94) respectively[34]. Therefore the test result for "NashTest" was not quite as impressive; and we need several independent and international studies to prove the usefulness of the "NashTest".

The "FibroTest" (in the United States it is marketed as "FibroSure") is a hepatic damage score that is

Table 1 Some of these panels depend on a dozen or more variables to derive the scores while others depend only on three or four parameters

Noninvasive test	Parameters	Disease	AUC	Ref.
APRI	AST, platelet count	Fibrosis, cirrhosis in mixed patient population	0.82	Adler *et al*[40], *Hepatology* (2008)
Enhanced liver fibrosis (ELF) test	Hyaluronic acid, tissue inhibitor of matrix metalloproteinase-1, amino terminal propeptide of procollagen type Ⅲ	NAFLD in children	0.92 to 0.99	Nobili *et al*[50], *Gastroenterology* (2009)
		Chronic liver disease	0.80	Nobili *et al*[50], *Gastroenterology* (2009)
HAIR	Hypertension, ALT, IR index	NAFLD	0.90	Dixon *et al*[31], *Gastroenterology* (2001)
NashTest A commercial panel from Biopredictive, France	Alpha2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, fasting glucose, triglycerides, cholesterol, ALT, AST, age, gender, weight, height	NAFLD	0.79	Poynard *et al*[34], *BMC Gastroenterology* (2006)
FIB-4	Platelets, ALT, AST and age	HCV fibrosis	0.76	Vallet-Pichard *et al*[38], *Hepatology* 2006
		NAFLD fibrosis	0.80	Shah *et al*[39], *Clinical Gastroenterology and Hepatology* (2009)
FibroTest/FibroSure A commercial panel from Biopredictive, France	α2-macroglobulin , apolipoprotein A1, haptoglobin, total bilirubin, GGT	NAFLD fibrosis	0.86	Ratziu *et al*[37], *BMC gastroenterology* (2006)
FibroQ	Age, AST, platelet Count, PT-INR	HCV fibrosis (F2-4)	0.783	Hsieh *et al*[41], *Chang Gung Med J* (2009)
Lok index	Platelet count, PT-INR, AST, ALT	HCV fibrosis	0.78	Lok *et al*[42], *Hepatology* (2005)
Forns Score	Age, platelet count, GGT, cholesterol	HCV fibrosis	0.86	Forns X *et al*, *Hepatology* (2002)
		Fibrosis from all causes	0.76	Adler *et al*[40], *Hepatology* (2008)
BARD Score	Body-mass index, AST/ALT ratio, type 2 diabetes mellitus	NAFLD fibrosis	0.67	Ruffillo *et al*[47], *Journal of Hepatology* (2011)
NAFLD fibrosis score	Age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio	NAFLD fibrosis	0.82	Angulo *et al*[45], *Hepatology* (2007)
			0.68	Ruffillo *et al*[47], *Journal of Hepatology* (2011)
Fibrometer	Platelets, prothrombin index, aspartate aminotransferase, α2-macroglobulin (A2M), hyaluronate, urea, and age	Viral and alcoholic chronic liver diseases fibrosis	0.883	Calès *et al*[43], *Hepatology* (2005)
		NAFLD	0.943	Calès *et al*[44], *Journal of Hepatology* (2009)

NashTest, FibroTest/FibroSure. SteatoTest and FibroMax are products from Biopredictive, France. FibroMax is the combination of NashTest, FibroTest/FibroSure and SteatoTest. AUC: Area under the curve; APRI: AST-to-platelet ratio index; AST: Aspartate transaminase; ALT: Alanine transaminase; NAFLD: Nonalcoholic fatty liver disease; GGT: Gamma-glutamyl transpeptidase; HCV: Hepatitis C virus.

useful in a variety of diseases involving the liver. It is derived from age, gender and five serum markers[33]. The markers are α-2-macroglobulin, haptoglobin, apolipoprotein a1 (APOA1), GGT, total bilirubin. ALT is used in another sub-test called ActiTest, for measuring necro inflammatory activity in patients with chronic hepatitis C or B. The patented formula for calculating the FibroTest score logistic regression coefficient is the following or a variant which can be found in the public domain[35].

The "FibroTest" is well standardized, reproducible and commercially available. According to a report by Imbert-Bismut *et al*[36] the impact of parameter analytical variability on Fibrotest and Actitest results was less than 10% and intra-patient reproducibility was within acceptable limits[36]. FibroTest was evaluated in two groups, group 1 from a reference center and group 2 was a multicenter study. The ROCs for the diagnosis of advanced fibrosis (F2F3F4): 0.86 in group 1 and 0.75 in group 2[37].

Biopredictive also offers the "SteatoTest" which combines α 2-macroglobulin, haptoglobin, APOA1, total bilirubin, GGT, fasting glucose, triglycerides, cholesterol and ALT, parameters adjusted for patient's age, gender, weight and height according to the company's website. Fibromax is the combination of FibroTest, SteatoTest and NashTest, available from the same company, "Biopredictive"[33].

FIB-4 is "an inexpensive and accurate marker of fibrosis in HCV infection in comparison with liver biopsy and Fibrotest" according to Vallet-Pichard *et al*[38] in a paper published in 2006. FIB-4, depends common clinical parameters-platelets, ALT, AST and age. According to the authors, "FIB-4 value < 1.45 or > 3.25 (64.6% of the cases) was concordant with the FibroTest results in 92.1% and 76%, respectively" and AUC was 0.76. A 2009 study by Shah *et al*[39] compared the performance of the FIB4 index with six other non-invasive markers of fibrosis in patients with NAFLD. They found that the FIB4 index is superior to the other noninvasive markers of fibrosis in patients with NAFLD [the AUC was greatest for FIB4 (AUC = 0.802)]. The authors however highlighted the need for even better noninvasive markers for NAFLD.

The AST-to-platelet ratio index (APRI) was developed as a simple, easy to use method in clinics to predict, severe fibrosis or cirrhosis in both HCV mono-infected and co-infected (HCV and HIV) patients. According to a meta-analysis of twenty two studies with 4,266 subjects, the summary AUCs of the APRI for significant fibrosis and cirrhosis were 0.76 and 0.82, respectively. For significant fibrosis, an APRI threshold of 0.5 was 81% sensitive and 50% specific. The Forns Index is mathematically derived from four simple parameters, age, GGT, cholesterol and platelet count. This index is best studied in HCV related fibrosis and it is useful with AUC of 0.750 and 0.760 respectively for the prediction of significant fibrosis (F/S2-4) in HCV and fibrosis from all causes. Comparable values for FibroTest are AUC of 0.794 and 0.800 respectively[40].

AST level, platelet count and prothrombin time (PT) international normalized ratio (INR) and the at onset are the variables considered in "FibroQ", another test for predicting fibrosis in HCV developed by a group in Taiwan in 2009. According to these investigators, FibroQ performed better than APRI, but was similar to ALT/AST ratio, in the prediction of significant fibrosis (it was possible to distinguish between patients with or without fibrosis in 77% of the patient population)[41].

Lok *et al*[42] proposed another simple formula for predicting fibrosis in chronic hepatitis C (CHC). The Lok index was based on platelet count, PT-INR, serum AST and ALT levels. Lok *et al*[42] studied a cohort of 1141 patients with CHC and reported an AUROC of 0.78-0.81 to detect cirrhosis. Calès *et al*[43] in 2005 reported a test which they named the "Fibrometer" to characterize different fibrosis parameters in viral and alcoholic chronic liver diseases. This test is based on the values platelets, prothrombin index, aspartate aminotransferase, α2-macroglobulin, hyaluronate, urea, and age. The AUC for Fibrometer was 0.883 compared with 0.808 for the Fibrotest. Recently the same group used Fibrometer to measure fibrosis in NAFLD. They found that it was superior to NAFLD fibrosis score (NFSA) and APRI. AUC for Fibrometer was 0.943 and for NFSA and APRI the values were 0.884 and 0.866, respectively[44]. The NAFLD fibrosis score was introduced by Angulo *et al*[45] in 2007 and includes routine clinical/lab variables such as age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio. This scoring was efficient in predicting fibrosis and had an AUC of 0.82 in the validation group. Harrison *et al*[46] proposed an index, referred to as the BRAD score, which included- body-mass index (BMI), AST/ALT ratio (AAR), and presence of type 2 diabetes mellitus. They scored these variables as follows-BMI $\geqslant$ 28 kg/m^2 = 1 point, BMI < 28 kg/m^2 = 0 point; AST/ALT ratio $\geqslant$ 0.8 = 2 points, AST/ALT ratio < 0.8 = 0 points; freshly recognized or preexisting diabetes = 1 point. A total of 2-4 points meant significant fibrosis[46]. Ruffillo *et al*[47] evaluated the diagnostic accuracy of this score in NAFLD patients and concluded that this score is useful in identifying patients without severe fibrosis.

A total of 2411 patients with compensated CLD (HCV = 75.1%, HBV = 10.5%, NASH = 7.9%, HIV/HCV = 6.5%) were evaluated by APRI, Forns index, Lok index, AST/ALT ratio, Fib-4, platelets and Fibrotest-Fibrosure against liver biopsy, in a multicenter study. This study concluded that the diagnostic performance is better for significant fibrosis for CHC compared with NAFLD patients, but accuracy was relatively poor among CHC patients with ALT[48].

Enhanced liver fibrosis (ELF) is a modified version of the original European Liver Fibrosis panel[49]. The original panel includes hyaluronic acid, tissue inhibitor of matrix metalloproteinases-1, amino terminal propeptide of procollagen type Ⅲ (which are involved in the synthesis and degradation of extracellular matrix) and age. Later the parameter "age" was removed from the panel establishing the enhanced liver fibrosis (ELF) test[49,50]. The test was effective in predicting NAFLD in children (AUC ranging from 0.92 to 0.99, from fibrosis stage 1 to stage 3)[50].

HOW IMPORTANT IS EXACT STAGE INFORMATION IN THE MANAGEMENT OF NAFLD?

It seems, rather than patient management, exact stage information is more important in academic research and in clinical trials (especially where different drugs are being tried on a limited number of patients). There may be only subtle differences between some of these drugs, which can only be better identified if there exists a good scoring system to evaluate the progress or regression of steatohepatitis.

The appearance and persistence of inflammation is an important turning point in the history of fatty liver disease. The presence of inflammation in "fatty liver" needs to be taken quite seriously because it can progress to fibrosis depending on the patient's genome and epigenome over time[1-4]. The major deficiency of most of the panels is the inability to identify this critical point effectively. Current panels are not reliable in distinguishing fatty liver disease from NASH accurately, although they are good at deciding fibrosis. Identification of fatty liver disease is important because of the associated liver, cardiovascular and cerebrovascular risk[3,51]. However when it comes to the disease staging, it is hitherto not clear whether accurate staging of the disease has a role in the management and what is its implication in practice.

The usefulness of accurate staging and grading of steatosis, inflammation and fibrosis in the management of NAFLD is controversial because of the following reasons. Firstly, pharmacological treatment is not warranted for simple fatty liver (fatty liver without inflammation). Secondly, there are no approved drugs for NASH[5]. Finally, to date, anti-fibrotic treatment

of fibrosis represents, an unsuccessful area, by and large, for drug development[52]. Currently therapy for NAFLD aims at achieving good control of diabetes, hypertension and body mass in diabetic, hypertensive and overweight/obese NASH patients through pharmacological, surgical or non-pharmacological methods such as lifestyle modification. There is no clear "evidence-based treatment" for NAFLD[53,54]. A literature search, didn't reveal to date, any definite guidelines from professional organizations other than what is described (vide-supra) for management of inflammation and fibrosis associated with NAFLD in a stage specific manner. It is therefore difficult to decide the usefulness of staging information on steatosis, inflammation and fibrosis in the currently available treatment methods for NAFLD. This implies, as far as treatment and benefit to the patients is concerned, small differences in efficiency (calculated often in terms of AUC by ROC analysis) between sophisticated, proprietary and costly/commercial tests and scoring algorithms versus simple, inexpensive, easily available non-proprietary tests and scoring systems may be insignificant (Table 1). A simple criteria such as presence of diabetes over five years, metabolic syndrome, obesity, obstructive sleep apnea, AST/ALT ratio > 0.8 or ferritin levels > 1.5 times normal in patients with over six months history of raised ALT and/or ultrasonological evidence of fat in liver would identify patients who need special care and personalized treatment depending on the comorbidities and etiology of NAFLD.

CONCLUSION

Despite the extensive research, development and investment in the field of biomarkers for NAFLD, it is doubtful how much benefit this has brought to the patients. Commercial panels and scoring systems have not improved upon the simpler, widely available, cost effective tests and clinical parameters and they offer little benefit in the management of NAFLD. The performance to date of biomarkers depends very much on the patient, the etiology of NAFLD and the stages of the disease and cannot be considered as a replacement for liver biopsy. Biomarkers, therefore, should serve as a tool to optimize the selection of patients with NAFLD for liver biopsy. There is no clear evidence that liver biopsy and detailed staging of the disease significantly influences the management decisions and benefits the patient. After all, there is no "evidence based medicine" for NAFLD except the management of associated morbidities such as components of the "metabolic syndrome" or (the largely symptomatic management) of cirrhosis.

ACKNOWLEDGMENTS

The manuscript was edited by Mr. Alan Alfieri and proof read by both A.Alfieri and R.Khan.

REFERENCES

1 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]

2 **Sanal MG**. The blind men 'see' the elephant-the many faces of fatty liver disease. *World J Gastroenterol* 2008; **14**: 831-844 [PMID: 18240340 DOI: 10.3748/wjg.14.831]

3 **Sanal MG**. Nonalcoholic fatty liver disease: the concept and confusion. *Minerva Gastroenterol Dietol* 2011; **57**: 419-426 [PMID: 22105730]

4 **Thampanitchawong P**, Piratvisuth T. Liver biopsy: complications and risk factors. *World J Gastroenterol* 1999; **5**: 301-304 [PMID: 11819452]

5 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384 [PMID: 20494470 DOI: 10.1016/j.jhep.2010.04.008]

6 **Cusi K**, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, Ortiz-Lopez C, Hecht J, Feldstein AE, Webb A, Louden C, Goros M, Tio F. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **60**: 167-174 [PMID: 23973932 DOI: 10.1016/j.jhep.2013.07.042]

7 **Parsian H**, Alizadeh M, Yahyapour Y. Clinical Application of Non-Invasive Markers of Liver Fibrosis. InTechOpen: Published on, 2013

8 **Patel K**, Shackel NA. Current status of fibrosis markers. *Curr Opin Gastroenterol* 2014; **30**: 253-259 [PMID: 24671009 DOI: 10.1097/MOG.0000000000000059]

9 **Pearce SG**, Thosani NC, Pan JJ. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. *Biomark Res* 2013; **1**: 7 [PMID: 24252302]

10 **Lee HH**, Seo YS, Um SH, Won NH, Yoo H, Jung ES, Kwon YD, Park S, Keum B, Kim YS, Yim HJ, Jeen YT, Chun HJ, Kim CD, Ryu HS. Usefulness of non-invasive markers for predicting significant fibrosis in patients with chronic liver disease. *J Korean Med Sci* 2010; **25**: 67-74 [PMID: 20052350 DOI: 10.3346/jkms.2010.25.1.67]

11 **Castera L**, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 666-675 [PMID: 24061203]

12 **Vizzutti F**, Arena U, Nobili V, Tarquini R, Trappoliere M, Laffi G, Marra F, Pinzani M. Non-invasive assessment of fibrosis in non-alcoholic fatty liver disease. *Ann Hepatol* 2009; **8**: 89-94 [PMID: 19502649]

13 **Cheung O**, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, Kellum JM, Min H, Luketic VA, Sanyal AJ. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. *Hepatology* 2008; **48**: 1810-1820 [PMID: 19030170 DOI: 10.1002/hep.22569]

14 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379 [PMID: 18510618 DOI: 10.1111/j.1572-0241.2007.01774.x]

15 **Fielding CA**, Jones GW, McLoughlin RM, McLeod L, Hammond VJ, Uceda J, Williams AS, Lambie M, Foster TL, Liao CT, Rice CM, Greenhill CJ, Colmont CS, Hams E, Coles B, Kift-Morgan A, Newton Z, Craig KJ, Williams JD, Williams GT, Davies SJ, Humphreys IR, O'Donnell VB, Taylor PR, Jenkins BJ, Topley N, Jones SA. Interleukin-6 signaling drives fibrosis in unresolved inflammation. *Immunity* 2014; **40**: 40-50 [PMID: 24412616 DOI: 10.1016/j.immuni.2013.10.022]

16 **Scheller J**, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* 2011; **1813**: 878-888 [PMID: 21296109 DOI: 10.1016/j.bbamcr.2011.01.034]

17 **CDC**. National Center for Health Statistics. Available from: URL: http://www.cdc.gov/nchs/fastats/obesity-overweight.htm accessed

9/30/2014
18 PTX3 pentraxin 3, long [Homo sapiens (human)]. Available from: URL: http://www.ncbi.nlm.nih.gov/gene/5806accessedon9/30/2014
19 **Kowdley KV**, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, Sanyal AJ, Nelson JE. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 77-85 [PMID: 21953442 DOI: 10.1002/hep.24706]
20 **Matsuda M**, Shimomura I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obes Res Clin Pract* 2013; **7**: e330-e341 [PMID: 24455761 DOI: 10.1016/j.orcp.2013.05.004]
21 **Feldstein AE**, Lopez R, Tamimi TA, Yerian L, Chung YM, Berk M, Zhang R, McIntyre TM, Hazen SL. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *J Lipid Res* 2010; **51**: 3046-3054 [PMID: 20631297]
22 **Yesilova Z**, Yaman H, Oktenli C, Ozcan A, Uygun A, Cakir E, Sanisoglu SY, Erdil A, Ates Y, Aslan M, Musabak U, Erbil MK, Karaeren N, Dagalp K. Systemic markers of lipid peroxidation and antioxidants in patients with nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2005; **100**: 850-855 [PMID: 15784031 DOI: 10.1111/j.1572-0241.2005.41500.x]
23 **Chalasani N**, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2004; **99**: 1497-1502 [PMID: 15307867 DOI: 10.1111/j.1572-0241.2004.30159.x]
24 **Sánchez-Gómez FJ**, Espinosa-Díez C, Dubey M, Dikshit M, Lamas S. S-glutathionylation: relevance in diabetes and potential role as a biomarker. *Biol Chem* 2013; **394**: 1263-1280 [PMID: 24002664 DOI: 10.1515/hsz-2013-0150]
25 **Dalle-Donne I**, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006; **52**: 601-623 [PMID: 16484333 DOI: 10.1373/clinchem.2005.061408]
26 **Cermelli S**, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS One* 2011; **6**: e23937 [PMID: 21886843]
27 **Yamada H**, Suzuki K, Ichino N, Ando Y, Sawada A, Osakabe K, Sugimoto K, Ohashi K, Teradaira R, Inoue T, Hamajima N, Hashimoto S. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. *Clin Chim Acta* 2013; **424**: 99-103 [PMID: 23727030 DOI: 10.1016/j.cca.2013.05.021]
28 **Ji J**, Zhang J, Huang G, Qian J, Wang X, Mei S. Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation. *FEBS Lett* 2009; **583**: 759-766 [PMID: 19185571 DOI: 10.1016/j.febslet.2009.01.034]
29 **Venugopal SK**, Jiang J, Kim TH, Li Y, Wang SS, Torok NJ, Wu J, Zern MA. Liver fibrosis causes downregulation of miRNA-150 and miRNA-194 in hepatic stellate cells, and their overexpression causes decreased stellate cell activation. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G101-G106 [PMID: 19892940 DOI: 10.1152/ajpgi.00220.2009]
30 **Alisi A**, Da Sacco L, Bruscalupi G, Piemonte F, Panera N, De Vito R, Leoni S, Bottazzo GF, Masotti A, Nobili V. Mirnome analysis reveals novel molecular determinants in the pathogenesis of diet-induced nonalcoholic fatty liver disease. *Lab Invest* 2011; **91**: 283-293 [PMID: 20956972]
31 **Dixon JB**, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; **121**: 91-100 [PMID: 11438497 DOI: 10.1053/gast.2001.25540]
32 **Palekar NA**, Naus R, Larson SP, Ward J, Harrison SA. Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2006; **26**: 151-156 [PMID: 16448452 DOI: 10.1111/j.1478-3231.2005.01209.x]
33 **NashTest**. The NashTest is diagnostic for non-alcoholic steato hepatitis (NASH) in patients with metabolic steatosis (overweight, diabetes, hyperlipidemia). Available from: URL: http://www.biopredictive.com/services/tests-OLD/nashtest/nashtest-en/view?set_language=enaccessedon10/06/2014
34 **Poynard T**, Ratziu V, Charlotte F, Messous D, Munteanu M, Imbert-Bismut F, Massard J, Bonyhay L, Tahiri M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholo steato hepatitis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 34 [PMID: 17096854]
35 **Poynard T**, inventor; Assistance Publique-Hopitaux De Paris (Ap-Hp), assignee. Diagnosis method of inflammatory, fibrotic or cancerous disease using biochemical markers. United States patent US: 6631330, 2003 Oct 7
36 **Imbert-Bismut F**, Messous D, Thibault V, Myers RB, Piton A, Thabut D, Devers L, Hainque B, Mercadier A, Poynard T. Intra-laboratory analytical variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) and reference ranges in healthy blood donors. *Clin Chem Lab Med* 2004; **42**: 323-333 [PMID: 15080567 DOI: 10.1515/CCLM.2004.058]
37 **Ratziu V**, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L, Tahiri M, Munteanu M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V, Poynard T. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 6 [PMID: 16503961]
38 **Vallet-Pichard A**, Mallet V, Pol S. FIB-4: a simple, inexpensive and accurate marker of fibrosis in HCV-infected patients. *Hepatology* 2006; **44**: 769; author reply 769-770 [PMID: 16941681 DOI: 10.1002/hep.21334]
39 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]
40 **Adler M**, Gulbis B, Moreno C, Evrard S, Verset G, Golstein P, Frotscher B, Nagy N, Thiry P. The predictive value of FIB-4 versus FibroTest, APRI, FibroIndex and Forns index to noninvasively estimate fibrosis in hepatitis C and nonhepatitis C liver diseases. *Hepatology* 2008; **47**: 762-773; author reply 763 [PMID: 18220307 DOI: 10.1002/hep.22085]
41 **Hsieh YY**, Tung SY, Lee IL, Lee K, Shen CH, Wei KL, Chang TS, Chuang CS, Wu CS, Lin YH. FibroQ: an easy and useful noninvasive test for predicting liver fibrosis in patients with chronic viral hepatitis. *Chang Gung Med J* 2009; **32**: 614-622 [PMID: 20035640]
42 **Lok AS**, Ghany MG, Goodman ZD, Wright EC, Everson GT, Sterling RK, Everhart JE, Lindsay KL, Bonkovsky HL, Di Bisceglie AM, Lee WM, Morgan TR, Dienstag JL, Morishima C. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 2005; **42**: 282-292 [PMID: 15986415 DOI: 10.1002/hep.20772]
43 **Calès P**, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konaté A, Gallois Y, Ternisien C, Chevailler A, Lunel F. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005; **42**: 1373-1381 [PMID: 16317693]
44 **Calès P**, Lainé F, Boursier J, Deugnier Y, Moal V, Oberti F, Hunault G, Rousselet MC, Hubert I, Laafi J, Ducluzeaux PH, Lunel F. Comparison of blood tests for liver fibrosis specific or not to NAFLD. *J Hepatol* 2009; **50**: 165-173 [PMID: 18977552]
45 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509]
46 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]
47 **Ruffillo G**, Fassio E, Alvarez E, Landeira G, Longo C, Domínguez

N, Gualano G. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. *J Hepatol* 2011; **54**: 160-163 [PMID: 20934232 DOI: 10.1016/j.jhep.2010.06.028]

48 **Sebastiani G**, Castera L, Halfon P, Pol S, Mangia A, Di Marco V, Pirisi M, Voiculescu M, Bourliere M, Alberti A. The impact of liver disease aetiology and the stages of hepatic fibrosis on the performance of non-invasive fibrosis biomarkers: an international study of 2411 cases. *Aliment Pharmacol Ther* 2011; **34**: 1202-1216 [PMID: 21981787 DOI: 10.1111/j.1365-2036.2011.04861.x]

49 **Parkes J**, Guha IN, Roderick P, Harris S, Cross R, Manos MM, Irving W, Zaitoun A, Wheatley M, Ryder S, Rosenberg W. Enhanced Liver Fibrosis (ELF) test accurately identifies liver fibrosis in patients with chronic hepatitis C. *J Viral Hepat* 2011; **18**: 23-31 [PMID: 20196799 DOI: 10.1111/j.1365-2893.2009.01263.x]

50 **Nobili V**, Parkes J, Bottazzo G, Marcellini M, Cross R, Newman D, Vizzutti F, Pinzani M, Rosenberg WM. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 160-167 [PMID: 18992746 DOI: 10.1053/j.gastro.2008.09.013]

51 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

52 **Schuppan D**, Kim YO. Evolving therapies for liver fibrosis. *J Clin Invest* 2013; **123**: 1887-1901 [PMID: 23635787 DOI: 10.1172/JCI66028]

53 **de Alwis NM**, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. *J Hepatol* 2008; **48** Suppl 1: S104-S112 [PMID: 18304679 DOI: 10.1016/j.jhep.2008.01.009]

54 **Chitturi S**, Farrell GC, Hashimoto E, Saibara T, Lau GK, Sollano JD. Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. *J Gastroenterol Hepatol* 2007; **22**: 778-787 [PMID: 17565630 DOI: 10.1111/j.1440-1746.2007.05001.x]

P- Reviewer: Fierbinteanu-Braticevici C, Gong ZJ, Roncucci L
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3232

World J Gastroenterol 2015 March 21; 21(11): 3232-3238
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Mitochondrial uncoupling protein 2 and pancreatic cancer: A new potential target therapy

Massimo Donadelli, Ilaria Dando, Elisa Dalla Pozza, Marta Palmieri

Massimo Donadelli, Ilaria Dando, Elisa Dalla Pozza, Marta Palmieri, Department of Life and Reproduction Sciences, Biochemistry Section, University of Verona, 37134 Verona, Italy

Author contributions: All authors contributed to the conceptual design of the manuscript and data interpretation; Donadelli M reviewed and summarized aspects of the literature that formed the basis of the manuscript; Palmieri M and Dando I carefully revised the manuscript; Dando I and Dalla Pozza E created illustrations; all authors also provided critical analysis of the discussed literature and gave final approval of the manuscript.

Supported by grants from Associazione Italiana Ricerca Cancro, Milan, Italy; Fondazione CariPaRo, Padova, Italy; and Ministero dell'Istruzione, dell'Università e della Ricerca, Rome, Italy.

Conflict-of-interest: The authors declare that they have no conflict of interest

Correspondence to: Massimo Donadelli, PhD, Professor, Department of Life and Reproduction Sciences, Biochemistry Section, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy. massimo.donadelli@univr.it
Telephone: +39-45-8027281
Fax: +39-45-8027170

Received: October 14, 2014
Peer-review started: October 14, 2014
First decision: December 11, 2014
Revised: December 18, 2014
Accepted: February 5, 2015
Article in press: February 5, 2015
Published online: March 21, 2015

Abstract

Overall 5-years survival of pancreatic cancer patients is nearly 5%, making this cancer type one of the most lethal neoplasia. Furthermore, the incidence rate of pancreatic cancer has a growing trend that determines a constant increase in the number of deceases caused by this pathology. The poor prognosis of pancreatic cancer is mainly caused by delayed diagnosis, early metastasis of tumor, and resistance to almost all tested cytotoxic drugs. In this respect, the identification of novel potential targets for new and efficient therapies should be strongly encouraged in order to improve the clinical management of pancreatic cancer. Some studies have shown that the mitochondrial uncoupling protein 2 (UCP2) is over-expressed in pancreatic cancer as compared to adjacent normal tissues. In addition, recent discoveries established a key role of UCP2 in protecting cancer cells from an excessive production of mitochondrial superoxide ions and in the promotion of cancer cell metabolic reprogramming, including aerobic glycolysis stimulation, promotion of cancer progression. These observations together with the demonstration that UCP2 repression can synergize with standard chemotherapy to inhibit pancreatic cancer cell growth provide the molecular rationale to consider UCP2 as a potential therapeutic target for pancreatic cancer. In this editorial, recent advances describing the relationship between cancer development and mitochondrial UCP2 activity are critically provided.

Key words: Uncoupling protein 2; Target therapy; Reactive oxygen species; Metabolism; Pancreatic cancer

Core tip: The dramatic poor prognosis of pancreatic cancer forces towards the identification of novel efficient therapeutic targets against this neoplasia. Overexpression of uncoupling protein 2 (UCP2) and its functional involvement in cancer development, reactive oxygen species production, and cancer metabolic reprogramming may represent the rationale and the

starting point for future drug design projects focused on the identification of specific UCP2 inhibitors as innovative therapeutic tool against pancreatic cancer.

Donadelli M, Dando I, Dalla Pozza E, Palmieri M. Mitochondrial uncoupling protein 2 and pancreatic cancer: A new potential target therapy. *World J Gastroenterol* 2015; 21(11): 3232-3238 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3232.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3232

INTRODUCTION

Pancreatic cancer (PC) ranks amongst the most lethal cancers and has a mortality rate that nearly equals the incidence rate and an overall 5-years survival of approximately 5%[1,2]. Dismally, its mortality rate has been increasing in the last years with a prediction for next years having the same trend. In contrast, in the last decades, an overall reduction in cancer-related mortality in Western countries has been observed for lung, breast, colorectal, and prostate cancers[3]. The reduced cancer mortality for the latter tumors is likely the result of several strategies, including development of early detection, prevention programs, and the discovery of new therapeutic targets and drugs. In the case of PC, because of its low incidence, population-based screening has been considered not feasible[4]. Indeed, the worldwide incidence of all the types of pancreatic cancers (85% of which are adenocarcinomas) ranges from 1 to 10 cases per 100000 people and is generally higher in developed countries and among men. Furthermore, PC has been classified as the eighth leading cause of death for cancer in men and the ninth in women[5]. Pancreatic adenocarcinoma (PDAC), the most aggressive and frequent PC, possesses a variety of hallmarks that include: (1) high rate of KRAS activating mutations; (2) progression from distinct types of precursor lesions; (3) propensity for both local invasion and distant metastasis; (4) extensive stromal reaction (desmoplasia) resulting in a hypovascular and hypoxic microenvironment; (5) reprogramming of cellular metabolism; and (6) tumor immune evasion[6]. More than 90% of all grades pancreatic intraepithelial neoplasia possess KRAS mutations[7]. Instead, the mutational inactivation of the CDKN2A, p53, and SMAD4 tumor suppressors has been detected with increasing frequency in type Ⅱ and type Ⅲ lesions of pancreatic intraepithelial neoplasia, suggesting that they may represent rate-limiting events for tumor progression, while KRAS mutations would contribute to its inception[8]. The epidermal growth factor receptor, the nuclear factor κB, the antiapoptotic protein Bcl-xL, and mitogen-activated protein kinase pathways have also been shown to contribute to KRAS-mediated pancreatic adenocarcinoma, suggesting alternative combinatorial tumorigenic strategies[9-11].

Several efforts made to identify pancreatic tumor biomarkers[12,13] have brought to the identification of the carcinoembryonic antigen and the carbohydrate antigen 19-9, which, however, are considered low sensitive and specific for screening pancreatic cancer at early stages. Despite these advances, more than 90% of patients who have received a diagnosis of pancreatic cancer die from the disease as a result of extensive metastasis (70%) or of bulky primary tumors with limited metastatic disease (30%)[14]. Thus, delayed diagnosis, early metastasis, and resistance to almost all the classes of cytotoxic drugs are considered the main causes of the extremely poor prognosis of PC. For all these reasons, research is now focused on the identification of new prognostic and diagnostic biomarkers and efficient therapeutic targets in order to improve the clinical management of PC. In this respect, we here provide critical comments on the possible usage of the antioxidant mitochondrial uncoupling protein 2 (UCP2) as a new potential target for PC treatment. Several studies have indeed shown that UCP2 is broadly over-expressed in various cancer types and its over-expression is strictly related with the regulation of reactive oxygen species (ROS) and cell metabolism (including autophagy), both processes known to be generally altered in cancer cells.

Uncoupling protein superfamily

Mitochondrial ATP production occurs by coupling the electron transport chain (ETC) with the phosphorylation of ADP into ATP, the so-called oxidative phosphorylation. These two processes are not always efficiently coupled, mainly because of the presence in the inner membrane of mitochondrial transporters, such as uncoupling proteins (UCPs). The UCPs belong to the superfamily of anion transport carriers of the mitochondrial inner membrane[15] and some of them are involved in thermogenesis and regulation of mitochondrial ROS. UCP1 was first discovered and cloned in 1986[16] and is involved in the non-shivering thermogenesis activity of brown adipose tissue (BAT)[17]. Since then, the discovery of UCPs has grown rapidly, UCP1 homologues being found across mammalian species (UCP2 and UCP3) but also in other eukaryotes from plants to animals[18,19]. UCP1, UCP2, and UCP3 are thought to differ in the nature of their uncoupling activity[20,21] and of their potential physiological roles[22]. A rapid overview of data collected on UCP1, 2 and 3 highlights how these proteins differ from each other. First, while UCP1 tissue expression is localized and abundant in BAT, UCP2 has been found in several tissues, including liver, brain, pancreas, adipose tissue, immune cells, spleen, kidney, and the central nervous system[23-25], and UCP3 is mainly present in the skeletal muscle[18]. Also, the physiological role of UCP1 is restricted to thermogenesis, which is unlikely to be the role for UCP2 and 3, as shown by their respective knock-out models[26,27]. UCP2 and 3 have

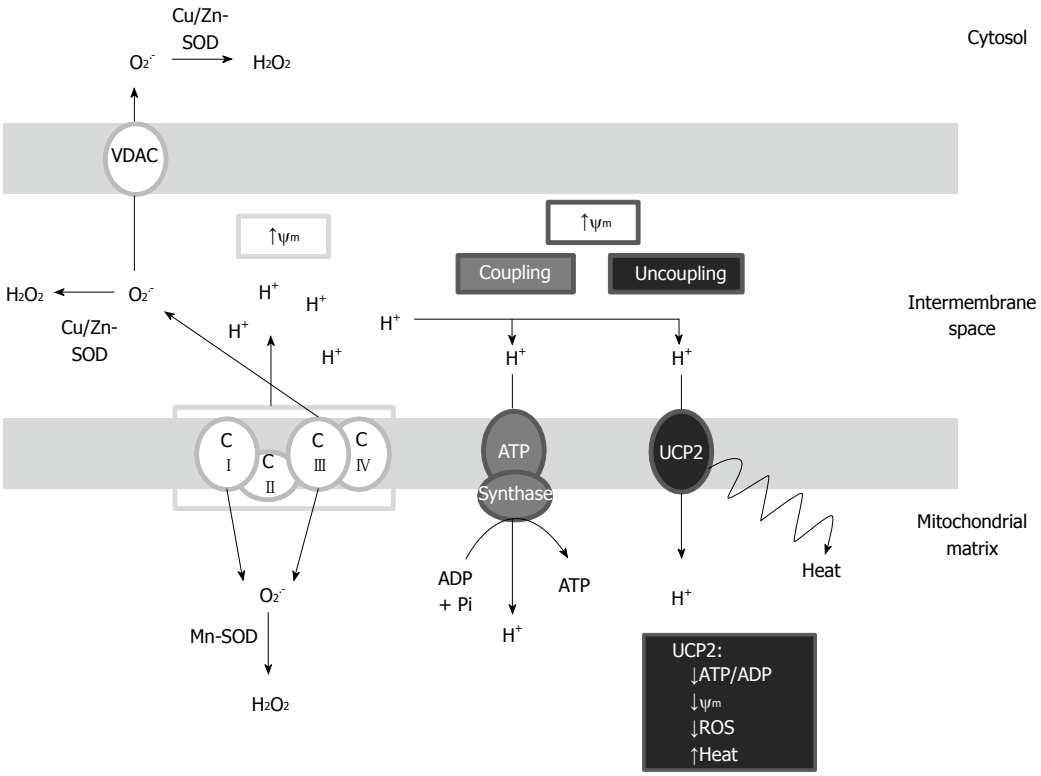


Figure 1 Uncoupling protein 2 uncoupling activity in oxidative phosphorylation. ROS: Reactive oxygen species; UCP2: Uncoupling protein 2; SOD: Superoxide dismutase; Mn-SOD: Manganese-superoxide dismutase.

been involved in a number of postulated functions in energy regulation, including regulation of insulin secretion[28] or ROS production and control of the immune response[26]. The other two members of the UCP superfamily, UCP4 and UCP5, are expressed in a tissue-specific manner and are involved in mitochondrial membrane potential reduction[29].

UCP2 and reactive oxygen species

The cellular antioxidant systems include a large set of enzymes and low-molecular-weight compounds that sequester excessively generated ROS or prevent their production by aerobic respiration. Some antioxidant systems can be energetically expensive because of their dependence on both ATP and NADPH usage. The UCP system represents an acute and energetically costly mechanism to decrease ROS production in mitochondria[30]. Indeed, as shown in Figure 1, the uncoupling of oxidative phosphorylation is a short circuit in which the transport of protons from the intermembrane space to the matrix bypasses ATP synthase resulting in a decrease of: (1) mitochondrial inner membrane potential; (2) leakage of electrons from ETC; and (3) consequently, ROS generation. The existence of a strong correlation between mitochondrial membrane potential and ROS production is well known[31]. Minor increases in membrane potential induce ROS formation, whereas slight decreases can substantially diminish their production, without greatly lowering the efficiency of oxidative phosphorylation. Hence, the mild uncoupling of mitochondrial oxidative phosphorylation may represent the first line of defense against oxidative stress[32]. According to this pattern, UCP2 can dissipate the proton gradient to prevent the proton-motive force from becoming excessive, thus decreasing ROS produced by electron transport[33]. Overall, it is estimated that 0.2%-2% of the O_2 consumed in mitochondria is reduced to superoxide by electron leakage. Mitochondrial superoxide ion is considered the initial and leading molecule of ROS signaling and is generally converted into hydrogen peroxide (H_2O_2) by superoxide dismutases. In addition, upon reaction with H_2O superoxide ion can generate hydroxyl radicals ($^{\bullet}HO$) implicated in lipid damage and protein oxidation[34,35]. The electron leakage causing superoxide production can occur both at complex Ⅰ (CⅠ) and complex Ⅲ (CⅢ) of the respiratory chain[36]: at CⅠ, superoxide has been shown to be exclusively directed toward the mitochondrial matrix and converted into membrane-permeable H_2O_2 by manganese-superoxide dismutase (Mn-SOD), while at CⅢ, superoxide is released to both the matrix and the intermembrane space where it can be converted into H_2O_2 by copper/zinc-superoxide dismutase (Cu/Zn-SOD) (Figure 1)[37]. In addition, some evidence indicates that mitochondrial matrix-directed superoxide can be released from mitochondria through voltage dependent anion channels causing an increase of cytosolic ROS[38]. Therefore, UCP2 acts as a sensor of mitochondrial oxidative stress and

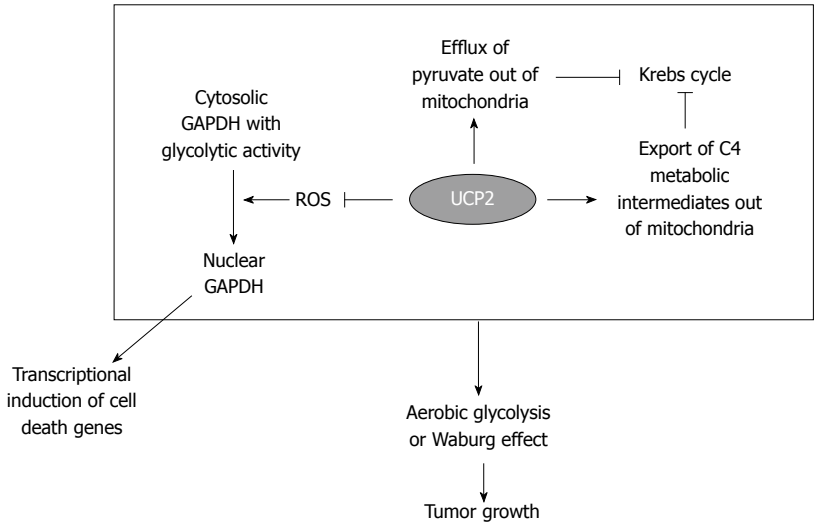


Figure 2 The regulation of cancer metabolism by uncoupling protein 2. ROS: Reactive oxygen species; UCP2: Uncoupling protein 2.

constitutes an important component of local feedback mechanisms generally implicated in cyto-protective activities controlling the production of mitochondrial ROS and regulating redox-sensitive cytosolic signaling pathways.

UCP2 and cancer metabolism

In 1956, Warburg *et al*[39] proposed that cancer was caused by defects in mitochondria, forcing cells to shift to energy production through glycolysis despite aerobic conditions. This characteristic of cancers is described as the "Warburg effect." Warburg statement is based on the observation that the irreversible injury to mitochondrial respiration is followed by a long fight for existence in which a part of the cells perishes for lack of energy while another part succeeds in replacing the lost respiration energy by developing aerobic glycolysis. The Warburg effect, considered now a hallmark of cancer, plays an important role in the growth of tumors, including gastrointestinal cancers, by remodeling the metabolic profile in order to allow tumor cell survival under adverse conditions[40]. More recently, some scientists tried to create a cellular model of the Warburg effect by developing an epithelial cell line lacking mitochondrial DNA (rho^0)[41]. Among the regulated genes, UCP2 expression was predominantly higher in rho^0 cells suggesting that UCP2 may inhibit ROS accumulation and protect the cells from excessive ROS production induced by mitochondrial defects linked to Warburg effect. In this respect, UCP2 may function as a potential diagnostic marker of cancer associated with the Warburg effect[42]. In addition to its antioxidant role, UCP2 acts as a direct metabolic regulator contributing to the Warburg phenotype. Indeed, as schematically reported in Figure 2, UCP2 has been proposed to function as a uniporter for pyruvate, which promotes pyruvate efflux from mitochondria, restricts mitochondrial respiration, and increases the rate of glycolysis in cancer cells[43]. Furthermore, UCP2 catalyzes the exchange of intramitochondrial C4 metabolites for cytosolic phosphate by an H^+-assisted mechanism, which is stimulated by both the electrical potential (negative inside) and pH gradient (acidic outside) existing across the inner mitochondrial membrane of respiring cells[44]. In particular, by exporting oxaloacetate and related C4 compounds from mitochondria, UCP2 negatively controls the oxidation of acetyl-CoA-producing substrates *via* the Krebs cycle, thus lowering the redox pressure on the mitochondrial respiratory chain, the ATP: ADP ratio, and ROS production. Notably, the mitochondrial concentration of oxaloacetate is usually very low, and its availability regulates the entry of acetyl-CoA into the Krebs cycle. Thus, UCP2 prevents mitochondrial glucose oxidation and favors a higher glucose utilization by aerobic glycolysis. In this context, our research group further confirmed the pro-glycolytic effect of UCP2 demonstrating for the first time that UCP2 can stabilize the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in the cytoplasm of cancer cells[45]. Accordingly, in response to oxidative stress, GAPDH has been demonstrated to undergo protein oxidation of redox-sensitive cysteine residues that stimulates its translocation to cell nuclei[46], where the enzyme favors transcriptional induction of cell death-related genes[47,48]. Thus, the antioxidant effect of UCP2 can inhibit both GAPDH oxidation and nuclear translocation supporting the glycolytic flux and preventing cancer cells from stimulating cell death mechanisms (Figure 2).

UCP2 and pancreatic cancer

A careful analysis of the recent scientific literature concerning the role of UCP2 in tumor development reveals that UCP2 and cancer may have a double relationship. Indeed, a dual regulation of UCP2 expression, depending on the stages of cancer development, has been observed in many tumor types.

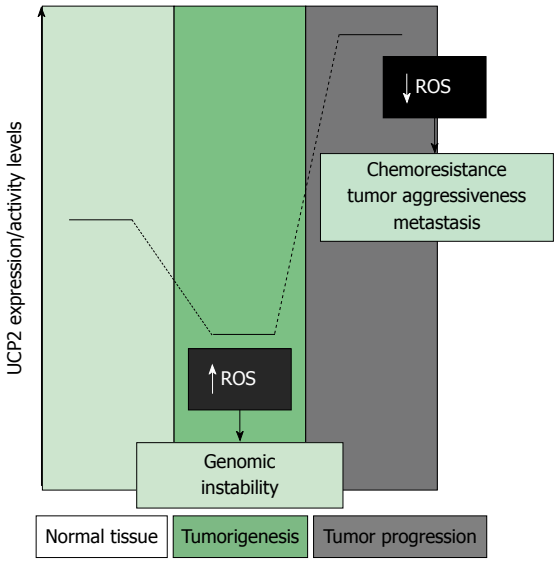


Figure 3 Relation between cancer development and uncoupling protein 2 expression or activity. ROS: Reactive oxygen species; UCP2: Uncoupling protein 2.

A number of studies have established the key role that UCP2 has in tumorigenesis and in chemoresistance. The generally accepted thesis envisages that, during the first stages of tumorigenesis, UCP2 is repressed to allow ROS accumulation and genomic instability, while it is triggered or over-expressed in the following stages of cancer development, determining chemoresistance and tumor aggressiveness by defending cancer cells from apoptosis through the negative regulation of mitochondrial ROS production (Figure 3)[49-51]. Accordingly, UCP2-null mice have a predisposition for enhanced tumorigenesis in the proximal colon, providing the first *in vivo* confirmation of a link between mitochondrial uncoupling proteins and cancer[52], while highly expressed UCP2 is associated with metastatic colon cancer and tumor aggressiveness[53]. The dual and opposite regulation of UCP2 expression in various stages of tumor development has also been demonstrated in breast cancer. In this system, the repression of UCPs by estrogens, a major risk factor for breast cancer initiation, may play a key role in estrogen-induced breast carcinogenesis[54]. On the contrary, the enhanced expression of UCP2 has been correlated to breast cancer progression. Indeed, a significant correlation between UCP2 levels and tumor grade-associated functional phenotypes has been found in a large number of breast cancer patients (n = 234)[55]. Concerning PC, some studies have shown that the protein level of UCP2 is significantly higher in human PC samples than in the adjacent normal tissues, suggesting that UCP2 may promote tumor growth in this tumor type[56]. An extensive study on Oncomine data sets addressed to analyze the UCP2 expression level in a number of cancer types, including pancreatic cancer, has revealed that UCP2 is over-expressed in ovarian, bladder, esophageal, testicular, kidney, colorectal, lung, breast, leukemia, prostate, as well as pancreas cancers[42]. This study has concluded that UCP2 over-expression is a general phenomenon linked to the progression of human cancers. Along this line of evidence, our research group has demonstrated that increased expression of UCP2 mRNA directly correlates with resistance to gemcitabine treatment, in a panel of pancreatic adenocarcinoma cell lines, and that the *UCP2* gene is induced by gemcitabine, demonstrating that the antioxidant effect of UCP2 plays a critical role in pancreatic cancer cell resistance to standard chemotherapy. We have also shown that UCP2 inhibition has a synergistic antiproliferative effect with gemcitabine in pancreatic adenocarcinoma cell growth[57]. Despite the availability of the above described data on the relationship between UCP2 expression/activity and PC, we believe that further studies need to be performed in order to better clarify the functional role of UCP2 in PC tumorigenesis and progression. Of crucial importance will be analyses on proteome and metabolic profiles of pancreatic cancer cells after knock-down or over-expression of UCP2 and clinical studies correlating UCP2 expression with clinicopathological factors and prognosis outcome on PC patients.

CONCLUSION

UCP2 over-expression may be considered a strategy adopted by cancer cells to protect themselves from excessive ROS production and to support the Warburg effect by reprogramming cancer cell metabolism. Thus, UCP2 inhibition can represent a therapeutic opportunity, in association to radio- or chemo-therapy, to treat tumors resistant to traditional therapy, such as PC. For this reason, we believe that UCP2 may be considered a potential target therapy for this tumor type. However, an efficient and specific UCP2 inhibitor is not yet available. The tools currently used in research studies to inhibit UCP2 are the genetic repression of *UCP2* mRNA by a specific siRNA or the inhibition of UCP2 activity by genipin, a natural aglycon derived from geniposide, an iridoid glycoside extracted from the fruit of *gardenia jasminoides*. Genipin, however, has unspecific pharmacological properties including anti-inflammatory and antidepressant-like effects[58]. Thus, drug design research to identify or synthetize a specific and effective UCP2 inhibitor should be strongly encouraged in order to counteract progression of pancreatic cancer and of many other tumor types over-expressing this protein, which is crucial for their aggressive phenotype.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Siegel R**, Ward E, Brawley O, Jemal A. Cancer statistics, 2011:

the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 2011; **61**: 212-236 [PMID: 21685461 DOI: 10.3322/caac.20121]
3 **Malvezzi M**, Bertuccio P, Levi F, La Vecchia C, Negri E. European cancer mortality predictions for the year 2013. *Ann Oncol* 2013; **24**: 792-800 [PMID: 23402763 DOI: 10.1093/annonc/mdt010]
4 **Del Chiaro M**, Segersvärd R, Lohr M, Verbeke C. Early detection and prevention of pancreatic cancer: is it really possible today? *World J Gastroenterol* 2014; **20**: 12118-12131 [PMID: 25232247 DOI: 10.3748/wjg.v20.i34.12118]
5 **Ryan DP**, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med* 2014; **371**: 1039-1049 [PMID: 25207767 DOI: 10.1056/NEJMra1404198]
6 **Feig C**, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. *Clin Cancer Res* 2012; **18**: 4266-4276 [PMID: 22896693 DOI: 10.1158/1078-0432.CCR-11-3114]
7 **Kanda M**, Matthaei H, Wu J, Hong SM, Yu J, Borges M, Hruban RH, Maitra A, Kinzler K, Vogelstein B, Goggins M. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. *Gastroenterology* 2012; **142**: 730-733.e9 [PMID: 22226782 DOI: 10.1053/j.gastro.2011.12.042]
8 **Hustinx SR**, Leoni LM, Yeo CJ, Brown PN, Goggins M, Kern SE, Hruban RH, Maitra A. Concordant loss of MTAP and p16/CDKN2A expression in pancreatic intraepithelial neoplasia: evidence of homozygous deletion in a noninvasive precursor lesion. *Mod Pathol* 2005; **18**: 959-963 [PMID: 15832197 DOI: 10.1038/modpathol.3800377]
9 **Ling J**, Kang Y, Zhao R, Xia Q, Lee DF, Chang Z, Li J, Peng B, Fleming JB, Wang H, Liu J, Lemischka IR, Hung MC, Chiao PJ. KrasG12D-induced IKK2/β/NF-κB activation by IL-1α and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012; **21**: 105-120 [PMID: 22264792 DOI: 10.1016/j.ccr.2011.12.006]
10 **Corcoran RB**, Cheng KA, Hata AN, Faber AC, Ebi H, Coffee EM, Greninger P, Brown RD, Godfrey JT, Cohoon TJ, Song Y, Lifshits E, Hung KE, Shioda T, Dias-Santagata D, Singh A, Settleman J, Benes CH, Mino-Kenudson M, Wong KK, Engelman JA. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. *Cancer Cell* 2013; **23**: 121-128 [PMID: 23245996 DOI: 10.1016/j.ccr.2012.11.007]
11 **Navas C**, Hernández-Porras I, Schuhmacher AJ, Sibilia M, Guerra C, Barbacid M. EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* 2012; **22**: 318-330 [PMID: 22975375 DOI: 10.1016/j.ccr.2012.08.001]
12 **Cecconi D**, Palmieri M, Donadelli M. Proteomics in pancreatic cancer research. *Proteomics* 2011; **11**: 816-828 [PMID: 21229586 DOI: 10.1002/pmic.201000401]
13 **He XY**, Yuan YZ. Advances in pancreatic cancer research: moving towards early detection. *World J Gastroenterol* 2014; **20**: 11241-11248 [PMID: 25170208 DOI: 10.3748/wjg.v20.i32.11241]
14 **Iacobuzio-Donahue CA**, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Vilardell F, Wang Z, Keller JW, Banerjee P, Herman JM, Cameron JL, Yeo CJ, Halushka MK, Eshleman JR, Raben M, Klein AP, Hruban RH, Hidalgo M, Laheru D. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol* 2009; **27**: 1806-1813 [PMID: 19273710 DOI: 10.1200/JCO.2008.17.7188]
15 **Hughes J**, Criscuolo F. Evolutionary history of the UCP gene family: gene duplication and selection. *BMC Evol Biol* 2008; **8**: 306 [PMID: 18980678 DOI: 10.1186/1471-2148-8-306]
16 **Bouillaud F**, Weissenbach J, Ricquier D. Complete cDNA-derived amino acid sequence of rat brown fat uncoupling protein. *J Biol Chem* 1986; **261**: 1487-1490 [PMID: 3753702]
17 **Enerbäck S**, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 1997; **387**: 90-94 [PMID: 9139827 DOI: 10.1038/387090a0]
18 **Boss O**, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P, Giacobino JP. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett* 1997; **408**: 39-42 [PMID: 9180264]
19 **Fleury C**, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin MF, Surwit RS, Ricquier D, Warden CH. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat Genet* 1997; **15**: 269-272 [PMID: 9054939 DOI: 10.1038/ng0397-269]
20 **Couplan E**, del Mar Gonzalez-Barroso M, Alves-Guerra MC, Ricquier D, Goubern M, Bouillaud F. No evidence for a basal, retinoic, or superoxide-induced uncoupling activity of the uncoupling protein 2 present in spleen or lung mitochondria. *J Biol Chem* 2002; **277**: 26268-26275 [PMID: 12011051 DOI: 10.1074/jbc.M202535200]
21 **Mozo J**, Ferry G, Studeny A, Pecqueur C, Rodriguez M, Boutin JA, Bouillaud F. Expression of UCP3 in CHO cells does not cause uncoupling, but controls mitochondrial activity in the presence of glucose. *Biochem J* 2006; **393**: 431-439 [PMID: 16178820 DOI: 10.1042/BJ20050494]
22 **Criscuolo F**, Gonzalez-Barroso Mdel M, Bouillaud F, Ricquier D, Miroux B, Sorci G. Mitochondrial uncoupling proteins: new perspectives for evolutionary ecologists. *Am Nat* 2005; **166**: 686-699 [PMID: 16475085 DOI: 10.1086/497439]
23 **Donadelli M**, Dando I, Fiorini C, Palmieri M. UCP2, a mitochondrial protein regulated at multiple levels. *Cell Mol Life Sci* 2014; **71**: 1171-1190 [PMID: 23807210 DOI: 10.1007/s00018-013-1407-0]
24 **Kong D**, Vong L, Parton LE, Ye C, Tong Q, Hu X, Choi B, Brüning JC, Lowell BB. Glucose stimulation of hypothalamic MCH neurons involves K(ATP) channels, is modulated by UCP2, and regulates peripheral glucose homeostasis. *Cell Metab* 2010; **12**: 545-552 [PMID: 21035764 DOI: 10.1016/j.cmet.2010.09.013]
25 **Krauss S**, Zhang CY, Lowell BB. The mitochondrial uncoupling-protein homologues. *Nat Rev Mol Cell Biol* 2005; **6**: 248-261 [PMID: 15738989 DOI: 10.1038/nrm1572]
26 **Arsenijevic D**, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, Couplan E, Alves-Guerra MC, Goubern M, Surwit R, Bouillaud F, Richard D, Collins S, Ricquier D. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat Genet* 2000; **26**: 435-439 [PMID: 11101840 DOI: 10.1038/82565]
27 **Vidal-Puig AJ**, Grujic D, Zhang CY, Hagen T, Boss O, Ido Y, Szczepanik A, Wade J, Mootha V, Cortright R, Muoio DM, Lowell BB. Energy metabolism in uncoupling protein 3 gene knockout mice. *J Biol Chem* 2000; **275**: 16258-16266 [PMID: 10748196 DOI: 10.1074/jbc.M910179199]
28 **Zhang CY**, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, Zheng XX, Wheeler MB, Shulman GI, Chan CB, Lowell BB. Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell* 2001; **105**: 745-755 [PMID: 11440717]
29 **Hoang T**, Smith MD, Jelokhani-Niaraki M. Toward understanding the mechanism of ion transport activity of neuronal uncoupling proteins UCP2, UCP4, and UCP5. *Biochemistry* 2012; **51**: 4004-4014 [PMID: 22524567 DOI: 10.1021/bi3003378]
30 **Mailloux RJ**, Harper ME. Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radic Biol Med* 2011; **51**: 1106-1115 [PMID: 21762777 DOI: 10.1016/j.freeradbiomed.2011.06.022]
31 **Mailloux RJ**, Harper ME. Mitochondrial proticity and ROS signaling: lessons from the uncoupling proteins. *Trends Endocrinol Metab* 2012; **23**: 451-458 [PMID: 22591987 DOI: 10.1016/j.tem.2012.04.004]
32 **Brand MD**, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab* 2005; **2**: 85-93 [PMID: 16098826 DOI: 10.1016/j.cmet.2005.06.002]
33 **Garlid KD**, Jabůrek M, Jezek P, Varecha M. How do uncoupling proteins uncouple? *Biochim Biophys Acta* 2000; **1459**: 383-389 [PMID: 11004454]
34 **Cadenas E**, Davies KJ. Mitochondrial free radical generation,

oxidative stress, and aging. *Free Radic Biol Med* 2000; **29**: 222-230 [PMID: 11035250]
35 **Cohen G**. Enzymatic/nonenzymatic sources of oxyradicals and regulation of antioxidant defenses. *Ann N Y Acad Sci* 1994; **738**: 8-14 [PMID: 7832459]
36 **Lanciano P**, Khalfaoui-Hassani B, Selamoglu N, Ghelli A, Rugolo M, Daldal F. Molecular mechanisms of superoxide production by complex III: a bacterial versus human mitochondrial comparative case study. *Biochim Biophys Acta* 2013; **1827**: 1332-1339 [PMID: 23542447 DOI: 10.1016/j.bbabio.2013.03.009]
37 **Papa L**, Manfredi G, Germain D. SOD1, an unexpected novel target for cancer therapy. *Genes Cancer* 2014; **5**: 15-21 [PMID: 24955214]
38 **Lustgarten MS**, Bhattacharya A, Muller FL, Jang YC, Shimizu T, Shirasawa T, Richardson A, Van Remmen H. Complex I generated, mitochondrial matrix-directed superoxide is released from the mitochondria through voltage dependent anion channels. *Biochem Biophys Res Commun* 2012; **422**: 515-521 [PMID: 22613204 DOI: 10.1016/j.bbrc.2012.05.055]
39 **Warburg O**. On the origin of cancer cells. *Science* 1956; **123**: 309-314 [PMID: 13298683]
40 **Sawayama H**, Ishimoto T, Sugihara H, Miyanari N, Miyamoto Y, Baba Y, Yoshida N, Baba H. Clinical impact of the Warburg effect in gastrointestinal cancer (review). *Int J Oncol* 2014; **45**: 1345-1354 [PMID: 25070157 DOI: 10.3892/ijo.2014.2563]
41 **Kulawiec M**, Safina A, Desouki MM, Still I, Matsui S, Bakin A, Singh KK. Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. *Cancer Biol Ther* 2008; **7**: 1732-1743 [PMID: 19151587]
42 **Ayyasamy V**, Owens KM, Desouki MM, Liang P, Bakin A, Thangaraj K, Buchsbaum DJ, LoBuglio AF, Singh KK. Cellular model of Warburg effect identifies tumor promoting function of UCP2 in breast cancer and its suppression by genipin. *PLoS One* 2011; **6**: e24792 [PMID: 21935467 DOI: 10.1371/journal.pone.0024792]
43 **Baffy G**. Uncoupling protein-2 and cancer. *Mitochondrion* 2010; **10**: 243-252 [PMID: 20005987 DOI: 10.1016/j.mito.2009.12.143]
44 **Vozza A**, Parisi G, De Leonardis F, Lasorsa FM, Castegna A, Amorese D, Marmo R, Calcagnile VM, Palmieri L, Ricquier D, Paradies E, Scarcia P, Palmieri F, Bouillaud F, Fiermonte G. UCP2 transports C4 metabolites out of mitochondria, regulating glucose and glutamine oxidation. *Proc Natl Acad Sci USA* 2014; **111**: 960-965 [PMID: 24395786 DOI: 10.1073/pnas.1317400111]
45 **Dando I**, Fiorini C, Pozza ED, Padroni C, Costanzo C, Palmieri M, Donadelli M. UCP2 inhibition triggers ROS-dependent nuclear translocation of GAPDH and autophagic cell death in pancreatic adenocarcinoma cells. *Biochim Biophys Acta* 2013; **1833**: 672-679 [PMID: 23124112 DOI: 10.1016/j.bbamcr.2012.10.028]
46 **Dastoor Z**, Dreyer JL. Potential role of nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase in apoptosis and oxidative stress. *J Cell Sci* 2001; **114**: 1643-1653 [PMID: 11309196]
47 **Colell A**, Green DR, Ricci JE. Novel roles for GAPDH in cell death and carcinogenesis. *Cell Death Differ* 2009; **16**: 1573-1581 [PMID: 19779498 DOI: 10.1038/cdd.2009.137]
48 **Lee J**, Giordano S, Zhang J. Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. *Biochem J* 2012; **441**: 523-540 [PMID: 22187934 DOI: 10.1042/BJ20111451]
49 **Derdak Z**, Mark NM, Beldi G, Robson SC, Wands JR, Baffy G. The mitochondrial uncoupling protein-2 promotes chemoresistance in cancer cells. *Cancer Res* 2008; **68**: 2813-2819 [PMID: 18413749 DOI: 10.1158/0008-5472.CAN-08-0053]
50 **Robbins D**, Zhao Y. New aspects of mitochondrial Uncoupling Proteins (UCPs) and their roles in tumorigenesis. *Int J Mol Sci* 2011; **12**: 5285-5293 [PMID: 21954358 DOI: 10.3390/ijms12085285]
51 **Su WP**, Lo YC, Yan JJ, Liao IC, Tsai PJ, Wang HC, Yeh HH, Lin CC, Chen HH, Lai WW, Su WC. Mitochondrial uncoupling protein 2 regulates the effects of paclitaxel on Stat3 activation and cellular survival in lung cancer cells. *Carcinogenesis* 2012; **33**: 2065-2075 [PMID: 22847181 DOI: 10.1093/carcin/bgs253]
52 **Derdák Z**, Fülöp P, Sabo E, Tavares R, Berthiaume EP, Resnick MB, Paragh G, Wands JR, Baffy G. Enhanced colon tumor induction in uncoupling protein-2 deficient mice is associated with NF-kappaB activation and oxidative stress. *Carcinogenesis* 2006; **27**: 956-961 [PMID: 16401637 DOI: 10.1093/carcin/bgi335]
53 **Kuai XY**, Ji ZY, Zhang HJ. Mitochondrial uncoupling protein 2 expression in colon cancer and its clinical significance. *World J Gastroenterol* 2010; **16**: 5773-5778 [PMID: 21128330]
54 **Sastre-Serra J**, Valle A, Company MM, Garau I, Oliver J, Roca P. Estrogen down-regulates uncoupling proteins and increases oxidative stress in breast cancer. *Free Radic Biol Med* 2010; **48**: 506-512 [PMID: 19969066 DOI: 10.1016/j.freeradbiomed.2009.11.025]
55 **Sayeed A**, Meng Z, Luciani G, Chen LC, Bennington JL, Dairkee SH. Negative regulation of UCP2 by TGFβ signaling characterizes low and intermediate-grade primary breast cancer. *Cell Death Dis* 2010; **1**: e53 [PMID: 21364658 DOI: 10.1038/cddis.2010.30]
56 **Li W**, Nichols K, Nathan CA, Zhao Y. Mitochondrial uncoupling protein 2 is up-regulated in human head and neck, skin, pancreatic, and prostate tumors. *Cancer Biomark* 2013; **13**: 377-383 [PMID: 24440978 DOI: 10.3233/CBM-130369]
57 **Dalla Pozza E**, Fiorini C, Dando I, Menegazzi M, Sgarbossa A, Costanzo C, Palmieri M, Donadelli M. Role of mitochondrial uncoupling protein 2 in cancer cell resistance to gemcitabine. *Biochim Biophys Acta* 2012; **1823**: 1856-1863 [PMID: 22705884 DOI: 10.1016/j.bbamcr.2012.06.007]
58 **Araki R**, Hiraki Y, Yabe T. Genipin attenuates lipopolysaccharide-induced persistent changes of emotional behaviors and neural activation in the hypothalamic paraventricular nucleus and the central amygdala nucleus. *Eur J Pharmacol* 2014; **741**: 1-7 [PMID: 25084220 DOI: 10.1016/j.ejphar.2014.07.038]

P- Reviewer: Fu DL, Giovannetti E, Stefaniak T, Su WC
S- Editor: Yu J **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3239

World J Gastroenterol 2015 March 21; 21(11): 3239-3244
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Effects of urotensin-Ⅱ on cytokines in early acute liver failure in mice

Liang-Ming Liu, Liang Zhao, Dong-Yu Liang, Fang-Ping Yu, Chang-Gen Ye, Wen-Juan Tu, Tong Zhu

Liang-Ming Liu, Liang Zhao, Dong-Yu Liang, Fang-Ping Yu, Chang-Gen Ye, Wen-Juan Tu, Tong Zhu, Department of Hepatology, Songjiang Hospital Affiliated to the First People's Hospital, Shanghai Jiaotong University, Shanghai 201600, China
Author contributions: Liu LM designed research; Zhao L, Liang DY, Yu FP, Ye CG, Tu WJ and Zhu T performed research; Liu LM contributed new reagents/analytic tools; Liu LM and Zhao L analyzed data; and Liu LM and Zhao L wrote the paper.
Supported by National Natural Science Foundation of China, No. 81070357 and No. 30660066.

Correspondence to: Liang-Ming Liu, MD, PhD, Department of Hepatology, Songjiang Hospital Affiliated to the First People's Hospital, Shanghai Jiaotong University, No. 746 Mid-Zhongshan Road, Songjiang District, Shanghai 201600, China. liuliangming@hotmail.com
Telephone: +86-21-67720053
Fax: +86-21-67720053
Received: July 7, 2014
Peer-review started: July 8, 2014
First decision: September 15, 2014
Revised: October 23, 2014
Accepted: December 16, 2014
Article in press: December 16, 2014
Published online: March 21, 2015

Abstract

AIM: To investigate urotensin-Ⅱ (UⅡ) and its effects on tumor necrosis factor (TNF)-α and interleukin (IL)-1β in early acute liver failure (ALF).

METHODS: We investigated the time-dependent alteration in UⅡ levels and its effects on TNF-α and IL-1β in liver and blood in the early stage of lipopolysaccharide/D-galactosamine-induced ALF.

RESULTS: After lipopolysaccharide/D-galactosamine challenge, UⅡ rose very rapidly and reached a maximal level 0.5 h, and the level remained significantly elevated after 2 h ($P < 0.05$). Six hours after challenge, UⅡ began to degrade, but remained higher than at 0 h ($P < 0.05$). Pretreatment with urantide, an inhibitor of the UⅡ receptor, suppressed the degree of UⅡ increase in liver and blood at 6 h after challenge ($P < 0.05$ *vs* paired controls). In addition, liver and blood TNF-α increased from 1 to 6 h, and reached a peak at 1 and 2 h, respectively; however, IL-1β did not rise until 6 h after challenge. Urantide pretreatment inhibited the degree of TNF-α and IL-1β increase following downregulation of UⅡ post-challenge (all $P < 0.05$).

CONCLUSION: UⅡ plays a role in the pathogenesis and priming of ALF by triggering an inflammatory cascade and driving the early release of cytokines in mice.

Key words: Acute hepatic failure; Interleukin-1β; Mouse; Tumor necrosis factor α; Urantide; Urotensin-Ⅱ

Core tip: In this study, we found that urotensin-Ⅱ (UⅡ) increased before tumor necrosis factor (TNF)-α and interleukin (IL)-1β following lipopolysaccharide/D-galactosamine challenge. Furthermore, pretreatment with urantide, an inhibitor of the UⅡ receptor, blocked TNF-α and IL-1β increases following downregulation of UⅡ in liver and blood at different time points after challenge. Therefore, UⅡ may play a pivotal role in the pathogenesis and priming of acute liver failure by triggering the inflammatory cascade, and initiating and driving the early release of TNF-α and IL-1β in lipopolysaccharide/D-galactosamine-challenged mice.

Liu LM, Zhao L, Liang DY, Yu FP, Ye CG, Tu WJ, Zhu T. Effects of urotensin-Ⅱ on cytokines in early acute liver failure in mice. *World J Gastroenterol* 2015; 21(11): 3239-3244 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3239.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3239

INTRODUCTION

Urotensin-Ⅱ (UⅡ), a somatostatin-like cyclic vasoactive polypeptide, is released from numerous tissues[1,2], and exerts a wide range of actions in the progression of many diseases, including cardiovascular, endocrine, immune system, and kidney diseases[3]. A recent study found that UⅡ level in plasma and liver was upregulated in patients with acute liver failure (ALF)[4]. UⅡ and its G-protein coupled receptor, GPR14, are mainly expressed in Kupffer and endothelial cells in liver tissues during ALF[4]. Kupffer and endothelial cells are important immune inflammatory cells in organisms; thus, there seems to be an interrelationship between the high level of UⅡ polypeptide and immune-mediated hepatic inflammatory injury in ALF.

ALF is an inflammatory process caused by a variety of proinflammatory cytokines, including interleukin (IL)-1β and IL-6, and particularly tumor necrosis factor (TNF)-α[5,6]. The cascades of these cytokines induced by the early burst of TNF-α result in acute inflammation in liver tissues, and lead to ALF[7]. Our recent study showed that high UⅡ-mediated ALF was associated with the upregulation of proinflammatory cytokines[8]. However, the impact of UⅡ on these cytokines in patients with ALF remains unclear. Therefore, we investigated the time-dependent alteration in UⅡ level and its effects on TNF-α and IL-1β levels in the early stage of ALF.

MATERIALS AND METHODS

Materials

Lipopolysaccharide (LPS) (*Escherichia coli* strain O55: B5) and D-galactosamine (D-GalN) were obtained from Sigma-Aldrich (St. Louis, MO, United States). Urantide was purchased from Peptides (Louisville, KY, United States). Male BALB/c mice (6 wk of age) weighing 20-22 g were obtained from the Animal Center of the First People's Hospital Affiliated to Shanghai Jiaotong University, and maintained in specific pathogen-free air at a temperature of 22 ± 2 ℃ with a 12 h light/dark cycle and relative humidity of 50%. Animal care and treatment were humane and in compliance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Medical Scientific Research of the First People's Hospital, Shanghai Jiaotong University (No: 2013KY041). All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Table 1 Polymerase chain reaction primer sequences and product lengths

Gene	Primer sequences	Product size (bp)
UⅡ	Sense: 5′-GAGCATTCCCTTCATCGTAG-3′ Antisense: 5′-CATAGCGTTCACTGCTCATT-3′	385
TNF-α	Sense: 5′-GGCGGTGCCTATGTCTACG-3′ Antisense: 5′-GACAAGCCTGTAGCCCACC-3′	354
IL-1β	Sense: 5′-CCAGTGAAATGATGGCTTATTACAG-3′ Antisense: 5′-GTAGTGGTGGTCGTAGATTCGTA-3′	151
β-actin	Sense: 5′-CCTGGCACCCAGCACAAT-3′ Antisense: 5′-GGGCCGGACTCGTCATAC-3′	156
	Sense: 5′-ATATCGCTGCGCTGGTCGTC-3′ Antisense: 5′-AGGATGGCGTGAGGGAGAGC-3′	517

IL: Interleukin; TNF: Tumor necrosis factor; UⅡ: Urotensin-Ⅱ.

Experimental design

Mice were injected intraperitoneally with 800 mg/kg D-GalN and 50 μg/kg LPS dissolved in 200 μL of pyrogen-free normal saline[9]. The mice were randomly divided into two groups: non-urantide, which received an intravenous injection of 100 μL normal saline, or urantide pretreatment, with 0.6 mg/kg urantide dissolved in 100 μL normal saline 30 min before the LPS/D-GalN injection, as previously described[8]. The mice were then anesthetized and killed at 0.0, 0.5, 1.0, 2.0 and 6.0 h after the LPS/D-GalN injection (n = 6 at each time point per group), and blood and liver were collected for testing.

Reverse transcription-polymerase chain reaction

Total RNA was extracted from liver tissues with TRIzol reagent (Invitrogen of Thermo Fisher Scientific, Waltham, MA, United States) following the manufacturer's instructions. Two micrograms of total RNA were used for the synthesis of first-strand cDNA with an M-MLV reverse transcription (RT) kit (Fermentas of Thermo Fisher Scientific). The polymerase chain reaction (PCR) primers were designed by Primer Premier 6.0 software (PremierBiosoft, Palo Alto, CA, United States) from the reported sequences (GenBank accession number X66539 for TNF-α, NM031512 for IL-1β, NM011910 for UⅡ, and NM031144 for β-actin) (Table 1). PCR was performed with the following thermal cycling conditions: denaturation at 94 ℃ for 5 min followed by 32 cycles of denaturation at 94 ℃ for 1 min, primer annealing at 58 ℃ (for UⅡ) or 55 ℃ (for TNF-α and IL-1β) for 45 s, and primer extension at 72 ℃ for 45 s, with a final extension at 72 ℃ for 10 min.

Enzyme-linked immunosorbent assay

Serum cytokine levels, including TNF-α and IL-1β, were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Inc., Minneapolis, MN, United States) according to the manufacturer's protocol; and serum UⅡ levels were determined using

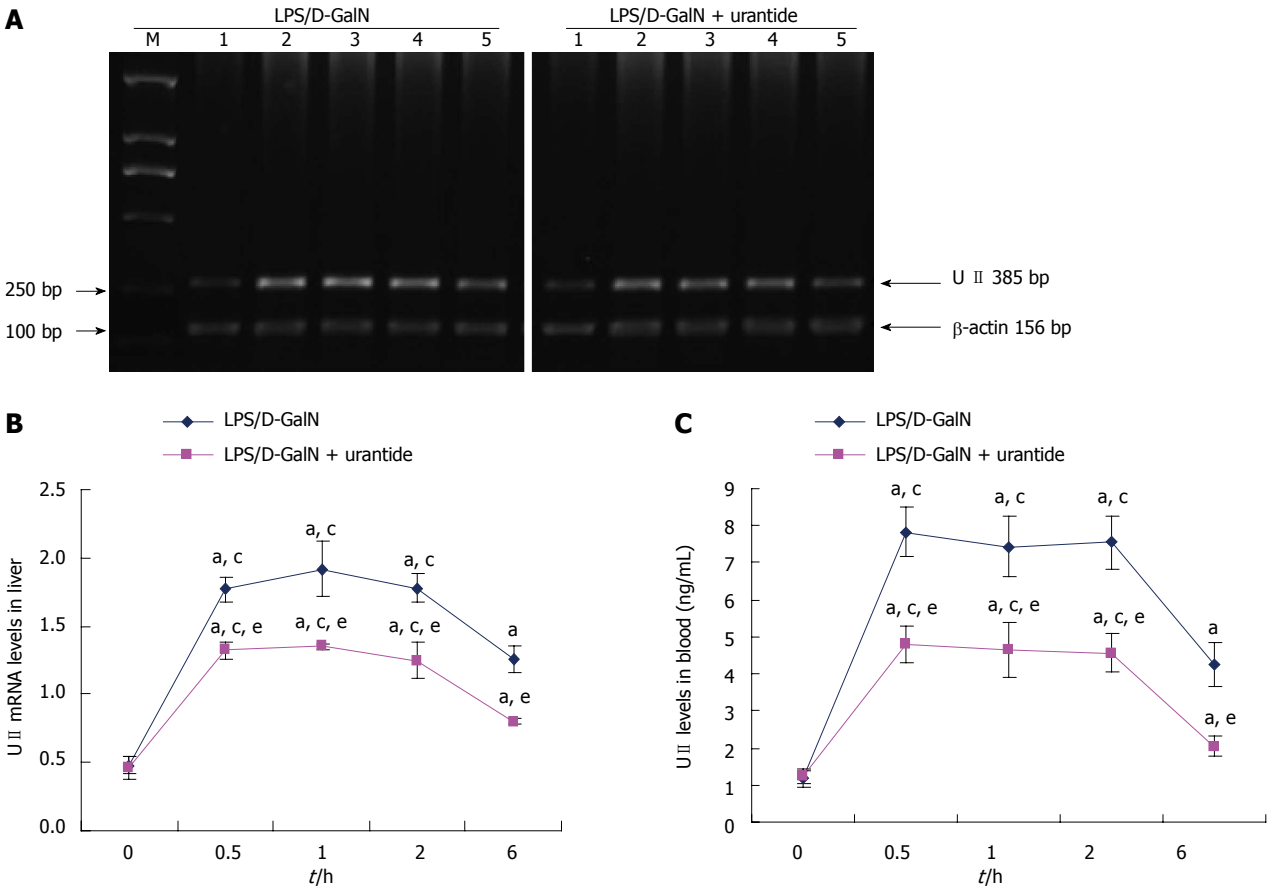


Figure 1 Time-dependent expression of urotensin-Ⅱ in the early stage of lipopolysaccharide/D-galactosamine challenge in mice with or without urantide treatment. A: Representative ethidium bromide-stained gel of reverse transcription-PCR products from liver (M: DNA marker; Lines 1, 2, 3, 4, and 5: 0.0, 0.5, 1.0, 2.0, and 6.0 h after LPS/D-GalN challenge, respectively); B: Relative expression levels of U Ⅱ mRNA in the liver (normalized to β-actin); C: Levels of U Ⅱ secretion in blood as assayed by ELISA. Values are mean ± standard deviation (n = 6); $^{a}P < 0.05$ *vs* 0 h; $^{c}P < 0.05$ *vs* 6 h; $^{e}P < 0.05$ *vs* mice without urantide pretreatment. U Ⅱ: Urotensin-Ⅱ; LPS: Lipopolysaccharide; D-GalN: D-galactosamine.

an enzyme immunoassay kit (Phoenix Biotech, Beijing, China), based on the principle of a "competitive" enzyme immunoassay[10], according to the manufacturer's guidelines.

Statistical analysis

SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, United States) was used in the study. The results are expressed as means ± standard deviation. A $P < 0.05$ was considered statistically significant.

RESULTS

Time course of U Ⅱ in the early stage of the LPS/D-GalN challenge in mouse liver and blood

A rapid increase in U Ⅱ level was observed in the very early stage of the LPS/D-GalN challenge in mice with or without urantide pretreatment. As shown in Figure 1, LPS/D-GalN induced a significant increase in U Ⅱ, which reached a peak from 0.5 to 2.0 h and remained elevated in liver and blood at 6 h (both $P < 0.05$). However, in urantide-pretreated mice, U Ⅱ levels were statistically lower from 0.5 to 6.0 h after challenge compared with the paired control (all $P < 0.05$).

Time-dependent expression of TNF-α in the early stage of the LPS/D-GalN challenge in mouse liver and blood

TNF-α levels were measured in the early stage of the LPS/D-GalN challenge in liver and blood in mice with or without urantide pretreatment. As shown in Figure 2, TNF-α increased and peaked at 1 and 2 h, and remained elevated at 6 h after drug administration in liver and blood (both $P < 0.05$). TNF-α levels in liver and blood were not significantly different between 0 and 0.5 h. However, TNF-α levels in liver and blood in urantide-pretreated mice were significantly lower than in paired control mice from 1 to 6 h after challenge (all $P < 0.05$).

Time-dependent alteration in IL-1β in the early stage of the LPS/D-GalN challenge in mouse liver and blood

The time-dependent alteration in IL-1β following urantide pretreatment was also determined in the early stage of the LPS/D-GalN challenge. As shown in Figure 3, IL-1β did not increase in liver and blood until 6 h after the LPS/D-GalN challenge ($P < 0.05$). IL-1β levels were not significantly different at 0.0, 0.5, 1.0, and 2 h. However, urantide pretreatment lowered IL-1β levels in liver and blood compared with paired control

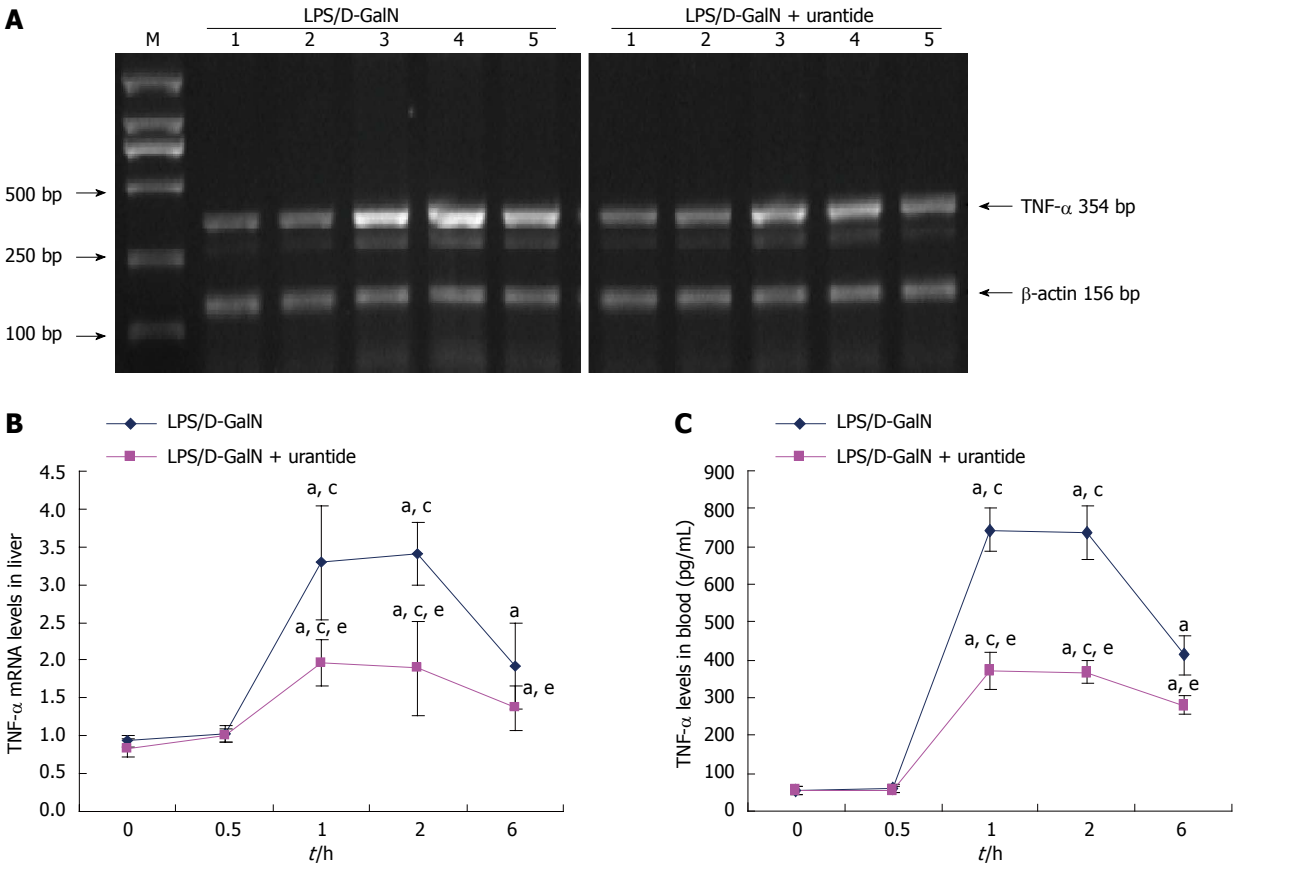


Figure 2 Time-dependent expression of tumor necrosis factor-α in the early stage of lipopolysaccharide/D-galactosamine challenge in mice with or without urantide pretreatment. A: Representative ethidium bromide-stained gel of reverse transcription-PCR products from liver (M: DNA marker; Lines 1, 2, 3, 4, and 5: 0.0, 0.5, 1.0, 2.0, and 6.0 h after LPS/D-GalN challenge, respectively); B: Relative expression levels of TNF-α mRNA in the liver (normalized to β-actin); C: Levels of TNF-α secretion in blood as assayed by ELISA. Values are mean ± standard deviation (n = 6); $^{a}P < 0.05$ *vs* 0 h; $^{c}P < 0.05$ *vs* 6 h; $^{e}P < 0.05$ *vs* mice without urantide pretreatment. TNF: Tumor necrosis factor; LPS: Lipopolysaccharide; D-GalN: D-galactosamine.

mice at 6 h after challenge ($P < 0.05$).

DISCUSSION

LPS induces lethal ALF in mice sensitized by D-GalN[11]. For more than 20 years, LPS/D-GalN-induced hepatitis in mice has been regarded as a well-established model for gaining insight into the mechanism of ALF[12,13]. Our previous reports showed that the simultaneous administration of LPS and D-GalN led to high mortality due to a severe hepatic inflammatory response followed by massive cell apoptosis and necrosis in mice[5,8,14]. Elevated levels of UⅡ were also observed in the liver and blood in this animal model, and blockade of the signal with urantide markedly suppressed liver apoptosis and acute inflammation[8].

UⅡ is a cyclic polypeptide that exerts a wide range of actions in health and disease[15,16], and has an important effect on inflammation-related diseases, such as hypertension[17], coronary atherosclerosis[18], chronic glomerulonephritis[19], and hepatic cirrhosis[20]. Patients with ALF also exhibit enhanced expression of UⅡ in the liver[4]. Watanabe *et al*[21] and Ban *et al*[22] showed that UⅡ is associated with endothelial dysfunction-related diseases and immune-driven inflammatory diseases. To validate the role of UⅡ signals in the pathogenesis of ALF, we serially tested the levels of UⅡ in liver and blood to investigate the sequence of events preceding acute liver damage, which was not fully apparent until 6 h after co-administration of LPS/D-GalN[5]. An early event prior to obvious injury may reveal the pathophysiologic mechanisms of ALF. We found that UⅡ is significantly induced in liver and plasma 6 h after LPS/D-GalN challenge, increasing and reaching a maximal level very rapidly. At 6 h after the challenge, UⅡ levels began to degrade, but remained high. In addition, we also observed that urantide pre-treatment suppresses the degree of this increase, suggesting an autocrine loop in the *in vivo* production of UⅡ. With positive feedback, early enhancement of UⅡ expression may be induced, finally leading to hepatic inflammatory injury after the LPS/D-GalN challenge. Therefore, UⅡ has cytokine-like activity.

To gain further mechanistic insights, we determined whether UⅡ has an effect on proinflammatory cytokines, including TNF-α and IL-1β, the pacing factors in the inflammatory response to hepatic injury. TNF-α in liver and blood increases following UⅡ upregulation, but not until 1 h after the LPS/D-GalN

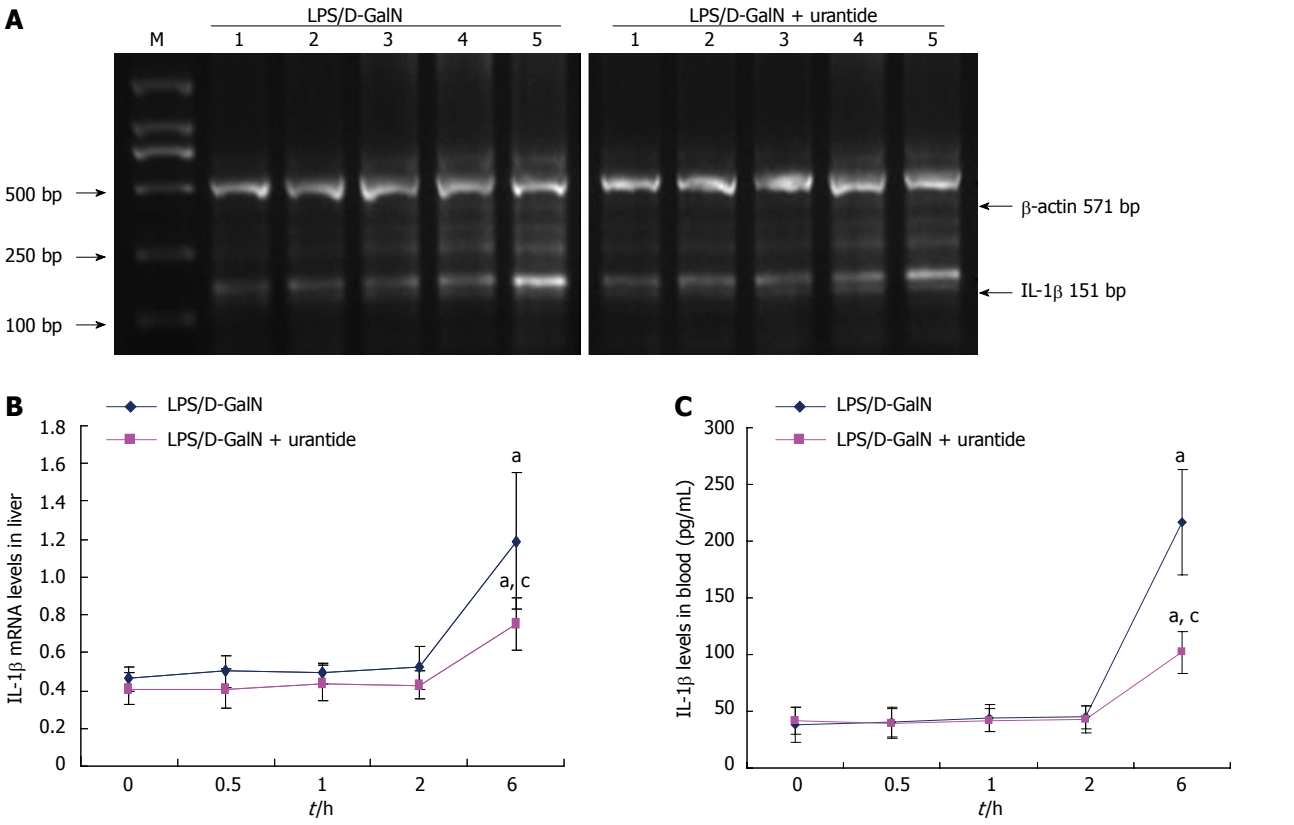


Figure 3 Time-dependent expression of interleukin-1β in the early stage of lipopolysaccharide/D-galactosamine challenge in mice with or without urantide pretreatment. A: Representative ethidium bromide-stained gel of reverse transcription-PCR products in liver (M: DNA marker; Lines 1, 2, 3, 4, and 5: 0.0, 0.5, 1.0, 2.0, and 6.0 h after LPS/D-GalN challenge, respectively); B: Relative expression levels of IL-1β mRNA in the liver (normalized to β-actin); C: Levels of IL-1β secretion in blood as assayed by ELISA. Values are mean ± standard deviation (n = 6); $^{a}P < 0.05$ *vs* 0 h; $^{c}P < 0.05$ *vs* 6 h; $^{e}P < 0.05$ *vs* mice without urantide pretreatment. IL: Interleukin; LPS: Lipopolysaccharide; D-GalN: D-galactosamine.

challenge. This is subsequently followed by elevations of liver and blood IL-1β levels, the secretion of which did not rise until 6 h after the LPS/D-GalN challenge. This time-dependent alteration suggests a causal relationship between UⅡ and both TNF-α and IL-1β in early ALF.

To confirm this deduction, the potency of the UⅡ receptor antagonist, urantide, was evaluated by the expression of TNF-α and IL-1β. We found that urantide pretreatment suppresses the increase in UⅡ, and reduces the degree of increase of TNF-α and IL-1β at different time points in the early stage of the LPS/D-GalN challenge. These results extend our previous finding on the inhibitory effect of urantide on the production of proinflammatory cytokines in mice with ALF[8]. From these results, we suggest that the sharp and rapid upregulation of UⅡ induces early expression and secretion of TNF-α and IL-1β.

TNF-α is known to play a central role in the pathogenesis of LPS/D-GalN-induced liver failure[23] by inducing the release of a variety of proinflammatory cytokines, including IL-1β[7]. Previous reports showed that UⅡ induced the expression of IL-6[24], and was upregulated by IFN-γ[25]. Therefore, UⅡ is involved in the vicious cycle of inflammatory cytokine release in immune-related tissue injury.

In conclusion, UⅡ can cause acute liver injury by triggering the inflammatory response, and by initiating and driving the early release of proinflammatory cytokines in LPS/D-GalN-challenged mice.

COMMENTS

Background

Urotensin-Ⅱ (UⅡ) plays a role in inflammation-related diseases and is upregulated in acute liver failure (ALF), an inflammatory process caused by proinflammatory cytokines including tumor necrosis factor (TNF)-α and interleukin (IL)-1β. However, the impact of UⅡ on these cytokines remains elusive.

Research frontiers

This study examines the mechanisms of immune-mediated inflammatory injury in acute liver failure, and the role of the urotensin system in tissue damage and inflammation.

Innovations and breakthroughs

This study demonstrated that UⅡ may cause acute liver injury by triggering the inflammatory response, and by initiating and driving the early release of proinflammatory cytokines in lipopolysaccharide/D-galactosamine-challenged mice.

Applications

The urotensin system may be a new research hotspot in mechanistic studies, and may provide a new drug target for the future treatment of ALF.

Terminology

ALF is a life-threatening clinical syndrome with a sudden loss of hepatic function in patients with no preexisting history of liver disease. The pathologic

feature of ALF is the death of a large number of parenchymal hepatocytes resulting from cell apoptosis and necrosis. Massive cell loss leads to functional impairment of the liver, and ultimately, multi-organ failure and death. Mortality is high in patients with ALF (approximately 90%). UⅡ, initially isolated from the teleost urophysis, is a somatostatin-like cyclic neuropeptide widely distributed in many tissues in many classes of vertebrates, including humans, and exerts biologic actions in both physiologic and pathologic conditions.

Peer-review

The paper investigated the role of UⅡ in modifying the levels of TNF-α and IL-1β in a well-established model of ALF. The major finding is the demonstration of the role of UⅡ in initiating the proinflammatory cascade in ALF.

REFERENCES

1 **Tostivint H**, Lihrmann I, Vaudry H. New insight into the molecular evolution of the somatostatin family. *Mol Cell Endocrinol* 2008; **286**: 5-17 [PMID: 18406049 DOI: 10.1016/j.mce.2008.02.029]

2 **Chen YH**, Yandle TG, Richards AM, Palmer SC. Urotensin II immunoreactivity in the human circulation: evidence for widespread tissue release. *Clin Chem* 2009; **55**: 2040-2048 [PMID: 19797715 DOI: 10.1373/clinchem.2009.131748]

3 **Vaudry H**, Do Rego JC, Le Mevel JC, Chatenet D, Tostivint H, Fournier A, Tonon MC, Pelletier G, Conlon JM, Leprince J. Urotensin II, from fish to human. *Ann N Y Acad Sci* 2010; **1200**: 53-66 [PMID: 20633133 DOI: 10.1111/j.1749-6632.2010.05514.x]

4 **Leifeld L**, Clemens C, Heller J, Trebicka J, Sauerbruch T, Spengler U. Expression of urotensin II and its receptor in human liver cirrhosis and fulminant hepatic failure. *Dig Dis Sci* 2010; **55**: 1458-1464 [PMID: 19582578 DOI: 10.1007/s10620-009-0875-4]

5 **Liu LM**, Zhang JX, Luo J, Guo HX, Deng H, Chen JY, Sun SL. A role of cell apoptosis in lipopolysaccharide (LPS)-induced nonlethal liver injury in D-galactosamine (D-GalN)-sensitized rats. *Dig Dis Sci* 2008; **53**: 1316-1324 [PMID: 17934810]

6 **Nowak M**, Gaines GC, Rosenberg J, Minter R, Bahjat FR, Rectenwald J, MacKay SL, Edwards CK, Moldawer LL. LPS-induced liver injury in D-galactosamine-sensitized mice requires secreted TNF-alpha and the TNF-p55 receptor. *Am J Physiol Regul Integr Comp Physiol* 2000; **278**: R1202-R1209 [PMID: 10801288]

7 **Liu D**, Li C, Chen Y, Burnett C, Liu XY, Downs S, Collins RD, Hawiger J. Nuclear import of proinflammatory transcription factors is required for massive liver apoptosis induced by bacterial lipopolysaccharide. *J Biol Chem* 2004; **279**: 48434-48442 [PMID: 15345713 DOI: 10.1074/jbc.M407190200]

8 **Liang DY**, Liu LM, Ye CG, Zhao L, Yu FP, Gao DY, Wang YY, Yang ZW, Wang YY. Inhibition of UII/UTR system relieves acute inflammation of liver through preventing activation of NF-κB pathway in ALF mice. *PLoS One* 2014; **8**: e64895 [PMID: 23755157 DOI: 10.1371/journal.pone.0064895]

9 **Gong X**, Luo FL, Zhang L, Li HZ, Wu MJ, Li XH, Wang B, Hu N, Wang CD, Yang JQ, Wan JY. Tetrandrine attenuates lipopolysaccharide-induced fulminant hepatic failure in D-galactosamine-sensitized mice. *Int Immunopharmacol* 2010; **10**: 357-363 [PMID: 20036342 DOI: 10.1016/j.intimp.2009.12.010]

10 **Porstmann T**, Kiessig ST. Enzyme immunoassay techniques. An overview. *J Immunol Methods* 1992; **150**: 5-21 [PMID: 1613258 DOI: 10.1016/0022-1759(92)90061-W]

11 **Yin X**, Gong X, Jiang R, Zhang L, Wang B, Xu G, Wang C, Wan J. Synthetic RGDS peptide attenuated lipopolysaccharide/D-galactosamine-induced fulminant hepatic failure in mice. *J Gastroenterol Hepatol* 2014; **29**: 1308-1315 [PMID: 24476051 DOI: 10.1111/jgh.12525]

12 **Kemelo MK**, Wojnarová L, Kutinová Canová N, Farghali H. D-galactosamine/lipopolysaccharide-induced hepatotoxicity downregulates sirtuin 1 in rat liver: role of sirtuin 1 modulation in hepatoprotection. *Physiol Res* 2014; **63**: 615-623 [PMID: 24908092]

13 **Chojkier M**, Fierer J. D-Galactosamine hepatotoxicity is associated with endotoxin sensitivity and mediated by lymphoreticular cells in mice. *Gastroenterology* 1985; **88**: 115-121 [PMID: 3880554]

14 **Liu LM**, Zhang JX, Wang XP, Guo HX, Deng H, Luo J. Pim-3 protects against hepatic failure in D-galactosamine (D-GalN)-sensitized rats. *Eur J Clin Invest* 2010; **40**: 127-138 [PMID: 20039932 DOI: 10.1111/j.1365-2362.2009.02235.x]

15 **Ross B**, McKendy K, Giaid A. Role of urotensin II in health and disease. *Am J Physiol Regul Integr Comp Physiol* 2010; **298**: R1156-R1172 [PMID: 20421634 DOI: 10.1152/ajpregu.00706.2009]

16 **Kiss RS**, You Z, Genest J, Behm DJ, Giaid A. Urotensin II differentially regulates macrophage and hepatic cholesterol homeostasis. *Peptides* 2011; **32**: 956-963 [PMID: 21376094 DOI: 10.1016/j.peptides.2011.02.016]

17 **Cheung BM**, Leung R, Man YB, Wong LY. Plasma concentration of urotensin II is raised in hypertension. *J Hypertens* 2004; **22**: 1341-1344 [PMID: 15201550]

18 **Hassan GS**, Douglas SA, Ohlstein EH, Giaid A. Expression of urotensin-II in human coronary atherosclerosis. *Peptides* 2005; **26**: 2464-2472 [PMID: 16026900]

19 **Balat A**, Karakök M, Yilmaz K, Kibar Y. Urotensin-II immunoreactivity in children with chronic glomerulonephritis. *Ren Fail* 2007; **29**: 573-578 [PMID: 17654320]

20 **Liu D**, Chen J, Wang J, Zhang Z, Ma X, Jia J, Wang Y. Increased expression of urotensin II and GPR14 in patients with cirrhosis and portal hypertension. *Int J Mol Med* 2010; **25**: 845-851 [PMID: 20428787]

21 **Watanabe T**, Arita S, Shiraishi Y, Suguro T, Sakai T, Hongo S, Miyazaki A. Human urotensin II promotes hypertension and atherosclerotic cardiovascular diseases. *Curr Med Chem* 2009; **16**: 550-563 [PMID: 19199921]

22 **Ban Y**, Watanabe T, Suguro T, Matsuyama TA, Iso Y, Sakai T, Sato R, Idei T, Nakano Y, Ota H, Miyazaki A, Kato N, Hirano T, Ban Y, Kobayashi Y. Increased plasma urotensin-II and carotid atherosclerosis are associated with vascular dementia. *J Atheroscler Thromb* 2009; **16**: 179-187 [PMID: 19638714]

23 **Fukuda T**, Mogami A, Tanaka H, Yoshikawa T, Hisadome M, Komatsu H. Y-40138, a multiple cytokine production modulator, protects against D-galactosamine and lipopolysaccharide-induced hepatitis. *Life Sci* 2006; **79**: 822-827 [PMID: 16626762]

24 **Johns DG**, Ao Z, Naselsky D, Herold CL, Maniscalco K, Sarov-Blat L, Steplewski K, Aiyar N, Douglas SA. Urotensin-II-mediated cardiomyocyte hypertrophy: effect of receptor antagonism and role of inflammatory mediators. *Naunyn Schmiedebergs Arch Pharmacol* 2004; **370**: 238-250 [PMID: 15549273]

25 **Birker-Robaczewska M**, Boukhadra C, Studer R, Mueller C, Binkert C, Nayler O. The expression of urotensin II receptor (U2R) is up-regulated by interferon-gamma. *J Recept Signal Transduct Res* 2003; **23**: 289-305 [PMID: 14753294]

P- Reviewer: Ma YJ **S- Editor**: Decsi T, Hashimoto N, Vezali E
L- Editor: AmEditor **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3245

World J Gastroenterol 2015 March 21; 21(11): 3245-3255
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

MicroRNA-1290 promotes esophageal squamous cell carcinoma cell proliferation and metastasis

Ming Li, Xiao-Yan He, Zhi-Mei Zhang, Shuo Li, Li-Hua Ren, Ri-Sheng Cao, Ya-Dong Feng, Yin-Lin Ji, Ye Zhao, Rui-Hua Shi

Ming Li, Xiao-Yan He, Zhi-Mei Zhang, Shuo Li, Li-Hua Ren, Ri-Sheng Cao, Ya-Dong Feng, Yin-Lin Ji, Ye Zhao, Rui-Hua Shi, Department of Gastroenterology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China
Ming Li, Department of Gastroenterology, Friendliness Hospital of Yangzhou, Yangzhou 225009, Jiangsu Province, China
Zhi-Mei Zhang, Department of Gastroenterology, First People's Hospital of Lianyungang, Lianyungang 222002, Jiangsu Province, China
Author contributions: Li M designed the research; He XY, Zhang ZM, Li S and Ren LH performed the research; Cao RS and Feng YD collected the clinical data; Ji YL, Shi RH and Zhao Y analyzed the data; and Li M wrote the paper.
Supported by grants from Innovative Team Project of Jiangsu Province, China, No. CXZZ11_0705.

Correspondence to: Rui-Hua Shi, MD, PhD, Department of Gastroenterology, The First Affiliated Hospital of Nanjing Medical University, No. 300 Guang Zhou Road, Gulou District, Nanjing 210029, Jiangsu Province. China. ruihuashi@126.com
Telephone: +86-25-83674636
Fax: +86-25-83718836
Received: August 8, 2014
Peer-review started: August 9, 2014
First decision: September 27, 2014
Revised: October 11, 2014
Accepted: December 16, 2014
Article in press: December 16, 2014
Published online: March 21, 2015

Abstract

AIM: To investigate the biological role of miR-1290 in esophageal squamous cell carcinoma (ESCC) progression and invasion and the underlying mechanism.

METHODS: Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to evaluate miR-1290 expression in ESCC tissue samples. The roles of miR-1290 in cell proliferation, migration and invasion were identified using miR-1290 mimic-transfected cells. In addition, the regulatory effect of miR-1290 on suppressor of cancer cell invasion (SCAI) was evaluated using qRT-PCR, Western blot analysis and a dual luciferase reporter assay.

RESULTS: miR-1290 was significantly upregulated in ESCC tissue samples compared with normal adjacent tissues (9.213 ± 1.150 *vs* 1.000 ± 0.0), ($P < 0.01$). Upregulation of miR-1290 was associated with tumor differentiation ($P = 0.021$), N classification ($P = 0.006$) and tumor-node-metastasis stage ($P = 0.021$) in ESCC patients. Moreover, ectopic miR-1290 expression potently promoted ESCC cell growth ($P < 0.01$), migration ($P < 0.01$) and invasion ($P < 0.01$) *in vitro*. miR-1290 overexpression in ESCC cell lines decreased SCAI expression at the translational level and reduced SCAI-driven luciferase-reporter activity ($P < 0.01$).

CONCLUSION: Our findings suggested that miR-1290 may play an oncogenic role in cellular processes of ESCC.

Key words: MicroRNA; MiR-1290; Esophageal squamous cell carcinoma; Suppressor of cancer cell invasion; Invasion; Metastasis

Core tip: In this study, we reported the clinical significance and biological effects of miR-1290 in esophageal squamous cell carcinoma (ESCC). We found that miR-1290 was significantly up-regulated in ESCC tissues. Moreover, we showed that ectopic expression of miR-1290 significantly promoted ESCC cell growth, migration and invasion. Further investigation revealed that suppressor of cancer cell invasion was a downstream target of miR-1290.

Li M, He XY, Zhang ZM, Li S, Ren LH, Cao RS, Feng YD, Ji YL, Zhao Y, Shi RH. MicroRNA-1290 promotes esophageal squamous cell carcinoma cell proliferation and metastasis. *World J Gastroenterol* 2015; 21(11): 3245-3255 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3245.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3245

INTRODUCTION

Esophageal cancer accounts for 2% of all human malignant tumors and is the sixth leading cause of cancer death. Esophageal cancer morbidity evidently varies with geographic location; for example, this cancer type is common in China, Japan and Africa in both males and females[1]. In China, esophageal squamous cell carcinoma (ESCC) is currently the major histologic subtype of esophageal cancer. Despite advances in therapeutic methods, ESCC remains one of the most common malignancies in China with an overall five-year survival rate of 20% after surgery[2].

Tumor proliferation and metastasis are main factors responsible for ESCC mortality. However, the molecular mechanism of proliferation and metastasis remains unclear[3]. Studies have shown that microRNAs (miRNAs) play a considerable role in tumor dissemination[4,5]. miRNAs are a group of endogenous small non-coding RNAs of approximately 22 nucleotides that inversely regulate gene expression by imprecisely binding to a complementary sequence in the 3′-untranslated region (UTR) of their target mRNAs[6]. miRNAs play an important role in gene regulation, cell differentiation, proliferation, apoptosis and tumor genesis[7]. These miRNAs function as either oncogenes or tumor suppressors[8,9]. A study has shown that patients in the same pathological stage of esophageal cancer receiving identical surgical therapy by the same surgeon but with differences in miRNA expression may have distinct prognoses[10].

Previous study have shown that miR-1290 is upregulated in colon cancer cells and osteosarcoma cells[11,12]. These studies have also demonstrated that miR-1290 functions as an oncogenic miRNA. However, the association between miR-1290 and ESCC has not been evaluated yet, and the biological value of miR-1290 in ESCC remains poorly understood.

Suppressor of cancer cell invasion (SCAI) is a tumor-suppressor gene that is downregulated in several human tumors. Decreased SCAI levels are tightly correlated with increased invasive cell migration[13]. Nevertheless, whether miR-1290 can regulate SCAI expression in human ESCC cells remains unknown.

In the present study, we investigated the relative miR-1290 expression level between tumor and normal tissues; we further studied the possible mechanism of miR-1290 in ESCC metastasis. The results showed that the expression level of miR-1290 in ESCC tissues was higher than that in normal adjacent tissues. Moreover, miR-1290 overexpression promoted colony formation, proliferation, migration and invasion; miR-1290 overexpression also reduced SCAI mRNA and protein levels in Eca109 and TE13 human ESCC cells *in vitro*. Understanding the molecular mechanisms of miR-1290 in the initiation and progression of human ESCC could provide the basis for developing a treatment strategy for ESCC.

MATERIALS AND METHODS

Patient samples and RNA extraction

A total of 24 matched human ESCC tumor tissues and normal adjacent tissues (NAT) were collected directly after surgical resection was performed at the First Affiliated Hospital, Nanjing Medical University (China). All of the tissue samples from patients with no prior neoadjuvant treatment were immediately frozen in liquid nitrogen and stored at -80 ℃ until miRNA was extracted. Clinicopathological information for all of the samples was available. ESCC tumors were graded according to the 2010 WHO classification of the tumors of digestive system[14]. Our research protocol was approved by the Ethics Review Committee of the Institutional Review Board of the hospital. Standard written consent was obtained from each patient. Total RNA was extracted from tissue samples and cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's protocol.

Cell lines and oligonucleotide transfection

Human ESCC cell lines Eca109 and TE13 were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). All of the cell lines were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (Invitrogen) with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, MD, United States) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen) at 37 ℃ in a humidified chamber with 5% CO_2. Hsa-miR-1290 mimic (sense 5′-UGGAUUUUUGGAUCAGGGA-3′, antiDallasense 5′-CCUGAUCCAAAAAUCCAUU-3′), negative control oligonucleotide (sense 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense 5′-ACGUGACACGUUCGGAGAATT-3′), has-miR-1290 inhibitor (sequence 5′-UCCCUGAUCCAAAAAUCCA-3′) and miRNA inhibitor negative control (sequence 5′

-CAGUACUUUUGUGUAGUACAA-3′) were synthesized by Genepharma (Shanghai, China). Ectopic miR-1290 expression in the cells was achieved by performing transfection with has-miR-1290 mimic using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions.

Quantitative real-time PCR

For quantitative real-time PCR (qRT-PCR), 2 μg of total RNA was reverse transcribed with Bulge-Loop™ miRNA-specific reverse transcription primers (RiboBio, Guangzhou, China). Quantitative PCR was performed with SYBR Premix Ex Taq (Takara, Dalian, China) reagents and Bulge-Loop™ primers (RiboBio, Guangzhou, China) in an ABI PRISM 7900 system (Applied Biosystems, Carlsbad, CA, United States) with small nuclear RNA U6 as a normalization control. The mRNA levels of SCAI were determined by qRT-PCR using SYBR Premix Ex Taq (Takara, Dalian, China) in an ABI PRISM 7900 Sequence Detection System and normalized to GAPDH levels. The SCAI primers used were: forward, 5′-AAGCAGTGGCAGTCCTATTTTG-3′ and reverse, 5′-GCTTCAAGCCATACCGATTATCC-3′. GAPDH primers were: forward, 5′-CGGAGTCAACGGATTTGGTCGTAT-3′ and reverse, 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′ (HuaGene, Shanghai, China). All of the samples were normalized to internal controls, and fold changes were calculated using $2^{-\Delta\Delta Ct}$.

Cell proliferation and apoptosis assays

For cell proliferation assays, cells were seeded in 96-well plates with 3000 cells for Eca109 and 2000 cells for TE13 per well on 0 d (24 h after has-miR-1290 mimic or has-miR-1290 inhibitor was transfected and negative control was administered). On 1, 2, 3, 4 and 5 d, cell viability was determined using cell counting kit-8 (CCK-8, Beyotime, Haimen, China). In brief, 10 μL of CCK8 solution was added to each well of the 96-well plate, and the plate was incubated for 2 h in an incubator. After the incubation was performed, the plates were washed with phosphate-buffered saline (PBS). The absorbance of each well was read at a wavelength of 450 nm and a proliferation curve on the basis of absorbance and time was constructed. To perform flow cytometry analysis (FCM) of cell apoptosis, an Annexin V-FITC/PI apoptosis detection kit (KeyGEN, Nanjing, China) was used. At 48 h after transfection, cells were harvested and washed with PBS twice, incubated with Annexin V-FITC and propidium iodide for 15 min in a dark room and analyzed by flow cytometry according to the manufacturer's instructions.

Colony formation assay

After transfection was performed, 300 cells were seeded in each well of a six-well culture plate and incubated for 14 d. Fresh culture medium was replaced at an interval of 3 d. The cells were fixed with 75% ethanol and stained with 0.5% crystal violet, and the number of colonies containing > 50 cells was counted.

Migration and invasion assays

To perform transwell migration or invasion assays, we placed Eca109 or TE13 cells (1×10^5 cells/well) transfected with has-miR-1290 mimic, has-miR-1290 inhibitor or negative control in 0.2 mL of RPMI-1640 without FBS in the upper chamber of each insert (8 μm pore size, BD Biosciences, United States) with or without 60 μL of 1 mg/mL Matrigel (BD, Biosciences, Bedford, MD, United States). The lower chamber was filled with 600 μL of RPMI1640 medium containing 10% FBS as a nutritional attractant. After 28 h, the cells on the top surface of the membrane were carefully removed using a cotton swab. Migrant cells attached to the lower surface were fixed with 75% methanol and stained with crystal violet for 30 min. The number of cells was counted in five different fields of view using an inverted microscope (magnification × 100).

miRNA target prediction

The putative targets of miRNA were predicted using TargetScan (www.targetscan.org) and miRanda (www.microRNA.org).

Dual luciferase reporter assays

The 3′-UTR of SCAI containing the predicted miR-1290 binding seed sequence was synthesized and directly cloned downstream of the firefly luciferase gene at XbaI sites to create a pGL3-SCAI-3′UTR-wt plasmid (5′-ACCCUGAGAAGAGUAAAUCAUUUAUUUUUGUAUA-AUGAGGU**AAAUCCA**ACUCUUAUACUUGGACCUAAGU-UAAAUGUCUGGAUUUGGA-3′; Invitrogen, Shanghai, China). The corresponding mutant reporter plasmid (pGL3-SCAI-3′UTR-mut; 5′-ACCCUGAGAA-GAGUAAAUCAUUUAUUUUUGUAUAAUGAGGU**UA-GUCUA**ACUCUUAUACUUGGACCUAAGUUAAAUGUCU-GGAUUUGGA-3′) was then synthesized. The pGL3-SCAI-3′UTR-wt/-mut reporter plasmid (800 ng) and phRL-TK vector (800 ng) expressing *Renilla* luciferase (Invitrogen) were co-transfected in Eca109 and TE13 cells with 80 ng has-miR-1290 mimic and negative control using Lipofectamine 2000 reagent (Invitrogen). After 48 h, cells were harvested and lysed with passive lysis buffer (Promega). Luciferase activity was determined using a dual-luciferase reporter assay system (Promega, Madison, WI, United States) according to the manufacturer's protocol. *Renilla* luciferase activities were used for normalization.

Western blot analysis

Western blot analysis was performed to detect the protein expression of SCAI in ESCC tissues and cell lines. The cells were lysed 48 h post-transfection with RIPA lysis buffer (Beyotime, Jiangsu, China) containing protease inhibitor; the proteins were then harvested. Total protein content was quantified by BCA assay

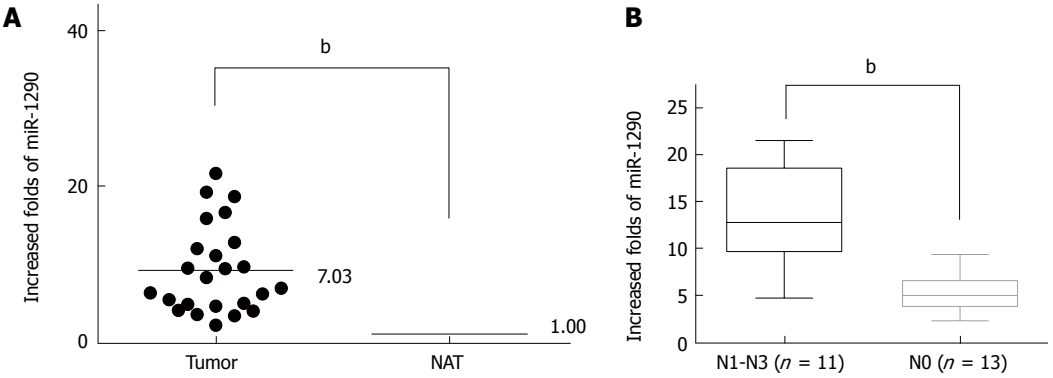


Figure 1 miR-1290 expression is upregulated in clinical specimens. A: qRT-PCR analysis showed that miR-1290 expression was upregulated in ESCC tissues compared with paired normal adjacent tissues; B: miR-1290 expression was significantly higher in ESCC patients with lymph node metastasis compared with patients without lymph metastasis $^{b}P < 0.01$ between groups. Small nuclear RNA U6 was used as a normalization control. NAT: Normal adjacent tissues; qRT-PCR: Quantitative real-time polymerase chain reaction; ESCC: Esophageal squamous cell carcinoma.

Table 1 Association of miR-1290 upregulation with clinicopathological characteristics of 24 patients with esophageal squamous cell carcinoma

Characteristic	Total (n = 24)	miR-1290 expression		P value
		High	Low	
Age (yr)				
< 60	12	5	7	0.682
⩾ 60	12	6	6	
Gender				
Female	10	5	5	0.729
Male	14	6	8	
Differentiation				
High	13	3	10	0.021
Middle + low	11	8	3	
T classification				
T1 + T2	10	3	7	0.240
T3 + T4	14	8	6	
N classification				
N0	13	3	10	0.006
N1-N3	11	8	3	
TNM Stage				
Ⅰ + Ⅱ	15	4	11	0.021
Ⅲ	9	7	2	

TNM: Tumor-node-metastasis.

(Beyotime). Equal amounts of protein extracts (30 to 40 ng) were separated using 8% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, United States). Afterwards, blots were blocked with 5% fat-free milk powder for 1 h. The membranes were incubated overnight at 4 ℃ in a 1:500 dilution of anti-human SCAI rabbit monoclonal antibody (Abcam, Cambridge, MA, United States). The blots were subsequently incubated with a horseradish peroxidase-conjugated secondary antibody (1:5000) and visualized using a super enhanced chemiluminescence detection reagent (Amersham Biosciences, Piscataway, NJ). Protein expression was assessed using Alpha Innotech imaging software (San Leandro, CA). GAPDH was used as an endogenous protein for normalization.

Statistical analysis

Data are presented as mean ± standard deviation (SD) from at least three independent experiments. Statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, United States). The difference between groups was analyzed using a two-tailed Student's *t*-test to compare two independent groups only and ANOVA followed by Student-Newman-Keuls *Q* test to compare two groups among three groups. The relationship between miR-1290 and SCAI expression was explored by Spearman's correlation analysis. Significant associations between miR-1290 changes and clinicopathological parameters were assessed using a χ^2 test. Two-sided *P*-values < 0.05 were considered statistically significant.

RESULTS

Relative miR-1290 expression level is specifically upregulated and correlated with lymph node metastasis and tumor-node-metastasis stage in patients with ESCC

Relative miR-1290 expression was detected by qRT-PCR between paired tumor tissues and normal adjacent tissues from 24 patients with ESCC. Our results demonstrated that the relative fold increases in miR-1290 expression were markedly upregulated in ESCC samples compared with the paired tumor-adjacent tissues (9.213 ± 1.150 *vs* 1.000 ± 0.0), (P < 0.01; Figure 1A). To evaluate the clinical value of miR-1290 in ESCC patients, we divided the patients into two groups according to the median value (6.6181) of miR-1290 level. The association between relative miR-1290 expression and clinicopathological information was then analyzed. A significant difference was observed between the two groups in terms of differentiation (P = 0.021), N classification (P = 0.006) and tumor-node-metastasis stage (P = 0.021) (Figure 1B, Table 1). No significant association was found between miR-1290 expression and other clinical characteristics, such as age, gender and T classification (Table 1). Hence, upregulated miR-1290 expression

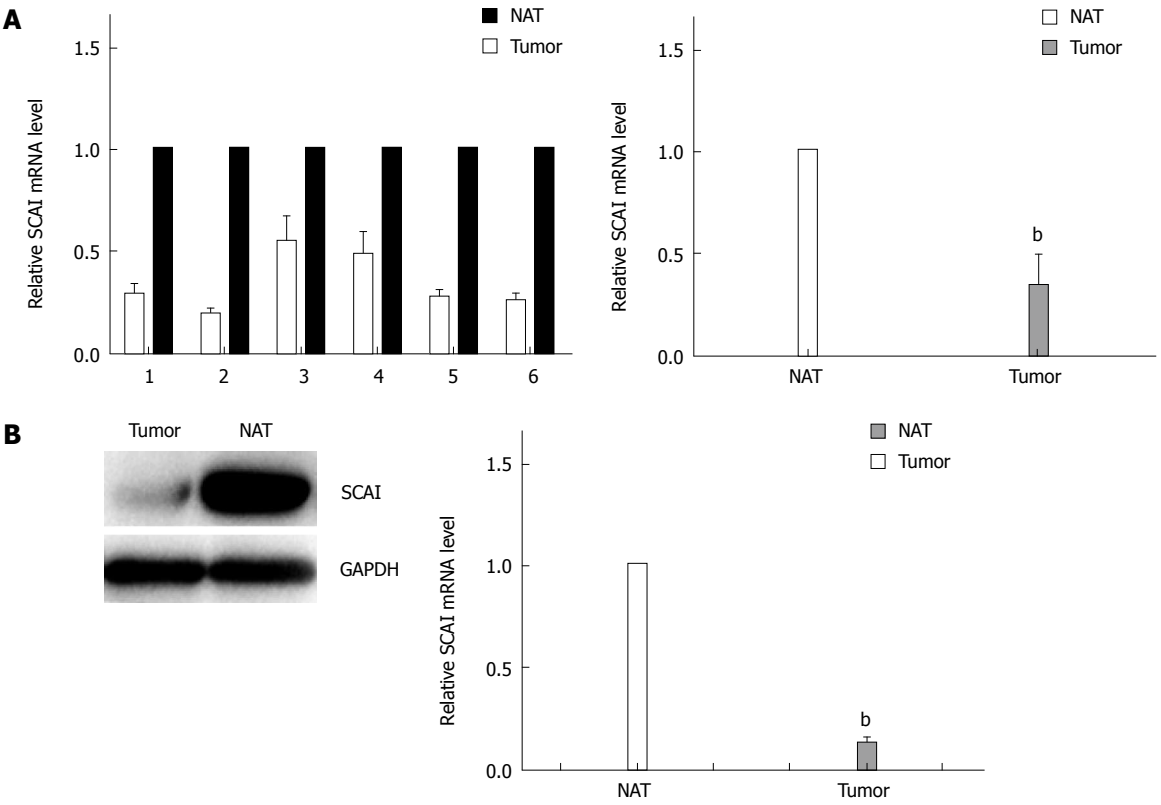


Figure 2 mRNA and protein expression of suppressor of cancer cell invasion is downregulated in clinical esophageal squamous cell carcinoma specimens. A: Quantitative real-time PCR analysis showed that the relative mRNA expression of SCAI was downregulated in ESCC tissues compared with that in paired adjacent normal tissues, $n = 6$; B: Protein expression level of SCAI was significantly decreased in ESCC compared with that in paired adjacent normal tissues, $n = 6$. $^{b}P < 0.01$ *vs* control. NAT: Normal adjacent tissues; SCAI: Suppressor of cancer cell invasion; PCR: Polymerase chain reaction; ESCC: Esophageal squamous cell carcinoma.

was closely related to ESCC metastasis.

mRNA and protein expression of SCAI is downregulated in ESCC tissues

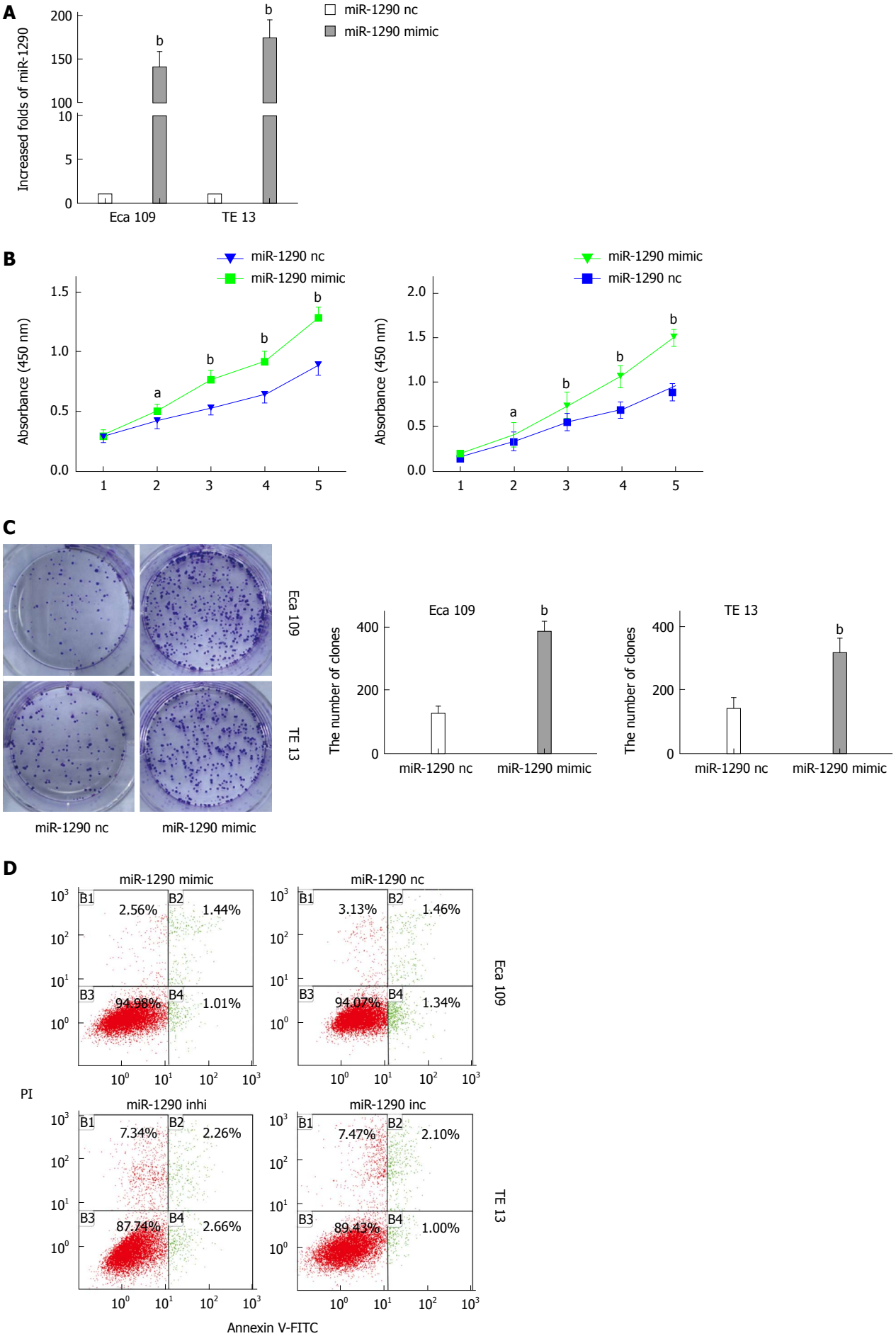
The mRNA and protein expression of SCAI in ESCC tissues was analyzed by qRT-PCR and Western blot analysis between paired tumor tissues and normal adjacent tissues from six patients with ESCC. These results showed that the relative mRNA and protein expression of SCAI was downregulated in ESCC tissues ($P < 0.01$; Figure 2A, B), which is in accordance with the results from a previous study[15].

miR-1290 promotes proliferation in ESCC cell lines

The significantly increased expression of miR-1290 in ESCC tissues prompted us to investigate the possible biological function of miR-1290 in tumorigenesis. qRT-PCR analysis results showed that miR-1290 expression increased by more than 100-fold in Eca109 and TE13 cells transfected with has-miR-1290 mimic compared with the control cells ($P < 0.01$; Figure 3A). A CCK8 staining assay revealed that miR-1290 promoted significant proliferation in Eca109 and TE13 cell lines transfected with has-miR-1290 mimic compared with the control cells ($P < 0.01$; Figure 3B). We also evaluated the ability of Eca109 and TE13 cell lines transfected with has-miR-1290 mimic to form colonies. Our data indicated that miR-1290 significantly stimulated Eca109 and TE13 cells to grow numerous and large colonies on soft agar ($P < 0.01$; Figure 3C). miR-1290 overexpression in Eca109 cells and miR-1290 under-expression in TE13 cells did not significantly change the apoptotic ability of cells (Figure 3D). The results revealed that miR-1290 enhanced the proliferation ability of ESCC cells.

miR-1290 overexpression promotes the migration and invasion of ESCC cell lines

To understand the biological effects of miR-1290 overexpression on the migration and invasion of ESCC cell lines *in vitro*, we performed transwell assays by transfecting Eca109 and TE13 cell lines with has-miR-1290 mimic or inhibitor. Matrigel-coated (for invasion) or uncoated (for migration) Transwell assays revealed that miR-1290 overexpression markedly promoted the invasion and migration of Eca109 and TE13 cells ($P < 0.01$; Figures 4A, C). The effect of miR-1290 under-expression was examined. As expected, miR-1290 under-expression remarkably decreased the invasion capabilities of Eca109 and TE13 cells ($P < 0.01$; Figure 4B, D). These observations suggested that miR-1290 significantly promoted the *in*


A
Increased folds of miR-1290
miR-1290 nc
miR-1290 mimic
Eca 109
TE 13
B
Absorbance (450 nm)
miR-1290 nc
miR-1290 mimic
C
Eca 109
TE 13
miR-1290 nc
miR-1290 mimic
The number of clones
D
miR-1290 mimic
miR-1290 nc
miR-1290 inhi
miR-1290 inc
PI
Annexin V-FITC
2.56%
1.44%
94.98%
1.01%
3.13%
1.46%
94.07%
1.34%
7.34%
2.26%
87.74%
2.66%
7.47%
2.10%
89.43%
1.00%

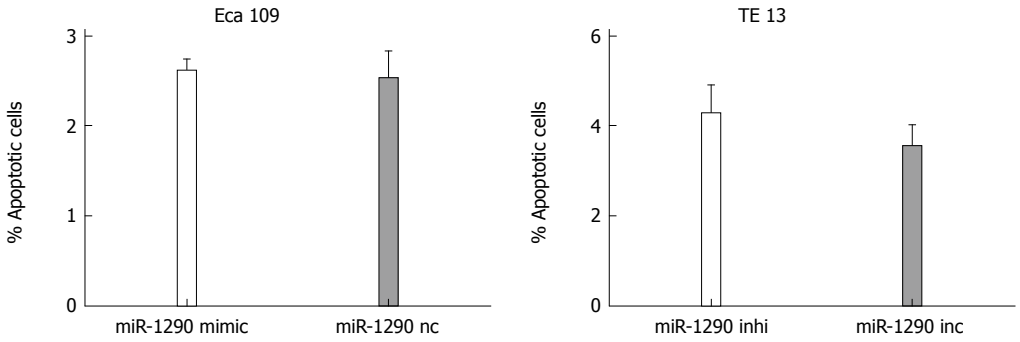
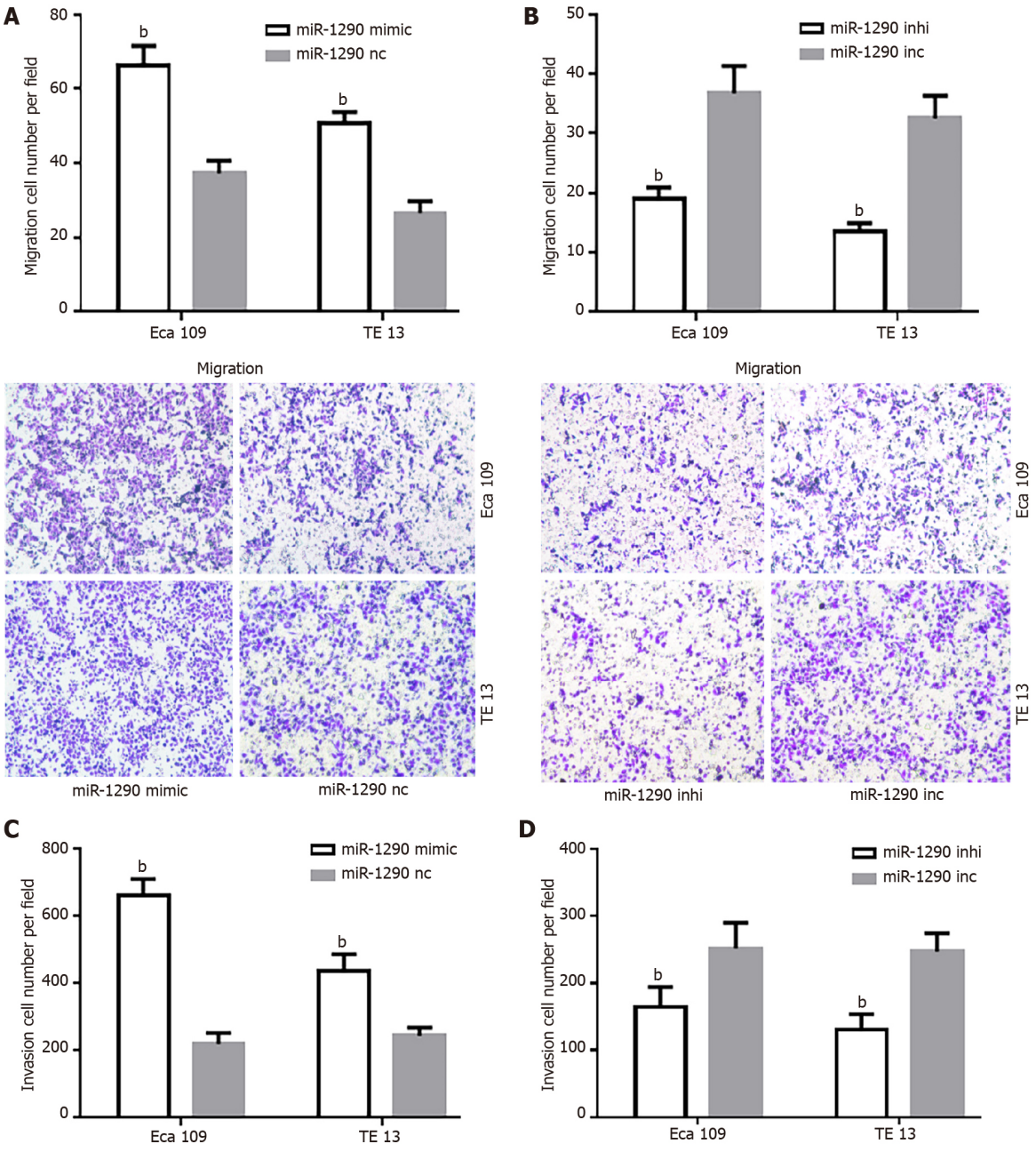


Figure 3 MiR-1290 overexpression promotes the growth of Eca109 and TE13 cells. A: miR-1290 expression was significantly higher in cells transfected with miR-1290 mimic than in negative control cells; B: CCK8 assay showed that miR-1290 promoted Eca109 and TE13 cell proliferation; C: Colony formation assay showed that miR-1290 enriched the colony formation of Eca109 and TE13 cells; D: miR-1290 overexpression in Eca109 cells and miR-1290 under-expression in TE13 cells did not significantly alter cell apoptosis ability. $^{b}P < 0.01$ *vs* control; $^{a}P < 0.05$ *vs* control. nc: Negative control; inhi: Inhibitor; inc: Inhibitor negative control.



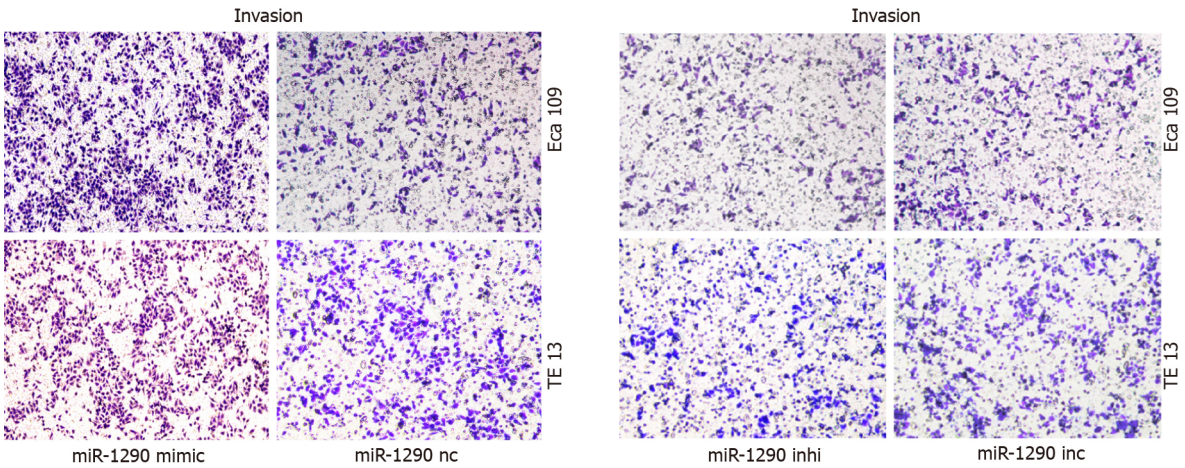


Figure 4 MiR-1290 enhances the migration and invasion of Eca109 and TE13 cells. Transwell migration assays were carried out in Eca109 and TE13 cells transfected with either miR-1290 mimic (A) or miR-1290 inhibitor (B). Representative fields of migrating cells on the membrane are presented. The histogram shows the number of migrating cells from three independent experiments. Overexpression (C) and under-expression of miR-1290 in Eca109 and TE13 cells (D) showed similar results in both invasion and migration assays. $^{b}P < 0.01$ *vs* control. nc: Negative control; inhi: Inhibitor; inc: Inhibitor negative control.

vitro migration and invasion of ESCC cell lines.

miR-1290 negatively regulates mRNA and protein expression of SCAI in vitro

To further observe the correlation of miR-1290, SCAI mRNA and protein expression in Eca109 and TE13 cell lines, we performed qRT-PCR and Western blot analyses. qRT-PCR analysis results indicated a decrease in the mRNA expression of SCAI in miR-1290-overexpressing cells, whereas miR-1290- under-expressing cells had increased mRNA levels of SCAI ($P < 0.01$; Figure 5A). Similar changes were found in SCAI protein levels from Western blot assays ($P < 0.01$; Figure 5B). These findings confirmed that miR-1290 overexpression downregulated the mRNA and protein expression of SCAI in Eca109 and TE13 cell lines.

miR-1290 directly binds to the 3'-UTR of SCAI

The putative miR-1290 target genes were predicted using the target prediction programs TargetScan and miRDB. SCAI was identified as a candidate miR-1290 target gene and sequence analysis results indicated that miR-1290 target sequence at 330 nt to 336 nt of the SCAI 3′-UTR was highly conserved across different species (Figure 6A). The relationship between the SCAI mRNA level and miR-1290 in 24 ESCC tissues was tested using qRT-PCR, and data showed an clearly negative relationship between expression SCAI and miR-1290 ($r = -0.842$, $P = 0.000$, Figure 6B). To validate whether SCAI is a valid target of miR-1290, we inserted wild-type or mutant SCAI 3′-UTR sequence in the downstream region of the luciferase reporter gene and co-expressed these sequences with either has-miR-1290 mimic or has-miR-1290 nc in Eca109 cells. miR-1290 overexpression caused an unambiguous decrease in relative luciferase activity ($P < 0.01$; Figure 5C); in contrast, activity did not decrease in the mutant 3′-UTR reporter, indicating that functionality depends on an intact seed sequence. Therefore, SCAI can be directly suppressed by miR-1290 *via* mRNA degradation and translation repression.

DISCUSSION

Evidence has shown anomalous miRNA expression in various types of human tumors[16,17]. Thus, studies have focused on tumor-associated miRNAs and specific target genes to elucidate biological mechanism. The identification of cancer-specific miRNAs and their target genes is necessary for understanding their role in tumor metastasis, which may be a requisite to define new therapeutic targets. miR-1290 is upregulated in several cancer forms[11,18]. Therefore, whether miR-1290 is upregulated in ESCC and whether miR-1290 can promote ESCC cell metastasis are postulated.

In the present study, miR-1290 expression in 24 pairs of human ESCC tumor tissues and normal adjacent tissues was determined by qRT-PCR. The miR-1290 expression was upregulated in ESCC. The upregulated miR-1290 in human ESCC may promote the tumor initiation and progression; this result indicates that miR-1290 may play an oncogenic role[11]. Before this study was conducted, however, the role of miR-1290 and its target genes was unclear in ESCC. Therefore, this study focused on the biological mechanisms of miR-1290 in human ESCC.

To characterize the functions of miR-1290 in human ESCC, we examined the changes in Eca109 and TE13 cell lines after miR-1290 was overexpressed and under-expressed. A CCK8 assay was performed to analyze the cell viability in Eca109 and TE13 cell lines; our results showed that miR-1290 enhanced cell viability. In the colony formation assay, however, miR-1290 promoted colony formation activities of Eca109 and TE13 cell lines. These findings showed that ectopic miR-1290 expression affected cell viability over a short time period and enhanced ESCC cell proliferation. Transwell assays with or without Matrigel were conducted to determine

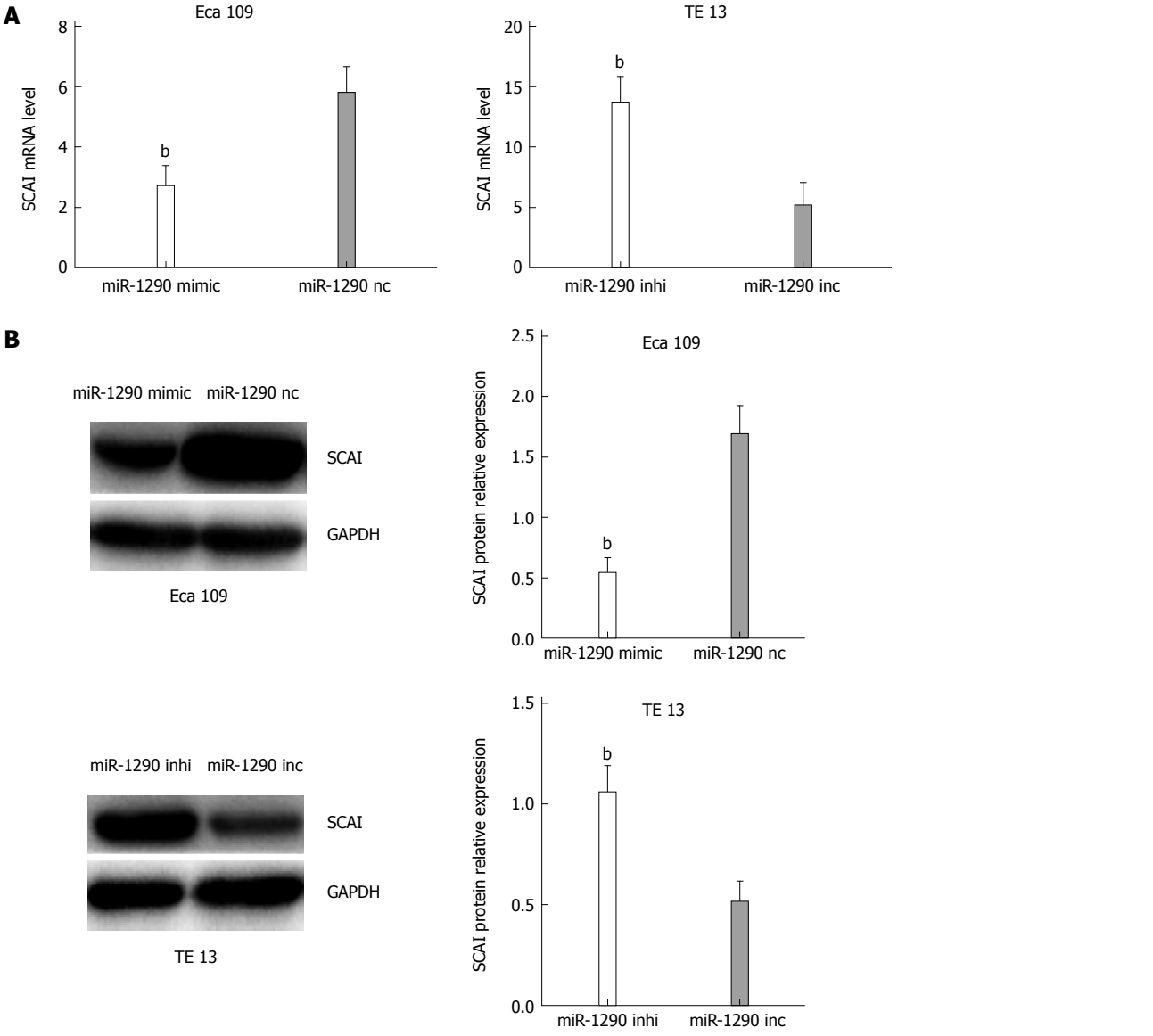


Figure 5 miR-1290 negatively regulates the mRNA and protein expression of suppressor of cancer cell invasion. A: mRNA expression of SCAI in Eca109 cells transfected with miR-1290 mimic decreased evidently compared with that in cells transfected with miR-1290 nc. Conversely, the mRNA expression of SCAI in TE13 cells transfected with miR-1290 inhibitor increased evidently compared with that in cells transfected with miR-1290 inc; B: Protein expression of SCAI exhibited a similar pattern in Eca109 cells transfected with miR-1290 mimic and miR-1290 nc or in TE13 cells transfected with miR-1290 inhibitor and miR-1290 inc. $^{b}P < 0.01$ *vs* control. nc: Negative control; inhi: Inhibitor; inc: Inhibitor negative control; SCAI: Suppressor of cancer cell invasion.

the functions of miR-1290 in the migration and invasion of Eca109 and TE13 cells. Our results demonstrated that miR-1290 overexpression significantly accelerated the migration and invasion of Eca109 and TE13 cells compared with the control group. Conversely, the migratory and invasive abilities were markedly decreased in Eca109 and TE13 cells transfected with has-miR-1290 inhibitor. In summary, miR-1290 promoted ESCC colony formation, migration and invasion in Eca109 and TE13 cell lines.

In this research, the role of miR-1290 in targeting SCAI in human Eca109 and TE13 cell lines was considered. Using bioinformatics technology, we confirmed SCAI as one of the target genes of miR-1290. SCAI functions in the RhoA-Dia1 signal transduction pathway and localizes in the nucleus, where it binds and inhibits myocardin-related transcription factor MAL by forming a ternary complex with the serum response factor (SRF)[13]. In a previous study[15], it was found that protein and mRNA expression of SCAI was significantly downregulated in glioma tissues and cell lines. SCAI silencing robustly promoted invasive and cancer stem cell-like phenotypes of glioma cells. Furthermore, SCAI downregulation activated Wnt/beta-catenin signaling and Wnt/beta-catenin pathway inhibition abrogated the effects of SCAI downregulation on glioma cell aggressiveness. SCAI acts as a transcriptional modulator to regulate cancer cell motility by suppressing MAL/SRF-dependent gene transcription[19]. These studies have suggested that SCAI may be involved in cancer development. Thus, a lower SCAI expression level was observed in ESCC than in adjacent normal tissues;

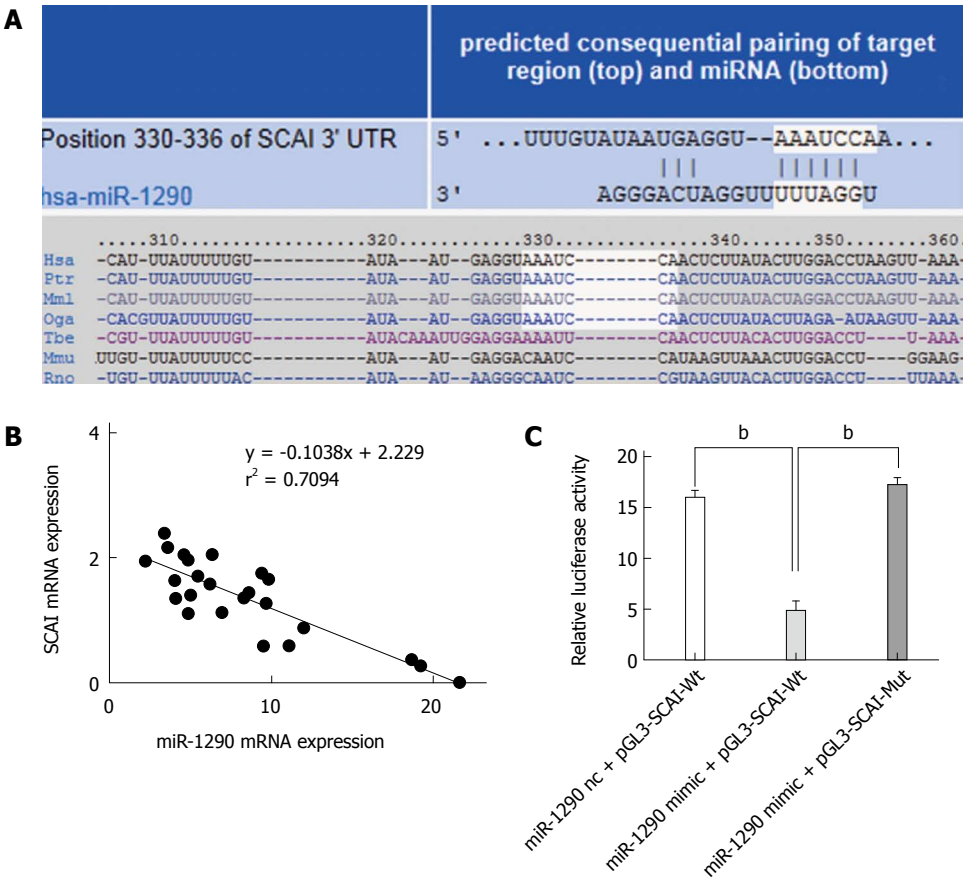


Figure 6 Suppressor of cancer cell invasion is a direct target of miR-1290. A: Putative miR-1290 binding sequence in the 3'-UTR of the mRNA of SCAI is shown; B: Inverse correlation between miR-1290 and mRNA levels of SCAI in tissue samples was illustrated; C: Dual-luciferase reporter assay showed a significant decrease in the relative luciferase activity of wt-*SCAI* (wild-type *SCAI* 3'-UTR) co-transfected with miR-1290 mimic compared with that of mut-*SCAI* (mutant-type *SCAI* 3'-UTR; $^{b}P < 0.01$ *vs* control) in Eca109 cells. SCAI: Suppressor of cancer cell invasion.

miR-1290 expression was inversely correlated with SCAI expression in tumor tissues. Furthermore, the relative fluorescence intensity of pGL3-SCAI-3′UTR-wt was specifically responsive to miR-1290 overexpression. A mutation in the miR-1290 binding site abolished the effect of miR-1290 on the regulation of fluorescence intensity. An increase in mRNA and protein levels of SCAI was found in Eca109 and TE13 cells transfected with a miR-1290 inhibitor. These results suggested that SCAI is a target of miR-1290 and is negatively regulated. SCAI may also exhibit anti-proliferative and anti-malignant transformation effects in Eca109 and TE13 cell lines.

In summary, miR-1290 expression was upregulated in ESCC tissues; miR-1290 elicited oncogenic effects, including the promotion of ESCC cell proliferation, migration and invasion, by targeting the anti-oncogene SCAI, highlighting the function of miR-1290 in tumor progression.

COMMENTS

Background

Esophageal cancer is the sixth leading cause of cancer-related deaths in China. Although recent developments in therapeutic strategies have helped cure many patients with early stage disease, the prognosis of patients with advanced disease and metastasis remains poor.

Research frontiers

miRNAs have been found to be involved in the regulation of multiple pathological processes that contribute to tumorigenesis and metastasis, such as tumor cell proliferation, differentiation, apoptosis, and invasion. In esophageal squamous cell carcinoma (ESCC), studies have indicated that miRNAs play important roles in regulating tumor invasion and metastasis. Previously, Zhang *et al* reported that miR-100 promoted migration and invasion through mammalian target of rapamycin in ESCC. However, the role of miR-1290 in ESCC progression and metastasis remains unclear and needs further exploration.

Innovations and breakthroughs

The authors found that the level of miR-1290 was significantly up-regulated in ESCC tissues compared with normal adjacent tissues. Ectopic expression of miR-1290 markedly promoted the proliferation, invasion and metastasis in ESCC cell lines. Further analysis indicated that the suppressor of cancer cell invasion (SCAI) was a direct downstream target of miR-1290. Collectively, these results demonstrated that miR-1290 promoted cell invasion and metastasis by targeting SCAI, thus providing a valuable target for cancer therapy.

Applications

The findings in this study indicated that miR-1290 was significantly up-regulated in ESCC with distant metastases. Further investigation identified that the SCAI was a direct target of miR-1290. Taken together, these data implicate that miR-1290 might be used a prognostic indicator and therapeutic target in ESCC patients.

Terminology

MicroRNAs: A group of small non-coding RNA molecules (approximately 22 nucleotides in length) found in plants, animals, and some viruses that function in transcriptional and post-transcriptional regulation of gene expression.

Peer-review

The authors used clinical samples for expression analyses, and a number of experimental state-of-the-art techniques to investigate the respective questions in an *in vitro* model. The different steps of the manuscript are logical, and the results present some very interesting findings about the role of miR-1290 in esophageal squamous cell carcinoma.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **McCann J**. Esophageal cancers: changing character, increasing incidence. *J Natl Cancer Inst* 1999; **91**: 497-498 [PMID: 10088616 DOI: 10.1093/jnci/91.6.497]

3 **Gupta GP**, Massagué J. Cancer metastasis: building a framework. *Cell* 2006; **127**: 679-695 [PMID: 17110329 DOI: 10.1016/j.cell.2006.11.001]

4 **Zhu M**, Zhang N, He S, Lui Y, Lu G, Zhao L. MicroRNA-106a targets TIMP2 to regulate invasion and metastasis of gastric cancer. *FEBS Lett* 2014; **588**: 600-607 [PMID: 24440352 DOI: 10.1016/j.febslet.2013.12.028]

5 **Yang MH**, Yu J, Jiang DM, Li WL, Wang S, Ding YQ. microRNA-182 targets special AT-rich sequence-binding protein 2 to promote colorectal cancer proliferation and metastasis. *J Transl Med* 2014; **12**: 109 [PMID: 24884732 DOI: 10.1186/1479-5876-12-109]

6 **Huntzinger E**, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat Rev Genet* 2011; **12**: 99-110 [PMID: 21245828 DOI: 10.1038/nrg2936]

7 **Sotillo E**, Thomas-Tikhonenko A. Shielding the messenger (RNA): microRNA-based anticancer therapies. *Pharmacol Ther* 2011; **131**: 18-32 [PMID: 21514318 DOI: 10.1016/j.pharmthera.2011.04.006]

8 **Peng Y**, Liu YM, Li LC, Wang LL, Wu XL. MicroRNA-338 inhibits growth, invasion and metastasis of gastric cancer by targeting NRP1 expression. *PLoS One* 2014; **9**: e94422 [PMID: 24736504 DOI: 10.1371/journal.pone.0094422]

9 **Fenger JM**, Bear MD, Volinia S, Lin TY, Harrington BK, London CA, Kisseberth WC. Overexpression of miR-9 in mast cells is associated with invasive behavior and spontaneous metastasis. *BMC Cancer* 2014; **14**: 84 [PMID: 24517413 DOI: 10.1186/1471-2407-14-84]

10 **Zhao BS**, Liu SG, Wang TY, Ji YH, Qi B, Tao YP, Li HC, Wu XN. Screening of microRNA in patients with esophageal cancer at same tumor node metastasis stage with different prognoses. *Asian Pac J Cancer Prev* 2013; **14**: 139-143 [PMID: 23534712 DOI: 10.7314/APJCP.2013.14.1.139]

11 **Wu J**, Ji X, Zhu L, Jiang Q, Wen Z, Xu S, Shao W, Cai J, Du Q, Zhu Y, Mao J. Up-regulation of microRNA-1290 impairs cytokinesis and affects the reprogramming of colon cancer cells. *Cancer Lett* 2013; **329**: 155-163 [PMID: 23142292 DOI: 10.1016/j.canlet.2012.10.038]

12 **Dai N**, Zhong ZY, Cun YP, Qing Y, Chen Ch, Jiang P, Li MX, Wang D. Alteration of the microRNA expression profile in human osteosarcoma cells transfected with APE1 siRNA. *Neoplasma* 2013; **60**: 384-394 [PMID: 23581410 DOI: 10.4149/neo_2013_050]

13 **Brandt DT**, Baarlink C, Kitzing TM, Kremmer E, Ivaska J, Nollau P, Grosse R. SCAI acts as a suppressor of cancer cell invasion through the transcriptional control of beta1-integrin. *Nat Cell Biol* 2009; **11**: 557-568 [PMID: 19350017 DOI: 10.1038/ncb1862]

14 **Montgomery E**, Field JK, Boffetta P, Daigo Y, Shimizu M, Shimoda T. Squamous cell carcinoma of the oesophagus. In: Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO classification of tumors of the digestive system. Lyon: IARC Press, 2010: 18-24

15 **Chen X**, Hu W, Xie B, Gao H, Xu C, Chen J. Downregulation of SCAI enhances glioma cell invasion and stem cell like phenotype by activating Wnt/β-catenin signaling. *Biochem Biophys Res Commun* 2014; **448**: 206-211 [PMID: 24785374 DOI: 10.1016/j.bbrc.2014.04.098]

16 **Zhang N**, Wang X, Huo Q, Sun M, Cai C, Liu Z, Hu G, Yang Q. MicroRNA-30a suppresses breast tumor growth and metastasis by targeting metadherin. *Oncogene* 2014; **33**: 3119-3128 [PMID: 23851509 DOI: 10.1038/onc.2013.286]

17 **Yu SJ**, Hu JY, Kuang XY, Luo JM, Hou YF, Di GH, Wu J, Shen ZZ, Song HY, Shao ZM. MicroRNA-200a promotes anoikis resistance and metastasis by targeting YAP1 in human breast cancer. *Clin Cancer Res* 2013; **19**: 1389-1399 [PMID: 23340296 DOI: 10.1158/1078-0432.CCR-12-1959]

18 **Li A**, Yu J, Kim H, Wolfgang CL, Canto MI, Hruban RH, Goggins M. MicroRNA array analysis finds elevated serum miR-1290 accurately distinguishes patients with low-stage pancreatic cancer from healthy and disease controls. *Clin Cancer Res* 2013; **19**: 3600-3610 [PMID: 23697990 DOI: 10.1158/1078-0432.CCR-12-3092]

19 **Kreßner C**, Nollau P, Grosse R, Brandt DT. Functional interaction of SCAI with the SWI/SNF complex for transcription and tumor cell invasion. *PLoS One* 2013; **8**: e69947 [PMID: 23936361 DOI: 10.1371/journal.pone.0069947]

P- Reviewer: Franzen T, Hummel R **S- Editor**: Ma YJ
L- Editor: Wang TQ **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3256

World J Gastroenterol 2015 March 21; 21(11): 3256-3265
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

High-mobility group box 1 expression and lymph node metastasis in intrahepatic cholangiocarcinoma

Yun-Fei Xu, Fu-Jun Ge, Bo Han, Xiao-Qing Yang, Hong Su, An-Cheng Zhao, Ming-Hong Zhao, Yu-Bao Yang, Jie Yang

Yun-Fei Xu, Department of Hepatobilliary Surgery, Shandong University Qilu Hospital, Jinan 250012, Shandong Province, China
Fu-Jun Ge, Yu-Bao Yang, Department of General Surgery, Affiliated Hospital of Shandong Medical School, Linyi 276000, Shandong Province, China
Bo Han, Xiao-Qing Yang, Hong Su, Department of Pathology, Shandong University Medical School, Jinan 250012, Shandong Province, China
An-Cheng Zhao, Ming-Hong Zhao, Department of Clinical Laboratory, Yishui Central Hospital, Linyi 276000, Shandong Province, China
Jie Yang, Department of Pediatrics, Shandong University Qilu Hospital, Jinan 250012, Shandong Province, China
Author contributions: Xu YF, Ge FJ and Han B contributed to this work equally; Xu YF and Yang J designed research; Ge FJ and Yang XQ performed immunohistochemistry; Su H, Zhao AC, Zhao MH and Yang YB performed the *in vitro* experiments; Xu YF, Han B and Yang J analyzed the data and wrote the paper.
Supported by National Natural Science Foundation of China No. 81072110 and No. 81171951; grants from Independent Innovation Foundation of Shandong University, No. 2010TB012; Scientific Research Foundation for Returned Scholars, Ministry of Education of China.
Ethics approval: This study was performed with prior patient consents and the approval of the Institutional Clinical Ethics Review Board of Shandong University.
Conflict-of-interest: The authors declare no conflicting interest.
Data sharing: No additional unpublished data are available

Correspondence to: Jie Yang, MD, PhD, Department of Pediatrics, Shandong University Qilu Hospital, No. 107 Wenhuaxi Road, Jinan 250012, Shandong Province, China. yang64662003@163.com
Telephone: +86-531-82169024
Fax: +86-531-82169024

Received: July 18, 2014
Peer-review started: July 20, 2014
First decision: August 15, 2014
Revised: September 2, 2014
Accepted: October 15, 2014
Article in press: October 15, 2014
Published online: March 21, 2015

Abstract

AIM: To evaluate the prognostic value of high-mobility group box 1 (HMGB1) expression in intrahepatic cholangiocarcinoma (IHCC) and the possible underlying mechanism.

METHODS: Tissue microarray was constructed from 65 IHCC patients. Immunohistochemistry was performed to validate expression of HMGB1 and Vascular endothelial growth factor C (VEGF-C). Real-time PCR and Western blot analyses were used to study transcript and protein levels. The interaction between HMGB1 and VEGF-C was evaluated by siRNA, real-time PCR, and enzyme-linked immuno assays. The correlation between HMGB1 expression and other clinicopathologic parameters was analyzed by χ^2 test, and the univariate as well as multivariate analyses were accomplished by Kaplan-Meier method and Cox-regression model, respectively.

RESULTS: Overall, overexpression of HMGB1 was found in 38/65 (58.8%) IHCCs, whereas VEGF-C overexpression was present in 30/65 (46.2%) cases. Overexpression of HMGB1 was significantly correlated with lymphatic microvessel density (P = 0.031, r = 0.268) and VEGF-C expression (P = 0.041, r = 0.254). With univariate analysis, both HMGB1 (P = 0.001) and VEGF-C (P = 0.004) were identified to be significantly associated with overall survival rate. Multivariate

analysis indicated that HMGB1 could be served as an unfavorable independent prognostic factor in IHCCs (P = 0.005). siRNA knockdown of HMGB1 inhibited transforming growth factor-β-induced epithelial-mesenchymal transition (EMT) by elevating E-Cadherin expression and reducing expression of N-Cadherin, Vimentin and Snail in RBE cells. Further *in vitro* study revealed that HMGB1 silencing significantly decreased the level of VEGF-C, whereas the recombinant HMGB1 increased the VEGF-C level in RBE cells (both $P < 0.05$), which suggested that HMGB1 could promote lymphatic microvessel density, and subsequently lymphatic invasion, *via* promoting VEGF-C expression.

CONCLUSION: Our results define an important role of HMGB1 in the progression of cholangiocarcinoma, and HMGB1 may serve as a prognostic marker for IHCC patients.

Key words: Epithelial-mesenchymal transition; High-mobility group box 1; Intrahepatic cholangiocarcinoma; Lymphatic microvessel density; Vascular endothelial growth factor C

Core tip: Cholangiocarcinoma is a lethal malignancy of the biliary tract, for which novel biomarkers are urgently needed for its management and treatment. This study shows that high-mobility group box 1 (HMGB1) is an independent prognostic factor in intrahepatic cholangiocarcinoma that positively correlates with lymphatic microvessel density and vascular endothelial growth factor C expression. Furthermore, HMGB1 enhances the secretion of vascular endothelial growth factor C and promotes epithelial-mesenchymal transition of RBE cells. Together, these results define an important role of HMGB1 in the progression of cholangiocarcinoma, which may serve as a prognostic marker for intrahepatic cholangiocarcinoma patients.

Xu YF, Ge FJ, Han B, Yang XQ, Su H, Zhao AC, Zhao MH, Yang YB, Yang J. High-mobility group box 1 expression and lymph node metastasis in intrahepatic cholangiocarcinoma. *World J Gastroenterol* 2015; 21(11): 3256-3265 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3256.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3256

INTRODUCTION

Cholangiocarcinoma (CCA) is the second most common cancer after hepatocellular carcinoma, and accounts for approximately 7%-10% of all primary hepatic cancers[1]. CCA is characterized by poor responsiveness to chemotherapy and radiotherapy in the majority of cases[2]. So far, surgical resection is the only potentially curative option. The morbidity and mortality of CCA, especially intrahepatic cholangiocarcinoma (IHCC), have been increasing worldwide in recent years[3]. IHCC is characterized by silent clinical signatures, early regional invasiveness, distant metastasis, and a poor prognosis[4]. Therefore, new insights into the biologic process of IHCCs and identification of novel biomarkers are urgently needed for cancer management and treatment.

High-mobility group box 1 (HMGB1) is a proinflammatory cytokine and chromatin-binding molecule[5], and is involved in a variety of biologic processes, including transcription, DNA repair, differentiation, and extracellular signal transduction[6]. Emerging data have suggested that HMGB1 could promote tumor progression *via* promoting proliferation and invasiveness of cancer cells[7]. Clinically, overexpression of HMGB1 has been reported in multiple malignancies including melanoma[8], gastric cancer[9], colorectal cancer[10], prostate cancer[11], and nasopharyngeal carcinoma[12]. However, to the best our knowledge, there has been no study so far to investigate the role of HMGB in IHCC.

Vascular endothelial growth factor C (VEGF-C) is a key mediator of lymphangiogenesis, acting *via* its receptors VEGF-R2 and VEGF-R3. Multiple studies have suggested that increased levels of VEGF-C correlate with lymphangiogenesis and distant metastasis[13]. Interestingly, Moriwaka *et al*[14] demonstrated a link between HMGB1 expression and lymph vessel density as well as VEGF-C expression in colon cancer. Additionally, HMGB1 has been suggested to promote lymphangiogenesis and invasive capacity of tumor cells through a VEGF-C-related pathway in oral squamous cell carcinoma[15].

Epithelial-mesenchymal transition (EMT), an early embryonic development program in which cells convert from the epithelial to the mesenchymal state, has been shown to play a critical role during cancer progression and metastasis[16]. During this process, the epithelial cancer cells lose epithelial characteristics and acquire mesenchymal properties resulting in reduction of adhesions and improvement of motility, thus promoting invasion and metastasis[17]. HMGB1 has been reported as a key regulator in the EMT process in mesothelial cells[18]. However, the link between HMGB1 and the EMT process remains unclear in the context of IHCC progression.

In this study, for the first time, we evaluated the expression and prognostic significance of HMGB1 in IHCCs. The roles of HMGB1 in EMT processes of IHCC, as well as the relationship between HMGB1 expression and VEGF-C were also investigated.

MATERIALS AND METHODS

Patients and follow-up

Our study consisted of specimens from 65 IHCC patients (32 male; 33 female) who underwent surgical resections between 2005 and 2011 at the Qilu Hospital of Shandong

University (Jinan, China). Detailed clinicopathologic profiles were obtained from medical records. The specimens were reviewed by two pathologists (Han B and Yang XQ). Tumor staging and histologic classification were assessed according to the 7th edition of American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) classification. The mean age of the patients was 56.9 ± 10.9 years (range, 28-83 years). Follow-up data were available for 57 patients, ranging from 3 to 96 mo (mean: 27.6 ± 27.3 mo). This study was approved by the Institutional Review Board at the School of Medicine of Shandong University and written consent was obtained from all patients.

Tissue microarray construction and immunohistochemistry (IHC)

The tissue microarray was constructed as previously described[19]. Two cores (1.0 mm in diameter) were taken from the representative areas of each tumor block and re-embedded into the recipient block. IHC was performed as previously described[20]. Briefly, 4 μm sections were deparaffinized and rehydrated. Sections were submerged into antigenic retrieval buffer (pH 6.0 citric acid) for heat-mediated retrieval by microwave for 15 min. The slides were incubated with primary antibodies for HMGB1 (GTX101277, 1:500; GeneTex Inc., Irvine, CA, United States) and VEGF-C (ab9546, 1:500; Abcam, Cambridge, United Kingdom) overnight at 4 ℃, then visualized using 3, 3-diaminobenzidine tetrahydrochloride as the chromogen. Slides incubated without primary antibody were considered as the negative control. The slides were then evaluated by two independent observers (Han B and Yang XQ) who were blind to the clinicopathologic data. The expression of HMGB1 and VEGF-C were evaluated with a semiquantitative scoring system based on intensity and distribution of positive-stained cells[21-23]. Briefly, the staining intensity (range, 0-3) and the percentage of positive cells (0, 0%-10%; 1, 11%-25%; 2, 26%-50%; 3, 51%-75%; 4, 76%-100%) were multiplied. Overexpression and non-overexpression were designated by a score of ⩾ 8 or < 8, respectively.

Assessment of lymphatic microvessel density

The quantitative vessel counts were performed according to the method described by Weidner *et al*[24]. Lymphatic microvessel density (LMVD) of the tumor was determined by using the D2-40 antibody (ab77854, 1:500; Abcam); the methodology and validation criteria were in compliance with the international consensus on evaluation of angiogenesis quantification in solid human tumors[25]. Regions with the highest LMVD were initially selected with low magnification (× 100) scanning, and then five "hotspot" fields in the corresponding area were selected and observed at a magnification of × 200. Any brown-stained, separated endothelial cell cluster was considered a single, countable lymphatic microvessel. The average amounts of lymphatic microvessels in the three fields were recognized as the value of LMVD[26]. The average score of LMVD of all samples was selected as the cut-off. The cut-off of LMVD was 12.7 and separated LMVD into high and low group[27].

Cell culture and reagents

The IHCC cell line RBE and perihilar cholangiocarcinoma cell line QBC939 were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China), The IHCC cell line HUCCT-1 was obtained from RIKEN Bioresource Center (Japan). All lines were cultured in RPMI-1640 medium supplemented with 10% FBS (Gibco of Thermo Fisher Scientific, Waltham, MA, United States). Human recombinant HMGB1 (rHMBG1) was purchased from Sino Biological Inc., Beijing, China (Cat No. 10326-H08H).

siRNA-mediated HMGB1 knockdown

Small interfering RNA (siRNA) was used to knockdown HMGB1 expression. Three specific siRNAs were designed and synthesized by Songon (Shanghai, China). The most effective single siHMGB1 (sense, 5'-CCUGUCCAUUGGUGAUGUUTT-3'; anti-sense, 5'-AACAUCACCAAUGGACAGGTT-3') was used for further experiments. A scrambled siRNA (scHMGB1) sequence was used as a control: sense, 5'-UUCUCCCAACGUGUCACG-3'; anti-sense, 5'-ACGUGACACGUUCGGAGAATT-3'.

Real-time PCR

Total RNA was extracted from the RBE cells and cDNA was synthesized by reverse transcription. A SYBR Green Realtime PCR Master Mix (Toyobo Co., Osaka, Japan) and ABI Prism 7700 Sequence Detection System (Applied Biosystems of Thermo Fisher Scientific) were used in this experiment. The primers of HMGB1 were as follows: sense, 5'-TTTAGATCTATGGCAAAGGAGATCCTAAGAAG-3'; anti-sense, 5'-TTTGAATTCTTATTCATCATCAT-CATCTTCTTCTTCATCT-3'. The relative HMGB1 expression was normalized to GAPDH (sense 5'-GAGTCAACGGATTTGGTCGT-3'; anti-sense, 5'-TTGATTTTGGAGGGATCTC-3'). PCR assays were performed in triplicate, and fold induction was calculated using the $2^{-\Delta CT}$ method[4].

Western blot

Cells were lysed and protein was extracted as previously described[20]. After SDS-PAGE, proteins were transferred to nitrocellulose membranes (BioTrace NT Nitocellulose; Pall Corp., Port Washington, NY, United States). The membrane was incubated with primary antibodies overnight at 4 ℃: Anti-HMGB1 (1:500), and antibodies against E-cadherin, N-cadherin, vimentin, slug, snail, claudin-1, ZO-1 and β-catenin (1:1000, #9782; Cell Signaling Technology Danvers, MA, United States). The secondary anti-rabbit antibody (Beyotime Company, China) was used at a dilution of 1:10000

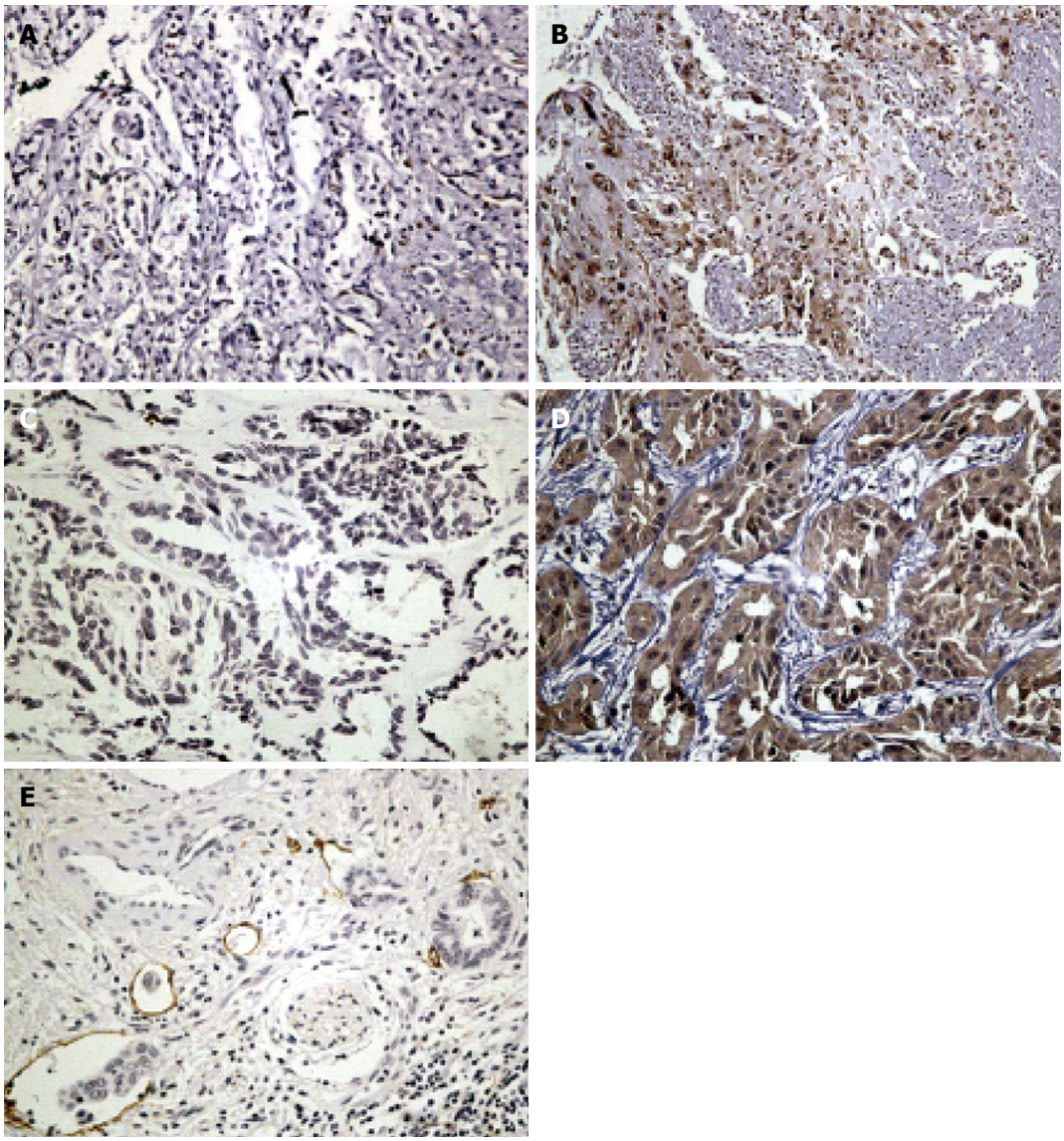


Figure 1 Representative immunohistochemistry in intrahepatic cholangiocarcinoma. A: Negative staining for high-mobility group box 1; B: Strong staining for high-mobility group box 1 was mainly localized in the nucleus, and effused to cytoplasm and extra milieu in inflamed or necrotic areas; C: Negative staining for vascular endothelial growth factor C; D: Strong staining for vascular endothelial growth factor C in cytoplasm; E: Representative staining for D2-40 in lymphatic endothelial cells. A cancer embolus in lymph-vessel is shown; magnification, × 200.

and the blot was developed with RapidStep ECL Reagent (Millipore Corp., Billerica, MA, United States). For the EMT assay, RBE cells were stimulated with 10 ng/mL transforming growth factor (TGF)-β for 72 h, and the cells were lysed and protein was extracted for further analysis.

Enzyme-linked immuno assay detection of secreted VEGF-C

RBE cells cultured in 12-well plate at a density of 2.5×10^4 cells per well were treated with scHMGB1, siHMGB1, or RPMI-1640 supplemented with 2 μg/mL rHMBG1. Cells treated with Lipofectamine 2000 alone served as a mock transfection group. The supernatants from each group were collected and centrifuged at 1000 rpm for 3 min after 48-h transfection/incubation. The levels of VEGF-C were detected using the human VEGF-C ELISA kit (Boster Systems Inc., Pleasanton, CA, United States) according to the manufacturer's instructions. Absorbance at a wavelength of 450 nm in every well was measured in spectrophotometer.

Statistical analysis

All the statistical analyses were performed by SPSS 17.0 software (SPSS, Chicago, IL, United States). The associations between HMGB1 expression and clinicopathologic parameters were assessed by a χ^2 test. Spearman's Rank correlation coefficient was used to identify the correlation between HMGB1 and LMVD. Cumulative overall survival rates were calculated by the Kaplan-Meier method and survival curves were compared by a log-rank test. For multivariate analysis, factors from univariate analysis were selected with a $P = 0.20$ cutoff[28]. Forward stepwise multivariate analysis

Table 1 Association of high-mobility group box 1 and vascular endothelial growth factor with clinicopathologic parameters in patients with intrahepatic cholangiocarcinoma *n* (%)

Parameters	HMGB1		*P* value	VEGF-C		*P* value
	Low	High		Low	High	
Age (yr)						
< 60	18 (42.9)	24 (57.1)		25 (59.5)	17 (40.5)	
⩾ 60	9 (39.1)	14 (60.9)	0.771	10 (43.5)	13 (56.5)	0.056
Gender						
Male	15 (46.9)	17 (63.6)		15 (46.9)	17 (53.1)	
Female	12 (36.4)	21 (63.6)	0.390	18 (54.5)	15 (45.5)	0.536
Tumor size (cm)						
< 5	8 (36.4)	14 (63.6)		10 (45.5)	12 (54.5)	
⩾ 5	19 (44.2)	24 (55.8)	0.545	23 (53.5)	20 (46.5)	0.540
Histologic classification						
Well	8 (47.1)	9 (52.9)		12 (70.6)	5 (29.4)	
Moderate	10 (32.3)	21 (67.7)		10 (32.3)	21 (67.7)	
Poor	9 (52.9)	8 (47.1)	0.329	12 (70.6)	5 (29.4)	0.016
Tumor stage						
Ⅰ + Ⅱ	21 (41.2)	30 (8.8)		25 (49.0)	26 (51.0)	
Ⅲ + Ⅳ	6 (42.9)	8 (57.1)	0.910	8 (57.1)	6 (42.9)	0.590
Node stage						
Negative	25 (50)	25 (50)		30 (60.0)	20 (40.0)	
Positive	2 (13.3)	13 (86.7)	0.011	3 (20.0)	12 (80.0)	0.007
UICC stage						
Ⅰ + Ⅱ	18 (48.6)	19 (51.4)		21 (56.8)	16 (43.2)	
Ⅲ + Ⅳ	9 (32.1)	19 (67.9)	0.181	12 (42.9)	16 (57.1)	0.267
Microvascular invasion						
Negative	20 (39.2)	31 (60.8)		26 (51.0)	25 (49.0)	
Positive	7 (50.0)	7 (50.0)	0.458	7 (50.0)	7 (50.0)	0.948
Perineural invasion						
Negative	22 (38.6)	35 (61.4)		30 (52.6)	27 (47.4)	
Positive	5 (62.5)	3 (37.5)	0.260[1]	3 (37.5)	5 (62.5)	0.475[a]
Satellite nodular						
Negative	24 (44.4)	30 (55.6)		27 (50)	27 (50)	
Positive	3 (27.3)	8 (72.7)	0.292	6 (54.5)	5 (45.5)	0.783
HBV infection						
Negative	24 (42.1)	33 (57.9)		30 (52.6)	27 (47.4)	
Positive	3 (37.5)	5 (62.5)	1.000[1]	3 (37.5)	5 (62.5)	0.475[a]
Calculus						
Negative	23 (40.4)	34 (59.6)		29 (51.8)	27 (48.2)	
Positive	4 (50.0)	4 (50.0)	0.712[1]	3 (37.5)	5 (62.5)	0.708[a]
LMVD						
Low	15 (57.7)	11 (42.3)		18 (69.2)	8 (30.8)	
High	12 (30.8)	27 (9.2)	0.031	15 (38.5)	24 (61.5)	0.015

[1]Analyzed by Fisher's exact test. HBV: Hepatitis B virus; LMVD: lymphatic microvessel density. HMGB1: High-mobility group box 1; VEGF-C: Vascular endothelial growth factor.

was used to identify independent prognostic factors. $P < 0.05$ was considered as significant.

RESULTS

Expression of HMGB1 and VEGF-C and correlations with clinicopathologic parameters

HMGB1 expression was mainly identified in the nucleus with slight penetration to cytoplasm or extracellular milieu, and the penetration tendency was manifest especially in area adjacent to necrosis. Overall, HMGB1 was overexpressed in 38/65 (58.8%) patients with IHCCs. VEGF-C expression was identified in the cytoplasm and overexpression was found in 32/65 (49.2%) IHCC cases. Representative IHC images of HMGB1 and VEGF-C are shown in Figure 1.

The relationship between HMGB1 overexpression and various clinicopathologic parameters are demonstrated in Table 1. Overall, overexpression of HMGB1 was significantly associated with lymph node metastasis ($P = 0.011$). No significant correlation was identified between HMGB1 overexpression and age, gender, tumor size, histologic classification, or microvascular or perineural invasion. Similarly, VEGF-C overexpression correlated with histologic classification ($P = 0.016$) and lymph node metastasis ($P = 0.007$).

Correlations of HMGB1 with LMVD and VEGF-C

The LMVD values of the 65 specimens ranged from 4 to 37 (mean: 12.7 ± 5.9), and tumors below or equal to 12.7 were classified as the low LMVD group ($n = 26$) and tumors above 12.7 were classified as the high LMVD group ($n = 39$). HMGB1 overexpression in high and low LMVD groups was compared by a χ^2 test, and

Table 2 Correlations of high-mobility group box 1 with lymphatic microvessel density and vascular endothelial growth factor C in patients with intrahepatic cholangiocarcinoma

HMGB1	LVD		*P* value	*r*	VEGF-C		*P* value	*r*
	Low	High			Negative	Positive		
Negative	15	12	0.031	0.268	18	9	0.03	0.268
Positive	11	27			15	23		

HMGB1: High-mobility group box 1; VEGF-C: Vascular endothelial growth factor; LVD: Lymphatic microvessel density.

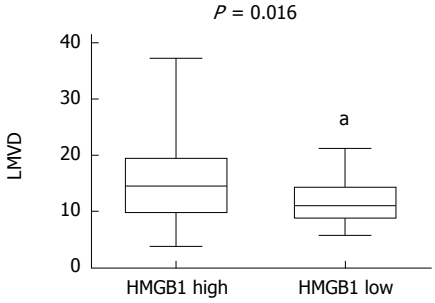


Figure 2 Distribution of lymphatic microvessel density categorized by high-mobility group box 1 expression. [a]*P* < 0.05 *vs* high expression. LMVD: Lymphatic microvessel density; HMGB1: High-mobility group box 1.

the difference were statistically significantly (*P* = 0.016) (Figure 2).

In Spearman's Rank correlation coefficient analysis, HMGB1 overexpression positively correlated with LMVD (*P* = 0.031, *r* = 0.268) and VEGF-C (*P* = 0.03, *r* = 0.268) (Table 2).

Prognostic value of HMGB1 expression in IHCC

Univariate analysis revealed that HMGB1 was an unfavorable prognostic factor (*P* = 0.001). Additionally, tumor size (*P* = 0.022), lymph node metastasis (*P* < 0.001), LMVD (*P* = 0.016), and VEGF-C (*P* = 0.004) were also significantly associated with overall survival. In a multivariate analysis, HMGB1 overexpression remained an independent prognostic factor (hazard ratio = 4.517, 95%CI: 1.458-13.992; *P* = 0.009) (Table 3). Notably, the IHCC patients who showed co-expression of HMGB1 and VEGF-C had the poorest survival compared with other subgroups when analyzed by the Kaplan-Meier method (Figure 3).

HMGB1 expression in CCA cell lines

Western blotting was utilized to determine the expression levels of HMGB1 in a panel of CCA cell lines. As shown in Figure 4A, the protein expression level of HMGB1 was substantially higher in RBE cells compared with HUCCT-1 and QBC939 cells.

In vitro effect of HMGB1 on EMT

We firstly confirmed the decreased mRNA and protein expression levels of HMGB1 in siHMGB1-treated RBE cells when compared with those of control groups (Figure 4B, C). As shown in Figure 4D, knockdown of HMGB1 suppressed the TGF-β-induced EMT, as indicated by decreased protein expression of N-cadherin, vimentin, snail, and E-cadherin. By contrast, no significant change was identified in the protein expressions of claudin-1, ZO-1, β-catenin, or slug after si-HMGB1 treatment.

Table 3 Multivariate analysis of prognostic factors in patients with intrahepatic cholangiocarcinoma

Parameters	HR	95%CI	*P* value
Node stage			
Negative	1.000		
Positive	3.166	1.108-9.046	0.031
Tumor size (cm)			
< 5	1.000		
⩾ 5	4.212	1.429-12.420	0.009
Microvascular invasion			
Negative	1.000		
Positive	2.730	1.088-6.850	0.032
HMGB1			
Negative	1.000		
Positive	4.517	1.458-13.992	0.009
VEGF-C			
Negative	1.000		
Positive	3.003	1.016-8.875	0.047

HMGB1: High-mobility group box 1; HR: Hazard ratio; VEGF-C: Vascular endothelial growth factor C.

Modulation of VEGF-C by HMGB1 in conditioned supernatants

The level of VEGF-C in conditioned medium was significantly decreased in the siHMGB1 group compared with the control groups (*P* < 0.005). In the rHMGB1-treated group, the VEGF-C level was significantly increased compared with the control (*P* < 0.001) (Figure 5).

DISCUSSION

Although the pathogenesis of CCA is poorly understood, a number of risk factors have been identified, including primary sclerosing cholangitis, liver fluke, biliary calculus, and chronic infection of hepatitis C virus[29]. Chronic inflammation induced by these risk factors together with partial bile obstruction form a complex tumor-promoting microenvironment. Within the tumor microenvironment, cytokines, chemokines, and reactive oxygen species induce the recruitment of inflammation-mediating immune cells and lead the necrosis of biliary epithelial cells, resulting in active secretion and/or passive releasing of HMGB1 to the extracellular

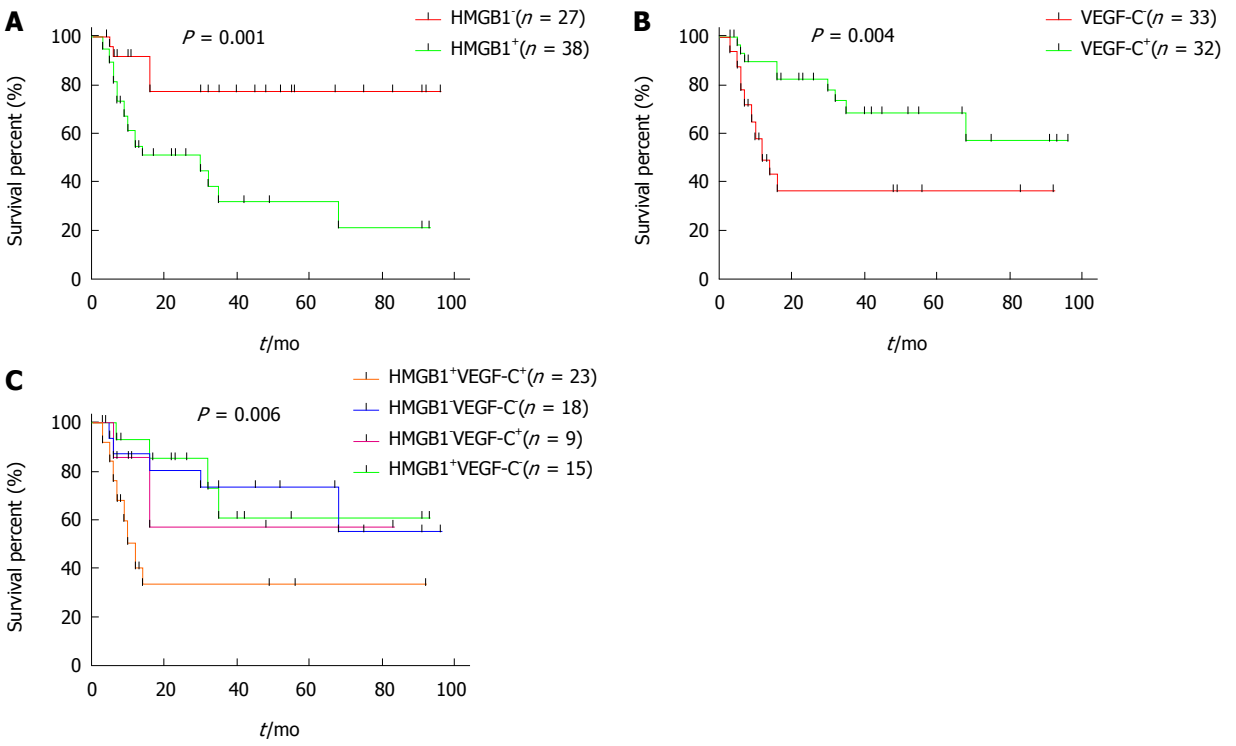


Figure 3 Survival curves of high-mobility group box 1 and vascular endothelial growth factor C in intrahepatic cholangiocarcinoma. Kaplan-Meier method univariate analyses of HMGB1 (A); VEGF-C (B); Survival curves of intrahepatic cholangiocarcinoma patients with and without expression of HMGB1 and VEGF-C co-expression (C). VEGF-C: Vascular endothelial growth factor C; HMGB1: High-mobility group box 1.

milieu. In turn, release of HMGB1 further stimulates inflammatory responses, hence, a feed-forward cycle of inflammation exists in the tumor microenvironment of CCA[30]. In the current study, HMGB1 expression was also identified in extracellular milieu, which suggests that HMGB1 can be actively secreted or passively released into extracellular space in the CCA.

Extracellular HMGB1 binding to its receptors (RAGE and Toll-like receptors) subsequently initiates a signaling cascade involving mitogen-activated protein kinase, nuclear factor-κB, phosphoinositide 3-kinase, and Cdc42 in the setting of cancer, may lead to tumor cell survival, expansion, and metastasis[31]. Elevated expression of HMGB1 has been reported in carcinomas of the stomach[32], liver[30], breast[33], and prostate[34]. Univariate and multivariate analyses revealed that HMGB1 overexpression is a poor prognosis factor in IHCC. Our results suggest that HMGB1 contributes to the development and progression of CCA.

Additionally, we show that overexpression of HMGB1 is associated with VEGF-C expression and high LMVD levels. Of note, VEGF-C is a prognostic factor and more importantly, the subset of IHCC patients with co-overexpression of HMGB1 and VEGF-C had the worst cancer-related survival. Previously, Chuangui *et al*[35] reported that the expression of HMGB1 is associated with lymph node metastasis and poor prognosis in esophageal squamous cell carcinoma. We thus speculate that HMGB1 playa a role in lymphangiogenesis through interaction with VEGF-C signaling pathways[36]. Further *in vitro* analysis showed that levels of VEGF-C in supernatants are significantly decreased with HMGB1 knockdown, and increased with rHMGB1 treatment. This indicates that HMGB1 expression in RBE cells promotes the secretion of VEGF-C. Alternatively, it is possible that HMGB1 binding to its receptors activates transduction signals such as Ras/mitogen-activated protein kinase and nuclear factor-κB, which stimulate the expression of VEGF-C, thereby, promoting lymphangiogenesis[37].

Metastasis is a central hallmark of malignancy during which cancer cells disseminate from the original site and transfer to distant organs[38]. EMT is considered as a critical step in this process[39]. The main molecular characteristic of EMT is the down-regulation of epithelial markers and the up-regulation of mesenchymal markers[40]. In the current study, for the first time, our data suggest that HMGB1 overexpression promotes EMT of RBE cells, and HMGB1 might endow CCA cells with the ability to metastasize *via* induction of EMT.

There are several limitations in our study. First, the IHCC cohort is relatively small. CCA is a rare malignancy, with only approximately 9760 new cases diagnosed annually in the United States[41], and IHCC only occupies about 20% of these cases[42]. Moreover, the majority of patients are diagnosed at an unresectable stage, which makes it difficult to obtain samples and perform a large randomized trial. Another limitation is the lack of *in vivo* data to further characterize the role of HMGB1 in IHCC. Although we have found that HMGB1 is an independent prognostic factor in IHCC, and HMGB1 expression is associated with lymph node metastasis, the underlying molecular

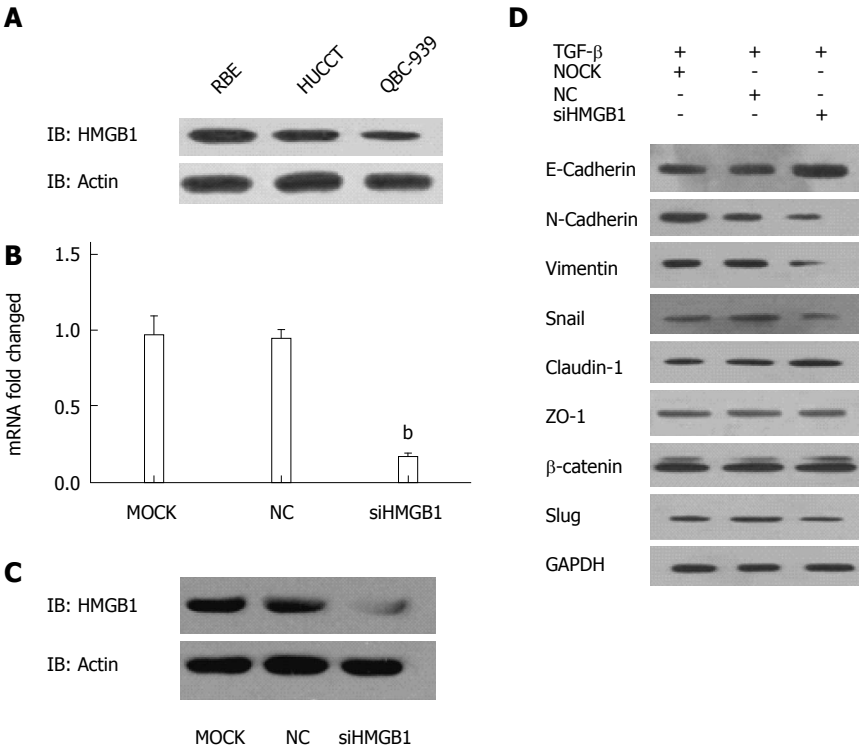


Figure 4 High-mobility group box 1 overexpression promotes epithelial-mesenchymal transition in RBE cell line. A: HMGB1 protein expression level in RBE, HUCCT-1, and QBC939 cell lines; B: mRNA expression level of HMGB1; C: Western blot; D: HMGB1 siRNA knockdown in RBE cells; $^{b}P < 0.01$ *vs* control. HMGB1: High-mobility group box 1.

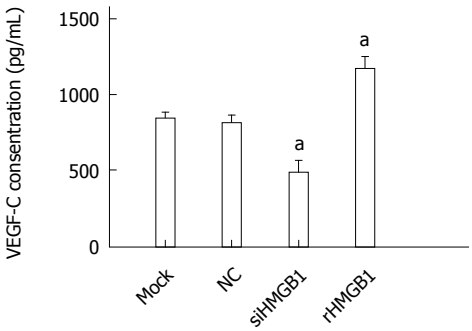


Figure 5 Vascular endothelial growth factor C levels in different groups. NC: Negative control; rHMGB1: Human recombinant high-mobility group box 1; siHMGB1: short interfering microRNA to high-mobility group box 1; $^{a}P < 0.05$ *vs* control. VEGF-C: Vascular endothelial growth factor C.

mechanism of how HMGB1 contributes to faster CCA progression still remains unclear. More experiments are needed to elucidate the receptor and downstream signaling pathways initiated by HMGB1, as well as the entire signaling network of HMGB1. In addition, animal models are an essential tool to study the role of biomarkers in cancer progression. We hope that our results *in vitro* trigger further investigation on the role of HMGB1 in IHCC *in vivo.*

In summary, this is the first study to systematically characterize HMGB1 expression in an IHCC cohort. HMGB1 is shown to be an independent poor prognostic factor in IHCC, and our data suggest a link between HMGB1 and VEGF-C in IHCC. That is, overexpression of HMGB1 might promote lymphangiogenesis through a VEGF-C-related pathway. On the other hand, HMGB1 overexpression promotes progression of IHCC by promoting angiogenesis and EMT.

COMMENTS

Background

Cholangiocarcinoma (CCA) is a lethal malignancy of the biliary tract with very few treatment options. CCA is characterized by poor responsiveness to chemotherapy and radiotherapy in the majority of cases. So far, surgical resection is the only potentially curative option. The morbidity and mortality of CCA, especially intrahepatic cholangiocarcinoma (IHCC), have been increasing worldwide in recent years. IHCC is characterized by silent clinical signatures, early regional invasiveness, distant metastasis, and a poor prognosis.

Research frontiers

High-mobility group box 1 (HMGB1) is involved in a variety of biologic processes, including transcription, DNA repair, differentiation, and extracellular signal transduction. Clinically, overexpression of HMGB1 has been reported in multiple malignancies, but there has been no study so far to investigate the role of HMGB in IHCC.

Innovations and breakthroughs

This study, for the first time, showed that HMGB1 is an independent prognostic factor in IHCC and positively correlates with lymphatic microvessel density and vascular endothelial growth factor C (VEGF-C) expression. *In vitro* analysis suggests that HMGB1 enhances the secretion of VEGF-C and promotes epithelial-mesenchymal transition of RBE cells.

Applications

This results defined an important role of HMGB1 in the progression of cholangiocarcinoma, and HMGB1 may serve as a prognostic marker for IHCC patients. These findings suggested that HMGB1 could be a potential molecular target in cholangiocarcinoma.

Peer-review

The authors evaluated HMBG1 as a possible novel prognostic marker for IHCC.

Additionally, mechanistic studies were performed investigating a presumed functional link between HMBG1 and epithelial-mesenchymal transition and VEGF-C-dependent lymphangiogenesis. This study deals with an interesting topic; some of the findings herein have not been reported in this particular setting and may indeed give some new insights.

REFERENCES

1 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 115-125 [PMID: 15192785 DOI: 10.1055/s-2004-828889]

2 **Kelley ST**, Bloomston M, Serafini F, Carey LC, Karl RC, Zervos E, Goldin S, Rosemurgy P, Rosemurgy AS. Cholangiocarcinoma: advocate an aggressive operative approach with adjuvant chemotherapy. *Am Surg* 2004; **70**: 743-748; discussion 748-749 [PMID: 15481288]

3 **Endo I**, Gonen M, Yopp AC, Dalal KM, Zhou Q, Klimstra D, D'Angelica M, DeMatteo RP, Fong Y, Schwartz L, Kemeny N, O'Reilly E, Abou-Alfa GK, Shimada H, Blumgart LH, Jarnagin WR. Intrahepatic cholangiocarcinoma: rising frequency, improved survival, and determinants of outcome after resection. *Ann Surg* 2008; **248**: 84-96 [PMID: 18580211 DOI: 10.1097/SLA.0b013e318176c4d3]

4 **Gatto M**, Bragazzi MC, Semeraro R, Napoli C, Gentile R, Torrice A, Gaudio E, Alvaro D. Cholangiocarcinoma: update and future perspectives. *Dig Liver Dis* 2010; **42**: 253-260 [PMID: 20097142 DOI: 10.1016/j.dld.2009.12.008]

5 **van Beijnum JR**, Nowak-Sliwinska P, van den Boezem E, Hautvast P, Buurman WA, Griffioen AW. Tumor angiogenesis is enforced by autocrine regulation of high-mobility group box 1. *Oncogene* 2013; **32**: 363-374 [PMID: 22391561 DOI: 10.1038/onc.2012.49]

6 **Ellerman JE**, Brown CK, de Vera M, Zeh HJ, Billiar T, Rubartelli A, Lotze MT. Masquerader: high mobility group box-1 and cancer. *Clin Cancer Res* 2007; **13**: 2836-2848 [PMID: 17504981 DOI: 10.1158/1078-0432.CCR-06-1953]

7 **Lotze MT**, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 2005; **5**: 331-342 [PMID: 15803152 DOI: 10.1038/nri1594]

8 **Poser I**, Golob M, Buettner R, Bosserhoff AK. Upregulation of HMG1 leads to melanoma inhibitory activity expression in malignant melanoma cells and contributes to their malignancy phenotype. *Mol Cell Biol* 2003; **23**: 2991-2998 [PMID: 12665595]

9 **Kuniyasu H**, Oue N, Wakikawa A, Shigeishi H, Matsutani N, Kuraoka K, Ito R, Yokozaki H, Yasui W. Expression of receptors for advanced glycation end-products (RAGE) is closely associated with the invasive and metastatic activity of gastric cancer. *J Pathol* 2002; **196**: 163-170 [PMID: 11793367 DOI: 10.1002/path.1031]

10 **Kuniyasu H**, Yano S, Sasaki T, Sasahira T, Sone S, Ohmori H. Colon cancer cell-derived high mobility group 1/amphoterin induces growth inhibition and apoptosis in macrophages. *Am J Pathol* 2005; **166**: 751-760 [PMID: 15743787 DOI: 10.1016/S0002-9440(10)62296-1]

11 **Gnanasekar M**, Kalyanasundaram R, Zheng G, Chen A, Bosland MC, Kajdacsy-Balla A. HMGB1: A Promising Therapeutic Target for Prostate Cancer. *Prostate Cancer* 2013; **2013**: 157103 [PMID: 23766911 DOI: 10.1155/2013/157103]

12 **Wu D**, Ding Y, Wang S, Zhang Q, Liu L. Increased expression of high mobility group box 1 (HMGB1) is associated with progression and poor prognosis in human nasopharyngeal carcinoma. *J Pathol* 2008; **216**: 167-175 [PMID: 18680137 DOI: 10.1002/path.2391]

13 **Mäkinen T**, Veikkola T, Mustjoki S, Karpanen T, Catimel B, Nice EC, Wise L, Mercer A, Kowalski H, Kerjaschki D, Stacker SA, Achen MG, Alitalo K. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3. *EMBO J* 2001; **20**: 4762-4773 [PMID: 11532940 DOI: 10.1093/emboj/20.17.4762]

14 **Moriwaka Y**, Luo Y, Ohmori H, Fujii K, Tatsumoto N, Sasahira T, Kuniyasu H. HMGB1 attenuates anti-metastatic defense of the lymph nodes in colorectal cancer. *Pathobiology* 2010; **77**: 17-23 [PMID: 20185963 DOI: 10.1159/000272950]

15 **Sasahira T**, Kirita T, Oue N, Bhawal UK, Yamamoto K, Fujii K, Ohmori H, Luo Y, Yasui W, Bosserhoff AK, Kuniyasu H. High mobility group box-1-inducible melanoma inhibitory activity is associated with nodal metastasis and lymphangiogenesis in oral squamous cell carcinoma. *Cancer Sci* 2008; **99**: 1806-1812 [PMID: 18616526 DOI: 10.1111/j.1349-7006.2008.00894.x]

16 **Nauseef JT**, Henry MD. Epithelial-to-mesenchymal transition in prostate cancer: paradigm or puzzle? *Nat Rev Urol* 2011; **8**: 428-439 [PMID: 21691304 DOI: 10.1038/nrurol.2011.85]

17 **Yang J**, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell* 2008; **14**: 818-829 [PMID: 18539112 DOI: 10.1016/j.devcel.2008.05.009]

18 **Qi F**, Okimoto G, Jube S, Napolitano A, Pass HI, Laczko R, Demay RM, Khan G, Tiirikainen M, Rinaudo C, Croce A, Yang H, Gaudino G, Carbone M. Continuous exposure to chrysotile asbestos can cause transformation of human mesothelial cells via HMGB1 and TNF-α signaling. *Am J Pathol* 2013; **183**: 1654-1666 [PMID: 24160326 DOI: 10.1016/j.ajpath.2013.07.029]

19 **Torhorst J**, Bucher C, Kononen J, Haas P, Zuber M, Köchli OR, Mross F, Dieterich H, Moch H, Mihatsch M, Kallioniemi OP, Sauter G. Tissue microarrays for rapid linking of molecular changes to clinical endpoints. *Am J Pathol* 2001; **159**: 2249-2256 [PMID: 11733374 DOI: 10.1016/S0002-9440(10)63075-1]

20 **Wang L**, Zhang J, Yang X, Chang YW, Qi M, Zhou Z, Zhang J, Han B. SOX4 is associated with poor prognosis in prostate cancer and promotes epithelial-mesenchymal transition in vitro. *Prostate Cancer Prostatic Dis* 2013; **16**: 301-307 [PMID: 23917306 DOI: 10.1038/pcan.2013.25]

21 **Moser B**, Janik S, Schiefer AI, Müllauer L, Bekos C, Scharrer A, Mildner M, Rényi-Vámos F, Klepetko W, Ankersmit HJ. Expression of RAGE and HMGB1 in thymic epithelial tumors, thymic hyperplasia and regular thymic morphology. *PLoS One* 2014; **9**: e94118 [PMID: 24705787 DOI: 10.1371/journal.pone.0094118]

22 **Weng H**, Deng Y, Xie Y, Liu H, Gong F. Expression and significance of HMGB1, TLR4 and NF-κB p65 in human epidermal tumors. *BMC Cancer* 2013; **13**: 311 [PMID: 23803172 DOI: 10.1186/1471-2407-13-311]

23 **Yang X**, Wang W, Wang C, Wang L, Yang M, Qi M, Su H, Sun X, Liu Z, Zhang J, Qin X, Han B. Characterization of EGFR family gene aberrations in cholangiocarcinoma. *Oncol Rep* 2014; **32**: 700-708 [PMID: 24927194 DOI: 10.3892/or.2014.3261]

24 **Weidner N**, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. *N Engl J Med* 1991; **324**: 1-8 [PMID: 1701519 DOI: 10.1056/NEJM199101033240101]

25 **Vermeulen PB**, Gasparini G, Fox SB, Colpaert C, Marson LP, Gion M, Beliën JA, de Waal RM, Van Marck E, Magnani E, Weidner N, Harris AL, Dirix LY. Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. *Eur J Cancer* 2002; **38**: 1564-1579 [PMID: 12142044]

26 **Hall FT**, Freeman JL, Asa SL, Jackson DG, Beasley NJ. Intratumoral lymphatics and lymph node metastases in papillary thyroid carcinoma. *Arch Otolaryngol Head Neck Surg* 2003; **129**: 716-719 [PMID: 12874070 DOI: 10.1001/archotol.129.7.716]

27 **Möbius C**, Demuth C, Aigner T, Wiedmann M, Wittekind C, Mössner J, Hauss J, Witzigmann H. Evaluation of VEGF A expression and microvascular density as prognostic factors in extrahepatic cholangiocarcinoma. *Eur J Surg Oncol* 2007; **33**: 1025-1029 [PMID: 17400419 DOI: 10.1016/j.ejso.2007.02.020]

28 **Shibahara H**, Tamada S, Higashi M, Goto M, Batra SK, Hollingsworth MA, Imai K, Yonezawa S. MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. *Hepatology* 2004; **39**: 220-229 [PMID: 14752841 DOI: 10.1002/hep.20031]

29 **Fujita T**. Analyzing risk factors for intrahepatic cholangiocarcinoma. *Hepatology* 2013; **58**: 1862-1863 [PMID: 23609480 DOI: 10.1002/hep.26448]

30 **Cheng BQ**, Jia CQ, Liu CT, Lu XF, Zhong N, Zhang ZL, Fan

W, Li YQ. Serum high mobility group box chromosomal protein 1 is associated with clinicopathologic features in patients with hepatocellular carcinoma. *Dig Liver Dis* 2008; **40**: 446-452 [PMID: 18294942 DOI: 10.1016/j.dld.2007.11.024]

31 **Kawai T**, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 2010; **11**: 373-384 [PMID: 20404851 DOI: 10.1038/ni.1863]

32 **Chung HW**, Lee SG, Kim H, Hong DJ, Chung JB, Stroncek D, Lim JB. Serum high mobility group box-1 (HMGB1) is closely associated with the clinical and pathologic features of gastric cancer. *J Transl Med* 2009; **7**: 38 [PMID: 19476625 DOI: 10.1186/1479-5876-7-38]

33 **Flohr AM**, Rogalla P, Meiboom M, Borrmann L, Krohn M, Thode-Halle B, Bullerdiek J. Variation of HMGB1 expression in breast cancer. *Anticancer Res* 2001; **21**: 3881-3885 [PMID: 11911263]

34 **Ishiguro H**, Nakaigawa N, Miyoshi Y, Fujinami K, Kubota Y, Uemura H. Receptor for advanced glycation end products (RAGE) and its ligand, amphoterin are overexpressed and associated with prostate cancer development. *Prostate* 2005; **64**: 92-100 [PMID: 15666359 DOI: 10.1002/pros.20219]

35 **Chuangui C**, Peng T, Zhentao Y. The expression of high mobility group box 1 is associated with lymph node metastasis and poor prognosis in esophageal squamous cell carcinoma. *Pathol Oncol Res* 2012; **18**: 1021-1027 [PMID: 22544356 DOI: 10.1007/s12253-012-9539-3]

36 **Liang X**, Yang D, Hu J, Hao X, Gao J, Mao Z. Hypoxia inducible factor-alpha expression correlates with vascular endothelial growth factor-C expression and lymphangiogenesis/angiogenesis in oral squamous cell carcinoma. *Anticancer Res* 2008; **28**: 1659-1666 [PMID: 18630523]

37 **van Beijnum JR**, Buurman WA, Griffioen AW. Convergence and amplification of toll-like receptor (TLR) and receptor for advanced glycation end products (RAGE) signaling pathways via high mobility group B1 (HMGB1). *Angiogenesis* 2008; **11**: 91-99 [PMID: 18264787 DOI: 10.1007/s10456-008-9093-5]

38 **Yang J**, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004; **117**: 927-939 [PMID: 15210113 DOI: 10.1016/j.cell.2004.06.006]

39 **Varnat F**, Duquet A, Malerba M, Zbinden M, Mas C, Gervaz P, Ruiz i Altaba A. Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for tumour growth, recurrence, metastasis and stem cell survival and expansion. *EMBO Mol Med* 2009; **1**: 338-351 [PMID: 20049737 DOI: 10.1002/emmm.200900039]

40 **Janda E**, Litos G, Grünert S, Downward J, Beug H. Oncogenic Ras/Her-2 mediate hyperproliferation of polarized epithelial cells in 3D cultures and rapid tumor growth via the PI3K pathway. *Oncogene* 2002; **21**: 5148-5159 [PMID: 12140765 DOI: 10.1038/sj.onc.1205661]

41 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**: 277-300 [PMID: 20610543 DOI: 10.3322/caac.20073]

42 **Khan SA**, Davidson BR, Goldin R, Pereira SP, Rosenberg WM, Taylor-Robinson SD, Thillainayagam AV, Thomas HC, Thursz MR, Wasan H. Guidelines for the diagnosis and treatment of cholangiocarcinoma: consensus document. *Gut* 2002; **51** Suppl 6: VI1-VI9 [PMID: 12376491]

P- Reviewer: Schmelzle M, Schmeding M **S- Editor**: Qi Y
L- Editor: AmEditor **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3266

World J Gastroenterol 2015 March 21; 21(11): 3266-3273
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Case Control Study

Hepatitis E in hemodialysis and kidney transplant patients in south-east Italy

Gaetano Scotto, Filippo Aucella, Giuseppe Grandaliano, Domenico Martinelli, Mario Querques, Antonio Gesuete, Barbara Infante, Paolo Delli Carri, Salvatore Massa, Giovanna Salatino, Fabio Bulla, Vincenzina Fazio

Gaetano Scotto, Microbiology and Clinical Microbiology, Master's Degree in Dentistry, University of Foggia, 71100 Foggia, Italy
Filippo Aucella, Antonio Gesuete, Nephrology and Dialysis Unit IRCCS "Casa Sollievo della Sofferenza" San Giovanni Rotondo (FG), 71013 San Giovanni Rotondo, Italy
Giuseppe Grandaliano, Barbara Infante, Nephrology and Dialysis Unit, University of Foggia, 71100 Foggia, Italy
Domenico Martinelli, Institute of Hygiene, University of Foggia, 71100 Foggia, Italy
Mario Querques, Paolo Delli Carri, Giovanna Salatino, Division of Nephrology, Hospital of Foggia, 71100 Foggia, Italy
Fabio Bulla, Clinic of Infectious Diseases, University of Foggia, 71100 Foggia, Italy
Salvatore Massa, Department of Agricultural, Food and Environmental Sciences, University of Foggia, 71100 Foggia, Italy
Vincenzina Fazio, Clinical Chemistry Laboratory, Unit of Virology, Hospital of Foggia, 71100 Foggia, Italy
Author contributions: All authors contributed equally to this work.
Ethics approval: The study was reviewed and approved by the Institutional Review Board of the University Hospital OORR, Foggia, Italy.
Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.
Conflict-of-interest: There is no conflict of interest with any financial, commercial, personal, political, intellectual, or religious organization regarding the material discussed in the manuscript.
Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at gaetano.scotto@unifg.it. Participants gave informed consent for data sharing.

Correspondence to: Gaetano Scotto, MD, Adjunct Professor, Chair of Microbiology and Clinical Microbiology, Master's Degree in Dentistry, University of Foggia, viale L. Pinto 1, 71100 Foggia, Italy. gaetano.scotto@unifg.it
Telephone: +39-33-37453981
Fax: +39-8-81732208
Received: October 3, 2014
Peer-review started: October 3, 2014
First decision: October 20, 2014
Revised: November 4, 2014
Accepted: January 8, 2015
Article in press: January 8, 2015
Published online: March 21, 2015

Abstract

AIM: To investigate the serovirological prevalence and clinical features of hepatitis E virus (HEV) infection in end-stage renal failure patients and in the healthy population.

METHODS: HEV infection is a viral disease that can cause sporadic and epidemic hepatitis. Previous studies unexpectedly showed a high prevalence of HEV antibodies in immunosuppressed subjects, including hemodialysis (HD) patients and patients who had undergone kidney transplant. A cohort/case-control study was carried out from January 2012 to August 2013 in two hospitals in southern Italy (Foggia and S. Giovanni Rotondo, Apulia). The seroprevalence of HEV was determined in 801 subjects; 231 HD patients, 120 renal transplant recipients, and 450 health individuals. All HD patients and the recipients of renal transplants were attending the Departments of Nephrology and Dialysis at two hospitals located in Southern Italy, and were included progressively in this study. Serum samples were tested for HEV antibodies (IgG/IgM); in the case of positivity they were confirmed by a Western blot assay and were also tested for HEV-RNA, and the HEV genotypes were determined.

RESULTS: A total of 30/801 (3.7%) patients were positive for anti-HEV Ig (IgG and/or IgM) and by Western blot. The healthy population presented with a prevalence of 2.7%, HD patients had a prevalence of 6.0%, and transplant recipients had a prevalence of 3.3%. The overall combined HEV-positive prevalence in the two groups with chronic renal failure was 5.1%. The rates of exposure to HEV (positivity of HEV-IgG/M in the early samples) were lower in the healthy controls, but the difference among the three groups was not statistically significant ($P > 0.05$). Positivity for anti-HEV/IgM was detected in 4/30 (13.33%) anti-HEV Ig positive individuals, in 2/14 HD patients, in 1/4 transplant individuals, and in 1/12 of the healthy population. The relative risk of being HEV-IgM-positive was significantly higher among transplant recipients compared to the other two groups (OR = 65.4, 95%CI: 7.2-592.7, $P < 0.001$), but the subjects with HEV-IgM positivity were numerically too few to calculate a significant difference. No patient presented with chronic hepatitis from HEV infection alone.

CONCLUSION: This study indicated a higher, but not significant, circulation of HEV in hemodialysis patients *vs* the healthy population. Chronic hepatitis due to the HEV virus was not observed.

Key words: Hepatitis E virus infection; Prevalence; Immunosuppressed subjects; Hemodialysis patients; Transplant recipients

Core tip: Hepatitis E, a single-stranded RNA virus, is the main aetiological agent of enteric non-A hepatitis. Previous seroprevalence surveys in developed countries showed variable rates of anti-hepatitis E virus (HEV) positivity in healthy populations, and several studies reported an unexpectedly high prevalence of antibodies against HEV in hemodialysis patients. The purpose of this survey was: (1) to compare the rate of HEV infection in renal transplant recipients and patients undergoing chronic hemodialysis to a control population; (2) to determine if these patients have an increased risk for HEV exposure; and (3) to evaluate the stage of liver disease.

Scotto G, Aucella F, Grandaliano G, Martinelli D, Querques M, Gesuete A, Infante B, Carri PD, Massa S, Salatino G, Bulla F, Fazio V. Hepatitis E in hemodialysis and kidney transplant patients in south-east Italy. *World J Gastroenterol* 2015; 21(11): 3266-3273 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3266.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3266

INTRODUCTION

Blood-borne viral hepatitis [hepatitis B virus (HBV) and hepatitis C virus (HCV)] infections represent relevant causes of liver disease in end stage renal failure patients on hemodialysis (HD)[1-5]. In recent years, preventive measures and extensive infection control guidelines guided a progressive decrease of HCV and HBV rates in these patients[2-9]. Nevertheless, a proportion of liver illnesses due to non A-B-C hepatitis occur in these individuals. Previous seroprevalence surveys in developed countries showed variable rates of anti-hepatitis E virus (HEV) positivity in healthy populations[10,11], and several studies reported an unexpectedly high prevalence of antibodies against HEV in hemodialysis patients[12-15]. The higher prevalence of HEV-IgG in chronic hemodialysis patients could be related to their impaired immunity, with an increased susceptibility to infections and decreased immune responses to antigenic stimuli (*e.g.*, HBV vaccination)[16-18]. Furthermore, they present a reduced response to HBV vaccination. In fact, these patients have an increased risk of contact with nosocomially-transmitted agents, and the role of enterically-transmitted hepatitis viruses in such cases needs to be defined.

Hepatitis E, a single-stranded RNA virus, is the main aetiological agent of enteric non-A hepatitis. In the recent past, it was believed to be present only in developing countries, where it was associated with epidemic outbreaks through the fecal-oral route from contaminated water supplies, but it is now recognized as a worldwide infection, sometimes related, in developed countries, to an asymptomatic zoonotic infection (as well as undercooked meat products)[19-21] or to parenteral/vertical transmission[22-24]. Furthermore, it has been recently noted that a variable rate of blood donors were positive for HEV-RNA[25-27]. There are scant reports on the prevalence and possible nosocomial transmission of HEV in HD patients. Some authors highlighted the high rates of anti-HEV antibodies in their HD patients and hypothesized other routes of transmission besides the fecal-oral route, although the real prevalence of HEV infection through the parenteral route, particularly via hemodialysis, is unknown[28]. Other investigators observed low rates of anti-HEV-positivity in their HD populations[15,29,30].

Previous seroprevalence studies showed anti-HEV/IgG positivity in 6%-16% of renal transplant recipients[31-33]; this variability is often because this virus is not routinely screened for in cases of acute hepatitis in recipients of solid-organ transplants. Recently, HEV infection has been presented as a chronic infection, sometimes with associated cirrhosis in immunosuppressed individuals. These cases included solid-organ (including kidney) transplant recipients receiving immunosuppressive therapy[33-36], patients with hematological malignancies[37-39], and subjects with HIV infection[40]. It is not known whether HEV can induce chronic hepatitis in subjects with defects of humoral and cellular immunity, such as in patients with end-stage renal failure requiring renal replacement therapy.

To our knowledge, few studies have examined the seroprevalence rate and clinical evolution of HEV infection among HD patients and in recipients of renal transplants in Italy. The purpose of this survey was (1) to compare the rate of HEV infection in renal transplant recipients and patients undergoing chronic hemodialysis to a control population; (2) to determine if these patients have an increased risk for HEV exposure; and (3) to evaluate the stage of liver disease.

MATERIALS AND METHODS

This observational study was carried out from January 2012 to August 2013. The seroprevalence of HEV was determined in 801 subjects (231 HD patients, 120 renal transplant recipients, and 450 individuals coming from the general population as controls). All of the HD patients and the recipients of renal transplants were attending the Departments of Nephrology and Dialysis at two hospitals located in southern Italy (Foggia and S. Giovanni Rotondo, Apulia), and were included progressively in this study. The controls were aged > 18 years and were identified from out-patient populations attending these hospitals for blood tests. Among the control patients, most were healthy, others had a range of acute/chronic general medical conditions, and some (approximately 6%) had a history of liver disease. All of the subjects included in the study were orally informed about the purpose of the study and invited to participate. Each patient gave informed consent. The research was conducted in accordance with the Declaration of Helsinki (as revised in 2008) and according to local guidelines and laws. Because this was a case-control study, the assent of the local Ethics Committee was not mandatory. At baseline, all study participants were requested to complete a questionnaire to obtain demographic, lifestyle, socio-economic, and clinical data in order to assess their previous exposure to viral hepatitis. These data included sexual orientation, ethnicity, and liver function tests; underlying nephrological diagnosis, previous transplantation (if on chronic hemodialysis), hemodialysis, and transplant vintage and previous/current immunosuppressive treatment data were obtained for HD and transplant patients. Routine HD techniques were performed with 0.75 h treatments three times a week. The history of blood transfusion requirements for each patient was evaluated. No patient admitted had a history of intravenous drug abuse. All enrolled subjects also received a full clinical examination and were treated according to their clinical situation. The demographic, clinical, and laboratory data of all patients are presented in Table 1.

The samples were investigated for the presence of anti-HEV immunoglobulin (IgG/IgM) using a commercial enzyme immunoassay (EIA) based on recombinant proteins (HEV IgG/IgM; DIA.PRO, Diagnostic BioProbes, Milan, Italy). If repeatedly positive (when sera gave an absorbance greater than the cut-off value), the results were confirmed by a Western blot assay (HEV-recomBlot, Nuclear Laser Medicine, Milan, Italy).

To determine HEV-RNA, a commercially-available assay was used (Qiamp viral RNA mini-kit, Qiagen, Chatsworth, CA). After RT-nested PCR, genotyping was performed using restriction endonuclease analysis; a technique in which DNA fragments obtained from digestion with restriction enzymes are compared to construct a restriction map showing the position of specific sites along a sequence of DNA[41]. Anti-HEV antibodies, Western blots, determination of HEV-RNA, and genotype assessments were performed using the same assays in a single laboratory (Foggia).

HBV markers were assayed by commercial immunoassay (Abbott-Auszyme Mc, Abbott Laboratories, North Chicago, IL, United States). The presence of antibodies to HCV was determined by the use of a third-generation enzyme-linked-immunoabsorbent assay (HCV-ELISA, Ortho Diagnostic System, Raritan, NJ, United States) and confirmed by a third-generation-recombinant-immunoblot assay (RIBA, Ortho Diagnostic Systems, Raritan, NJ, United States). To determine HBV-DNA and HCV-RNA, a commercially-available assay was used (Qiamp viral RNA, Qiagen, Chatsworth, CA). The presence of antibodies to HIV 1 and 2 was determined by a commercial immunoassay (Ortho Diagnostic Systems, Raritan, NJ, United States). To determine HIV-RNA, a commercially-available assay was used (Artus HIV virus 1, Rg RT-PCR kit, Qiagen, Chatsworth, CA, United States).

Serum alanine-amino-transferase (ALT) was quantified by ultraviolet-enzymatic-assay (normal range, 0-40 IU/L). Each patient's hepatic biochemical, epidemiological, and virological parameters were recorded, and a serum sample was taken and frozen at -70 ℃ prior to being tested for HEV by reverse transcriptase-polymerase chain reaction (RT-PCR), anti-HEV immunoglobulin G (IgG, IgM) immunoassays, and western blotting.

Statistical analysis

The χ^2 test was used to compare categorical variables (sex, positivity for anti-HEV IgG/M, Western blot test results for HEV antibodies, HCV antibodies, and HBV markers). When possible, odds ratio and 95%CIs were calculated. Continuous variables (age and ALT levels) were compared by Student's *t*-test for independent samples and ANOVA. Logistic-regression models were used to account for the confounding effects of patient demographics. *P* values < 0.05 were considered significant. The data were analyzed by STATA 10 MP software (Stata Corp., United States) for Mac OS X.

Table 1 Clinical characteristics, *n*

	Transplant patients	HD patients	General population
Total	120	231	450
Sex (male:female)	82:38	126:105	178:272
Median age (yr)	48	63	40
Causes of renal failure	Chronic glomerulonephritis: 43	Chronic glomerulonephritis: 73	
	Nephroangiosclerosis: 21	Nephroangiosclerosis: 27	
	Polycystic kidney disease: 16	Polycystic kidney disease: 32	
	Diabetic nephropathy: 14	Diabetic nephropathy: 44	
	Chronic interstitial nephritis: 11	Chronic interstitial nephritis: 26	
	Other aetiologies: 15	Other aetiologies: 29	
Median HD treatment	18 mo (range: 1-54 mo)	79 mo (range: 3-154 mo)	
	17 mo (range: 1-48 mo) HEV-negative	78.9 mo (range: 3-149 mo) HEV-negative	
	21 mo (range: 3-54 mo) HEV-positive	81 mo (range: 7-154 mo) HEV-positive	
Immunosuppressive treatment	All of the patients (120)	Yes: 2 (males)	

HEV: Hepatitis E virus; HD: Hemodialysis.

Table 2 Hepatitis E virus antibodies *n* (%)

	Transplant patients	HD patients	General population
Total	120	231	450
Anti-HEV Ig pos.	4 (3.3)	14 (6)	12 (2.7)
HEV IgM pos.	1	2	1
HEV RNA pos.	2	3	1

HEV: Hepatitis E virus; HD: Hemodialysis.

RESULTS

A total of 30/801 (3.7%) patients were anti-HEV Ig (IgG and/or IgM) and Western blot positive; almost none of the patients showed any clinical symptom that could be related to acute or chronic hepatitis. The prevalence in dialysis patients was 6.0% (14 patients); in transplant recipients the prevalence was 3.3% (4 individuals) and in the general population the prevalence was 2.7% (12 subjects). The combined overall HEV-positive prevalence in the two groups with chronic renal failure was 5.1%. The rates of exposure to HEV (positivity of HEV-IgG/M in the early samples) were lower in the healthy controls, but the difference among the three groups was not statistically significant ($P > 0.05$).

Positivity for anti-HEV/IgM was detected in 4/30 (13.33%) anti-HEV Ig positive individuals, in 2/14 HD patients, in 1/4 transplant individuals, and in 1/12 individuals from the healthy population (Table 2).

There was not a statistically significant difference between the rates in HD patients and healthy controls (0.98% *vs* 0.22%, $P > 0.05$). The relative risk of being HEV-IgM-positive was significantly higher in transplant recipients compared to the other two groups (OR = 65.4, 95%CI: 7.2-592.7, $P < 0.001$), but the subjects with HEV-IgM positivity were numerically too few to determine a significant difference. The origin of acute HEV infection (IgM positive and HEV-RNA detectable) in HD patients, transplant recipients, and the healthy population was uncertain.

HEV-RNA determination was positive in all IgM-positive patients and in two of the IgG positive patients (1 dialysis and 1 transplant, both HEV/HCV co-infected), who presented with hepatic fibrosis. Among dialysis and transplant patients with acute hepatitis (anti-HEV IgM), one carried genotype 1 (an immigrant) and two presented with genotype 3; among the general population the only anti-HEV IgM patient presented with genotype 3.

There was no significant correlation for either group between sex (males 7/14 in HD patients, 2/4 in renal transplants recipients, and 8/12 in the healthy population) and HEV-IgG/IgM and Western blot positivity ($P > 0.05$). The mean age in the transplant individuals and in the dialysis patients was not significantly different between subjects who were HEV-positive (age of transplant subjects: 48.5 ± 12.1 years; age of HD patients: 59.0 ± 6.7) *vs* HEV-negative (age of transplant subjects: 48.5 ± 18.9 years; age of HD patients: 62.9 ± 6.3; $P > 0.05$). Instead, the mean age of the healthy population was significantly higher in HEV-positive subjects (49.8 ± 12.1 years) *vs* HEV-negative (39.7 ± 18.9 years, $P < 0.05$). However, in logistic-regression models adjusted for age, sex, and group, the risk of anti-HEV positivity was not significantly higher in HD patients compared with the other two groups. The only statistically significant association in HD patients was with age (OR = 11.7, 95%CI: 5.9-23.2, $P < 0.001$). The cohort of patients > 45 years presented with HEV positivity more frequently than the groups aged 21-45 years. The risk of Western blot positivity was related to age and was higher in HD patients (OR = 12.3, 95%CI: 5.9-25.5, $P < 0.001$).

The hemodialysis vintage in HD patients ranged between 4-121 mo (median 79.2 mo) for anti-HEV positive patients and between 1-184 mo (median 79.4 mo) for the anti-HEV negative patients ($P > 0.05$). Among the patients with a functioning transplant, only 2/4 patients who were HEV positive had a prior history of HD treatment (median 21 mo *vs* 17 mo for HEV-negative patients). The length of hemodialysis treatment in these subjects also did not seem to be a significant risk factor for HEV IgG positivity; however,

the duration of HD treatment before renal transplant in HEV-positive individuals was lower compared to that of hemodialysis patients.

Five out of one hundred and thirty one (2.2%) HD patients and 1/120 (0.83%) transplant recipient presented with HBV infection (hepatitis B surface antigen positive), while 67/231 (29.0%) HD patients and 29/120 (24.2%) transplant recipients had serological parameters of previous HBV infection (anti-HBc and/or anti-HBs positivity). The individuals who had been immunized with hepatitis B vaccine were not included in this calculation of HBV infection. Co-infection with HBV/HEV was not present in any of the HD or transplant subjects.

Moreover, we observed 18/231 (7.8%) patients with an anti-HCV antibody among HD patients and 19/120 (15.8%) patients with an anti-HCV antibody among the transplant recipients. Co-infection with HEV/HCV was present in 1/14 (7.1%) of the HD patients and 1/4 (25%) of the transplant subjects. No co-infection with other hepatitis viruses was present in the HEV positive subjects from the general population.

Patients with chronic renal failure and HEV-IgG positivity were for the most part asymptomatic; only 7/18 (38.9%) reported moderate asthenia, whereas jaundice was present in 1/3 (33.3%) IgM-positive patients and hepato-splenomegaly and distended abdomen were observed in 2/3 (66.76%).

Among the HD patients and transplant recipients who were anti-HEV positive, approximately 67% of individuals had normal ALT values. Higher serum levels of ALT were observed in HEV-IgM positive *vs* HEV-IgM negative subjects (178.8 ± 131.1 *vs* 33.7 ± 14.5, $P < 0.001$). Among the HEV-IgG positive subjects, ALT levels were not significantly different among HD patients (31.59 ± 18.05 UI/mL) *vs* transplant individuals (28.8 ± 13.1) and the general population (54 ± 30 UI/mL), $P > 0.05$.

There is no connection between any stage of chronic hepatitis and HEV infection alone in any of the patients groups who presented with chronic renal failure. In fact, the two IgG positive patients (1 dialysis and 1 transplant patient) who presented with chronic active hepatitis with advanced fibrosis were HCV-HEV co-infected, and both patients presented with genotype HEV 3.

DISCUSSION

In the past few years, there has been increasing evidence that HEV, with the development of acute/chronic clinical disease (mainly in immune-compromised patients, HD patients, and transplant recipients), may occur in non-endemic areas, with the zoonotic pathway (porcine zoonosis) having been found to be the major reason for this infection[20,21,42,43]. There are few data on the prevalence of HEV infection and/or the prevalence of circulating HEV antibodies in end-stage renal failure patients for Italy as a whole or in just in southern Italy[11,44,45].

In this survey, we studied three cohorts of individuals (hemodialysis patients, kidney transplant recipients, and the general population as a control); the overall prevalence of circulating HEV-Ab was 3.7% with appreciable, but not significant, deviations between the general population (2.7%) and HD patients (6.0%), but not kidney transplant recipients (3.3%), compared to the general population.

There are few studies with conflicting results about HEV epidemiology among HD patients[10,11,14,15]. The different prevalence of HEV infection in the general population[42,43,46], the parameters for the inclusion of patients, the routes of HEV transmission[22,24], and the serological assays used[47,48] could partially explain the different findings. In our research in patients with defects in cellular and humoral immunity, we confirmed HEV-Ig positivity with western immunoblotting techniques, which validated both the acute-phase and chronic-phase with better sensitivity and specificity[49]. The seroprevalence of anti-HEV/IgG observed in our HD patients is lower than that reported in other recent studies in the United Kingdom[10], France[46], and Japan[15], and is consistent with HEV seroprevalence data in Greece[13,30], another study in Japan[29], Spain[31], and Saudi Arabia[50]. In all of these studies, there was a higher anti-HEV seroprevalence in HD patients *vs* controls.

A logistic regression analysis showed that neither length of HD, nor other variables related to HD, such as blood transfusion, were associated with HEV, while a significant link was reported between the presence of antibodies type IgG and older age (> 70 years).

The correlation of HEV/older age could reflect a cohort phenomenon due to infections acquired some decades ago. Antibodies for HEV/IgG can persist over the long-term, and it may be that water-borne hepatitis outbreaks of unknown aetiology occurred in Apulia earlier in the 20th century and became present as sporadic cases owing to the fecal pollution of drinking water with hepatitis E and not A (previously related to the high circulation of HAV in our region, which is approximately 60% of subjects older than 50 years).

HEV is usually associated with an enterically-transmitted infection, but the high prevalence of anti-HEV reported in some studies in HD patients indicated that the fecal-oral route may not be the only route of transmission of HEV and these individuals with a high risk for HBV and HCV could also have been infected by unknown HEV. In fact, experimental transmission of HEV in humans showed a transient phase of viremia preceding the onset of clinical symptoms, and prolonged viremia has been observed in some patients[50]. Therefore, a theoretical possibility of HEV parenteral transmission has been suggested, mainly in endemic areas[50,51]. In our study, only two patients presented with an association between HEV and HCV. Our data are probably different than that of regions

with high rates of HEV infection for two reasons: first, in Italy, there is a modest rate of anti-HEV and a low flow of HEV in our community, resulting in a reduced risk of chronic HCV co-infection; second, a relatively small number of patients were tested.

Another interesting and stimulating hypothesis was suggested by Harrison *et al*[52], claiming that the use of heparin, derived from porcine small intestine, in HD patients might be one possible cause of HEV infection[53]; HEV has been found in the porcine small intestine after experimental HEV infection in pigs[54]. It is not known whether heparin regularly used in humans is infected with HEV, but this might be a possible route of infection and merits further studies[52].

Acute HEV in HD and transplant patients is infrequent and possibly under-diagnosed. Only 0.9% (2/31) of HD patients and 1/120 (0.8%) of transplant recipients in the present survey were anti-HEV/IgM positive, and only one of the patients had any symptoms/signs suspected or diagnosed as acute hepatitis. The clinical events usually associated with acute hepatitis are often less evident in these patients (low grade or subclinical hepatitis); this factor might have contributed to the failure to recognize and document any clinical episodes of hepatitis in our patients. Serum levels of ALT are low in HD patients, and its elevation is usually less pronounced in these individuals, even in the presence of acute hepatitis. In fact, chronic uremic patients present with a reduced immune competence, and this situation can be responsible for the attenuated inflammatory reactions in the liver and consequently reduced hepatocyte destruction, and, therefore, the AST/ALT levels in HD patients are usually suppressed[50,55]. The ALT levels in the three patients found to be IgM anti-HEV positive in our study showed only mild abnormalities; the highest ALT levels, with a peak elevation within 1 ± 3 d of the onset of the illness followed by a decline, were less than twice the upper limit of normal levels.

We observed that the HD patients had a higher HEV seroprevalence than transplant recipients (6% *vs* 3.3%), suggesting that hemodialysis could represent a risk factor for HEV infection. However, in our study, renal transplant recipients with a prior history of HD had the same rate of HEV infection than that seen in transplants recipients without prior HD treatment. The different seroprevalence between the HD patients and transplant recipients is unexplained, although it is possible that anti-HEV serological assays perform poorly in transplant recipients receiving immunosuppressive treatment are the cause, thereby determining a percentage error with false negative results[56]. In the transplant recipients in our survey, the numbers and the doses of immunosuppressive drugs were decreased when they were diagnosed anti-HEV IgM and HEV-RNA positive. In fact, this is the first approach to control HEV infection in these patients[33], because these drugs decrease the synthesis of antibodies and inhibit the cell-cycle progression and differentiation of human B lymphocytes. The humoral immune response is necessary to clear HEV and prevent hepatitis[57,58].

However, in the past few years, many studies have reported a surprisingly high prevalence of chronic HEV hepatitis in immunosuppressed patients, including kidney transplant recipients[34-36]. In our survey, at 6 mo of follow-up, no patient remained chronically viremic and there was not a correlation between any stage of chronic hepatitis and HEV infection alone in any patient of the two groups with chronic renal failure. The only patients who presented with chronic active hepatitis were HCV-HEV co-infected.

Finally, this study showed a higher circulation of HEV in end-stage renal failure in the district of Foggia *vs* the healthy population, but this high prevalence is mainly related to hemodialysis patients.

There was no relationship between the duration of HD treatment and the risk of acquiring a HEV infection. None of the kidney transplant recipients or HD patients had any evidence of chronic HEV. Although no specific therapy is currently available, unexplained hepatitis in the dialysis setting and in kidney transplant recipients undergoing chronic immunosuppression calls for an evaluation of HEV infection, especially in kidney transplant candidates.

COMMENTS

Background

Hepatitis E is the main aetiological agent of enteric non-A hepatitis. In the recent past, it was believed to be present only in developing countries, where it was associated with epidemic outbreaks through the fecal-oral route from contaminated water supplies, but it is now recognized as a worldwide infection, sometimes related, in developed countries, to an asymptomatic zoonotic infection (as well as undercooked meat products).

Research frontiers

Recently, previous seroprevalence surveys in developed countries showed variable rates of anti-hepatitis E virus (HEV) positivity in healthy populations, and several studies reported an unexpectedly high prevalence of antibodies against HEV in hemodialysis patients.

Innovations and breakthroughs

There are scant reports on the prevalence and possible nosocomial transmission of HEV in hemodialysis (HD) patients. Some authors highlighted the high rates of anti-HEV antibodies in their HD patients and hypothesized other routes of transmission besides the fecal-oral route, although the real prevalence of HEV infection through the parenteral route, particularly via hemodialysis, is unknown. Other investigators observed low rates of anti-HEV-positivity in their HD populations.

Applications

This study showed a higher circulation of HEV in end-stage renal failure in the district of Foggia *vs* the healthy population, but this high prevalence is mainly related to hemodialysis patients. There was no relationship between the duration of HD treatment and the risk of acquiring a HEV infection. None of the kidney transplant recipients or HD patients had any evidence of chronic HEV. Although no specific therapy is currently available, unexplained hepatitis in the dialysis setting and in kidney transplant recipients undergoing chronic immunosuppression calls for an evaluation of HEV infection, especially in kidney transplant candidates.

Terminology

Hepatitis E, a single-stranded RNA virus, is the main aetiological agent of enteric non-A hepatitis. The prevalence of HEV-IgG in chronic hemodialysis patients and transplant recipients could be related to their impaired immunity, with an increased susceptibility to infections and decreased immune responses

to antigenic stimuli.

Peer-review

Scotto *et al* report the results from screening a large number of HD and control patients for prior and current HEV infection. This is of interest because chronic HEV has been shown to infect immunosuppressed populations, and hemodialysis patients are in some respects immunocompromised. There has also been a relatively high prevalence found in renal transplant patients that come from this population.

REFERENCES

1 **Tsianos EV**, Dalekos GN, Elisaf M, Zervou E, Siamopoulos KC. High frequency of antibodies to Hantaan virus and hepatitis C virus in chronic haemodialysis patients. Coincidence or cross-reaction? *J Intern Med* 1993; **234**: 607-610 [PMID: 8258753 DOI: 10.1111/j.1365-2796.1993.tb01021.x]

2 **Fabrizi F**, Lunghi G, Martin P. Hepatitis B virus infection in hemodialysis: recent discoveries. *J Nephrol* 2002; **15**: 463-468 [PMID: 12455711 DOI: 10.1111/j.1525-139x.2007.00311.x]

3 **Fabrizi F**, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. *Hepatology* 2002; **36**: 3-10 [PMID: 12085342 DOI: 10.1053/jhep.2002.34613]

4 **Wong PN**, Fung TT, Mak SK, Lo KY, Tong GM, Wong Y, Loo CK, Lam EK, Wong AK. Hepatitis B virus infection in dialysis patients. *J Gastroenterol Hepatol* 2005; **20**: 1641-1651 [PMID: 16246180 DOI: 10.1111/j.1440-1746.2005.03837.x]

5 **Mina P**, Georgiadou SP, Rizos C, Dalekos GN, Rigopoulou EI. Prevalence of occult hepatitis B virus infection in haemodialysis patients from central Greece. *World J Gastroenterol* 2010; **16**: 225-231 [PMID: 20066742 DOI: 10.3748/wjg.v16.i2.225]

6 **Alter MJ**, Favero MS, Maynard JE. Impact of infection control strategies on the incidence of dialysis-associated hepatitis in the United States. *J Infect Dis* 1986; **153**: 1149-1151 [PMID: 3701120 DOI: 10.1093/infdis/153.6.1149]

7 **Ayoola EA**, Huraib S, Arif M, al-Faleh FZ, al-Rashed R, Ramia S, al-Mofleh IA, Abu-Aisha H. Prevalence and significance of antibodies to hepatitis C virus among Saudi haemodialysis patients. *J Med Virol* 1991; **35**: 155-159 [PMID: 1725179 DOI: 10.1002/jmv.1890350303]

8 **Niu MT**, Alter MJ, Kristensen C, Margolis HS. Outbreak of hemodialysis-associated non-A, non-B hepatitis and correlation with antibody to hepatitis C virus. *Am J Kidney Dis* 1992; **19**: 345-352 [PMID: 1562024 DOI: 10.1016/s0272-6386(12)80452-5]

9 **Okuda K**, Hayashi H, Yokozeki K, Kobayashi S, Kashima T, Irie Y. Acute hepatitis C among renal failure patients on chronic haemodialysis. *J Gastroenterol Hepatol* 1998; **13**: 62-67 [PMID: 9737574 DOI: 10.1111/j.1440-1746.1998.tb00547.x]

10 **Dalton HR**, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. *Lancet Infect Dis* 2008; **8**: 698-709 [PMID: 18992406 DOI: 10.1016/s1473-3099(08)70255-x]

11 **Scotto G**, Martinelli D, Centra M, Querques M, Vittorio F, Delli Carri P, Tartaglia A, Campanale F, Bulla F, Prato R, Fazio V. Epidemiological and clinical features of HEV infection: a survey in the district of Foggia (Apulia, Southern Italy). *Epidemiol Infect* 2014; **142**: 287-294 [PMID: 23673019 DOI: 10.1017/s0950268813001167]

12 **Dalekos GN**, Zervou E, Elisaf M, Germanos N, Galanakis E, Bourantas K, Siamopoulos KC, Tsianos EV. Antibodies to hepatitis E virus among several populations in Greece: increased prevalence in an hemodialysis unit. *Transfusion* 1998; **38**: 589-595 [PMID: 9661693 DOI: 10.1046/j.1537-2995.1998.38698326339.x]

13 **Stefanidis I**, Zervou EK, Rizos C, Syrganis C, Patsidis E, Kyriakopoulos G, Sdrakas L, Tsianas N, Rigopoulou EI, Liakopoulos V, Dalekos GN. Hepatitis E virus antibodies in hemodialysis patients: an epidemiological survey in central Greece. *Int J Artif Organs* 2004; **27**: 842-847 [PMID: 15560678 DOI: 10.1016/j.jcv.2005.05.007]

14 **Sylvan SP**, Jacobson SH, Christenson B. Prevalence of antibodies to hepatitis E virus among hemodialysis patients in Sweden. *J Med Virol* 1998; **54**: 38-43 [PMID: 9443107 DOI: 10.1002/(sici)1096-9071(199801)54]

15 **Mitsui T**, Tsukamoto Y, Hirose A, Suzuki S, Yamazaki C, Masuko K, Tsuda F, Endo K, Takahashi M, Okamoto H. Distinct changing profiles of hepatitis A and E virus infection among patients with acute hepatitis, patients on maintenance hemodialysis and healthy individuals in Japan. *J Med Virol* 2006; **78**: 1015-1024 [PMID: 16789007 DOI: 10.1002/jmv.20657]

16 **Litjens NH**, Huisman M, van den Dorpel M, Betjes MG. Impaired immune responses and antigen-specific memory CD4+ T cells in hemodialysis patients. *J Am Soc Nephrol* 2008; **19**: 1483-1490 [PMID: 18480314 DOI: 10.1681/asn.2007090971]

17 **Edey M**, Barraclough K, Johnson DW. Review article: Hepatitis B and dialysis. *Nephrology* (Carlton) 2010; **15**: 137-145 [PMID: 20470270 DOI: 10.1111/j.1440-1797.2009.01268.x]

18 **Cohen G**, Haag-Weber M, Hörl WH. Immune dysfunction in uremia. *Kidney Int Suppl* 1997; **62**: S79-S82 [PMID: 9350688 DOI: 10.3390/toxins4110962]

19 **Reuter G**, Fodor D, Forgách P, Kátai A, Szucs G. Characterization and zoonotic potential of endemic hepatitis E virus (HEV) strains in humans and animals in Hungary. *J Clin Virol* 2009; **44**: 277-281 [PMID: 19217346 DOI: 10.1016/j.jcv.2009.01.008]

20 **Christensen PB**, Engle RE, Hjort C, Homburg KM, Vach W, Georgsen J, Purcell RH. Time trend of the prevalence of hepatitis E antibodies among farmers and blood donors: a potential zoonosis in Denmark. *Clin Infect Dis* 2008; **47**: 1026-1031 [PMID: 18781880 DOI: 10.1086/591970]

21 **Meng XJ**, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol* 2002; **40**: 117-122 [PMID: 11773103 DOI: 10.1128/jcm.40.1.117-122.2002]

22 **Mushahwar IK**. Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. *J Med Virol* 2008; **80**: 646-658 [PMID: 18297720 DOI: 10.1002/jmv.21116]

23 **Somani SK**, Aggarwal R, Naik SR, Srivastava S, Naik S. A serological study of intrafamilial spread from patients with sporadic hepatitis E virus infection. *J Viral Hepat* 2003; **10**: 446-449 [PMID: 14633178 DOI: 10.1046/j.1365-2893.2003.00458.x]

24 **Teshale EH**, Grytdal SP, Howard C, Barry V, Kamili S, Drobeniuc J, Hill VR, Okware S, Hu DJ, Holmberg SD. Evidence of person-to-person transmission of hepatitis E virus during a large outbreak in Northern Uganda. *Clin Infect Dis* 2010; **50**: 1006-1010 [PMID: 20178415 DOI: 10.1086/651077]

25 **Cleland A**, Smith L, Crossan C, Blatchford O, Dalton HR, Scobie L, Petrik J. Hepatitis E virus in Scottish blood donors. *Vox Sang* 2013; **105**: 283-289 [PMID: 23763589 DOI: 10.1111/vox.12056]

26 **Mansuy JM**, Bendall R, Legrand-Abravanel F, Sauné K, Miédouge M, Ellis V, Rech H, Destruel F, Kamar N, Dalton HR, Izopet J. Hepatitis E virus antibodies in blood donors, France. *Emerg Infect Dis* 2011; **17**: 2309-2312 [PMID: 22172156 DOI: 10.3201/eid1712.110371]

27 **Scotto G**, Giammario A, Centra M, Vittorio F, Martinelli D, Fazio V. Seroprevalence of hepatitis E virus among blood donors in a district of southern Italy. *Blood Transfus* 2012; **10**: 565-566 [PMID: 22682344 DOI: 10.2450/2012.0154-11]

28 **Mitsui T**, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. *J Med Virol* 2004; **74**: 563-572 [PMID: 15484278 DOI: 10.1002/jmv.20215]

29 **Kikuchi K**, Yoshida T, Kimata N, Sato C, Akiba T. Prevalence of hepatitis E virus infection in regular hemodialysis patients. *Ther Apher Dial* 2006; **10**: 193-197 [PMID: 16684223 DOI: 10.1111/j.1744-9987.2006.00363.x]

30 **Psichogiou M**, Vaindirli E, Tzala E, Voudiclari S, Boletis J, Vosnidis G, Moutafis S, Skoutelis G, Hadjiconstantinou V, Troonen H, Hatzakis A. Hepatitis E virus (HEV) infection in haemodialysis

patients. The Multicentre Haemodialysis Cohort Study on Viral Hepatitis. *Nephrol Dial Transplant* 1996; **11**: 1093-1095 [PMID: 8671974 DOI: 10.1093/oxfordjournals.ndt.a027461]

31 **Ibarra H**, Riedemann S, Reinhardt G, Ardiles L, Calvo M, Siegel F. Anti-HEV in dialysis and renal transplant patients in an endemic region in Chile. *Clin Nephrol* 1998; **50**: 267-268 [PMID: 9799076 DOI: 10.1016/s0140-6736(94)90315-8]

32 **Buffet C**, Laurent-Puig P, Chandot S, Laurian Y, Charpentier B, Briantais MJ, Dussaix E. A high hepatitis E virus seroprevalence among renal transplantation and haemophilia patient populations. *J Hepatol* 1996; **24**: 122-125 [PMID: 8834035 DOI: 10.1016/s0168-8278(96)80196-6]

33 **Kamar N**, Selves J, Mansuy JM, Ouezzani L, Péron JM, Guitard J, Cointault O, Esposito L, Abravanel F, Danjoux M, Durand D, Vinel JP, Izopet J, Rostaing L. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med* 2008; **358**: 811-817 [PMID: 18287603 DOI: 10.1056/nejmoa0706992]

34 **Gérolami R**, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. *N Engl J Med* 2008; **358**: 859-860 [PMID: 18287615 DOI: 10.1056/nejmc0708687]

35 **Gérolami R**, Moal V, Picard C, Colson P. Hepatitis E virus as an emerging cause of chronic liver disease in organ transplant recipients. *J Hepatol* 2009; **50**: 622-624 [PMID: 19157619 DOI: 10.1016/j.jhep.2008.12.008]

36 **Haagsma EB**, van den Berg AP, Porte RJ, Benne CA, Vennema H, Reimerink JH, Koopmans MP. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl* 2008; **14**: 547-553 [PMID: 18383084 DOI: 10.1002/lt.21480]

37 **Ollier L**, Tieulie N, Sanderson F, Heudier P, Giordanengo V, Fuzibet JG, Nicand E. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. *Ann Intern Med* 2009; **150**: 430-431 [PMID: 19293084 DOI: 10.7326/0003-4819-150-6-200903170-00026]

38 **Péron JM**, Mansuy JM, Récher C, Bureau C, Poirson H, Alric L, Izopet J, Vinel JP. Prolonged hepatitis E in an immunocompromised patient. *J Gastroenterol Hepatol* 2006; **21**: 1223-1224 [PMID: 16824086 DOI: 10.1111/j.1440-1746.2006.04209.x]

39 **Tamura A**, Shimizu YK, Tanaka T, Kuroda K, Arakawa Y, Takahashi K, Mishiro S, Shimizu K, Moriyama M. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. *Hepatol Res* 2007; **37**: 113-120 [PMID: 17300706 DOI: 10.1111/j.1872-034x.2007.00024.x]

40 **Dalton HR**, Bendall RP, Keane FE, Tedder RS, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. *N Engl J Med* 2009; **361**: 1025-1027 [PMID: 19726781 DOI: 10.1056/nejmc0903778]

41 **Gouvea V**, Hoke CH, Innis BL. Genotyping of hepatitis E virus in clinical specimens by restriction endonuclease analysis. *J Virol Methods* 1998; **70**: 71-78 [PMID: 9506814 DOI: 10.1016/s0166-0934(97)00172-9]

42 **Purcell RH**, Emerson SU. Hepatitis E: an emerging awareness of an old disease. *J Hepatol* 2008; **48**: 494-503 [PMID: 18192058 DOI: 10.1016/j.jhep.2007.12.008]

43 **Aggarwal R**, Naik S. Epidemiology of hepatitis E: current status. *J Gastroenterol Hepatol* 2009; **24**: 1484-1493 [PMID: 19686410 DOI: 10.1111/j.1440-1746.2009.05933.x]

44 **Gessoni G**, Manoni F. Hepatitis E virus infection in north-east Italy: serological study in the open population and groups at risk. *J Viral Hepat* 1996; **3**: 197-202 [PMID: 8871881 DOI: 10.1111/j.1365-2893.1996.tb00095.x]

45 **Fabrizi F**, Lunghi G, Bacchini G, Corti M, Pagano A, Locatelli F. Hepatitis E virus infection in haemodialysis patients: a seroepidemiological survey. *Nephrol Dial Transplant* 1997; **12**: 133-136 [PMID: 9027787 DOI: 10.1093/ndt/12.1.133]

46 **Mansuy JM**, Peron JM, Abravanel F, Poirson H, Dubois M, Miedouge M, Vischi F, Alric L, Vinel JP, Izopet J. Hepatitis E in the south west of France in individuals who have never visited an endemic area. *J Med Virol* 2004; **74**: 419-424 [PMID: 15368508 DOI: 10.1002/jmv.20206]

47 **Bendall R**, Ellis V, Ijaz S, Ali R, Dalton H. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. *J Med Virol* 2010; **82**: 799-805 [PMID: 20336757 DOI: 10.1002/jmv.21656]

48 **Drobeniuc J**, Meng J, Reuter G, Greene-Montfort T, Khudyakova N, Dimitrova Z, Kamili S, Teo CG. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. *Clin Infect Dis* 2010; **51**: e24-e27 [PMID: 20578874 DOI: 10.1086/654801]

49 **Haqshenas G**, Huang FF, Fenaux M, Guenette DK, Pierson FW, Larsen CT, Shivaprasad HL, Toth TE, Meng XJ. The putative capsid protein of the newly identified avian hepatitis E virus shares antigenic epitopes with that of swine and human hepatitis E viruses and chicken big liver and spleen disease virus. *J Gen Virol* 2002; **83**: 2201-2209 [PMID: 12185274]

50 **Ayoola EA**, Want MA, Gadour MO, Al-Hazmi MH, Hamza MK. Hepatitis E virus infection in haemodialysis patients: a case-control study in Saudi Arabia. *J Med Virol* 2002; **66**: 329-334 [PMID: 11793384 DOI: 10.1002/jmv.2149]

51 **Chauhan A**, Jameel S, Dilawari JB, Chawla YK, Kaur U, Ganguly NK. Hepatitis E virus transmission to a volunteer. *Lancet* 1993; **341**: 149-150 [PMID: 8093748 DOI: 10.1016/0140-6736(93)90008-5]

52 **Harrison A**, Scobie L, Crossan C, Parry R, Johnston P, Stratton J, Dickinson S, Ellis V, Hunter JG, Prescott OR, Madden R, Lin NX, Henley WE, Bendall RP, Dalton HR. Hepatitis E seroprevalence in recipients of renal transplants or haemodialysis in southwest England: a case-control study. *J Med Virol* 2013; **85**: 266-271 [PMID: 23169048 DOI: 10.1002/jmv.23463]

53 **Jaques LB**, Kavanagh LW, Kuo SH. Variation in commercial heparin and its relation to the problem of heparin standardization for clinical use. *Thromb Res* 1973; **3**: 295-306 [DOI: 10.1016/0049-3848(73)90055-8]

54 **Lee YH**, Ha Y, Ahn KK, Chae C. Localisation of swine hepatitis E virus in experimentally infected pigs. *Vet J* 2009; **179**: 417-421 [PMID: 18308595 DOI: 10.1016/j.tvjl.2007.10.028]

55 **Fabrizi F**, Lunghi G, Finazzi S, Colucci P, Pagano A, Ponticelli C, Locatelli F. Decreased serum aminotransferase activity in patients with chronic renal failure: impact on the detection of viral hepatitis. *Am J Kidney Dis* 2001; **38**: 1009-1015 [PMID: 11684554 DOI: 10.1053/ajkd.2001.28590]

56 **Legrand-Abravanel F**, Kamar N, Sandres-Saune K, Lhomme S, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Izopet J. Hepatitis E virus infection without reactivation in solid-organ transplant recipients, France. *Emerg Infect Dis* 2011; **17**: 30-37 [PMID: 21192851 DOI: 10.3201/eid1701.100527]

57 **Luo H**, Chen H, Daloze P, Chang JY, St-Louis G, Wu J. Inhibition of in vitro immunoglobulin production by rapamycin. *Transplantation* 1992; **53**: 1071-1076 [PMID: 1585470 DOI: 10.1097/00007890-199205000-00019]

58 **Aagaard-Tillery KM**, Jelinek DF. Inhibition of human B lymphocyte cell cycle progression and differentiation by rapamycin. *Cell Immunol* 1994; **156**: 493-507 [PMID: 7517796 DOI: 10.1006/cimm.1994.1193]

P- Reviewer: Kanda T, Kaplan DE, Tamori A **S- Editor**: Ma YJ
L- Editor: Rutherford A **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3274

World J Gastroenterol 2015 March 21; 21(11): 3274-3281
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Ratio of metastatic lymph nodes is more important for rectal cancer patients treated with preoperative chemoradiotherapy

In Ja Park, Chang Sik Yu, Seok-Byung Lim, Yong Sik Yoon, Chan Wook Kim, Tae Won Kim, Jong Hoon Kim, Jin Cheon Kim

In Ja Park, Chang Sik Yu, Seok-Byung Lim, Yong Sik Yoon, Chan Wook Kim, Jin Cheon Kim, Department of colon and rectal Surgery, University of Ulsan College of Medicine and Asan Medical Center, Seoul 138-736, South Korea
Tae Won Kim, Department of Medical Oncology, University of Ulsan College of Medicine and Asan Medical Center, Seoul 138-736, South Korea
Jong Hoon Kim, Department of Radiation Oncology, University of Ulsan College of Medicine and Asan Medical Center, Seoul 138-736, South Korea
Author contributions: Park IJ designed study and write manuscript; Yu CS designed study and provided critical comments on manuscript; Lim SB, Yoon YS, Kim CW, Kim TW, Kim JH and Kim JC provided clinical data and were also involved in editing the manuscript; Kim JC provided critical comments on manuscript.

Correspondence to: Chang Sik Yu, MD, PhD, Professor, Department of colon and rectal Surgery, University of Ulsan College of Medicine and Asan Medical Center, 86 Asanbyeongwon-gil, Songpa-gu, Seoul 138-736, South Korea. csyu@amc.seoul.kr
Telephone: +82-2-30103494
Fax: +82-2-4749027
Received: August 7, 2014
Peer-review started: August 8, 2014
First decision: August 27, 2014
Revised: October 6, 2014
Accepted: November 19, 2014
Article in press: November 19, 2014
Published online: March 21, 2015

Abstract

AIM: To evaluate the predictive value of the lymph node (LN) ratio (LNR, number of metastatic LNs/examined LNs) for recurrence in patients with rectal cancer and to compare its applicability according to preoperative chemoradiotherapy (PCRT).

METHODS: From 2000 to 2009, 967 patients with metastatic LNs after curative resection for locally advanced rectal cancer were identified. Patients were categorized according to PCRT (PCRT *vs* No PCRT). The cut-off LNR was determined based on the pN1 *vs* pN2 when the recommended number of LNs was harvested. The 5-year recurrence-free survival (RFS) rates using the Kaplan-Meier method were compared according to p/yp N stage and the LNR in each group.

RESULTS: Among patients with the same p/ypN stage, the 5-year RFS rate differed according to the LNR. In addition, the 5-year RFS rate was significantly different between pN and LNR groups in patients with No PCRT. In PCRT group, however, only LNR was associated with prognosis. On multivariate analysis, both pN and LNR were significant independent prognostic factors for 5-year RFS in the No PCRT group. In the PCRT group, only LNR category was found to be associated with RFS (HR = 2.36, 95%CI: 1.31-3.84, and P = 0.001).

CONCLUSION: The LNR is an important prognostic predictor of RFS in rectal cancer patients especially treated with PCRT. Current pN categories could not discriminate between prognostic groups of RFS after PCRT.

Key words: Rectal cancer; Preoperative chemora-

diotherapy; Lymph node ratio; Prognosis; pN

Core tip: The number of metastatic lymph node might show different prognosis according to the number of examined lymph node. Retrieved number of lymph node after preoperative chemoradiotherapy (PCRT) has been known fewer than those without PCRT. However, number of metastatic lymph nodes used in pathologic staging was same between patients treated with PCRT and those without PCRT. The present study suggests the metastatic lymph node ratio would be useful prognostic indicator and it is more prominent in patients treated with PCRT.

Park IJ, Yu CS, Lim SB, Yoon YS, Kim CW, Kim TW, Kim JH, Kim JC. Ratio of metastatic lymph nodes is more important for rectal cancer patients treated with preoperative chemoradiotherapy. *World J Gastroenterol* 2015; 21(11): 3274-3281 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3274.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3274

INTRODUCTION

The current staging system for colorectal cancer is based solely on the number of metastatic lymph nodes (LNs)[1]. To accurately stage patients using this system, a sufficient number of LNs-greater than 12 for colorectal cancer-must be examined to avoid underestimation of nodal stage[2]. The number of identified metastatic LNs can be influenced by the total number of LNs examined, and this can affect staging[3-5]. However, it remains unclear whether the prognostic significance of the number of metastatic LNs differs between patients who have only a small number of LNs retrieved compared to patients who have several LNs retrieved.

To overcome this limitation of the TNM staging system, a complementary LN metastasis stratification method is needed. The LNR, defined as the ratio of metastatic to examined LNs, has been shown to be useful in identifying prognostic subgroups within gastric and esophageal cancer patients[6,7]. The prognostic value of the LNR has also been demonstrated in colon and rectal cancer[8-10]. These previous studies have shown that the LNR can be used not only as a prognostic indicator, but also as a parameter for a more accurate stratification system than the metastatic LN absolute number-based staging system in colon and rectal cancer.

Preoperative chemoradiotherapy (PCRT) has been shown to induce shrinkage of tumors and provide lymphatic drainage, and is associated with improved local control[11-13]. However, the applicability of postoperative pathologic results in patients treated by PCRT has not been fully assessed. Furthermore, while a correlation between lymph node metastasis and poor oncologic outcome in patients treated with PCRT and radical resection has been suggested, the value of the LNR after PCRT remains controversial[14,15]. PCRT has been shown to result in a significant decrease in both the size and number of LNs available for examination after resection[16-20]. Consequently, the number of LNs examined could be below the recommended number in patients with rectal cancer. Therefore, for patients with rectal cancer treated with PCRT, a complementary LN metastasis stratification method may be needed than for those treated with upfront surgery.

It is unclear whether the impact of the LNR on prognosis differs between rectal cancer patients treated with PCRT and those treated with upfront surgical resection. The aim of this study was to evaluate the prognostic impact of the LNR in rectal cancer patients with metastatic LNs after radical resection.

MATERIALS AND METHODS

Patients, clinical staging, and treatment

We performed a retrospective consecutive cohort study of patients with biopsy-proven, locally advanced mid and low rectal cancer who were treated at Asan Medical Center between 2000 and 2009. Patients were identified from our institutional colorectal cancer patient database and tumor registry. Among the identified patients, 967 patients proved to have metastatic LNs on final pathologic examination. Patients with concurrent distant metastasis, concurrent inflammatory bowel disease, hereditary colorectal cancer syndromes, concurrent malignancy, emergent surgery, a prior history of radiotherapy to the pelvis, or a prior history of malignancy were excluded. Study approval was obtained from the Asan Medical Center Institutional Review Board.

Pretreatment clinical stage was assessed based on transrectal ultrasound (TUS), magnetic resonance imaging (MRI), or computed tomography (CT) findings. All patients also underwent full colonoscopic evaluation to exclude synchronous tumors, as well as digital rectal examination and proctoscopy to identify the tumor distance from the anal verge. Some patients were treated with PCRT, with a median radiotherapy dose of 50.4 Gy and concurrent fluoropyrimidine-based chemotherapy (mainly single-agent infusional 5-fluorouracil or capecitabine). For patients treated with PCRT, operations were generally performed 6 to 8 wk following the completion of PCRT using total mesorectal excision principles.

Standard pathologic tumor staging of the resected specimen was then performed. Postoperative follow-up consisted of routine physical examination with carcinoembryonic antigen (CEA) measurement every 3 to 6 mo, as well as colonoscopy every 2 to 3 years and

Table 1 Patient and tumor characteristics *n* (%)

	Non-PCRT, *n* = 724	PCRT, *n* = 243	*P* value
Age (mean ± SD) (yr)	54 ± 10.3	59.2 ± 11.3	< 0.001
< 50	154 (21.3)	80 (32.9)	
50-65	346 (47.8)	130 (53.5)	< 0.001
> 65	224 (30.9)	33 (13.6)	
Gender			0.005
Male	434 (59.9)	148 (60.9)	
Female	290 (40.1)	95 (39.1)	
Location[1]			< 0.001
6-10 cm	474 (65.5)	100 (41.2)	
⩽ 5 cm	250 (34.5)	143 (58.8)	
Sphincter preservation	600 (82.9)	177 (72.8)	< 0.001
Among patients with low rectum	133 (53.2)	81 (56.6)	0.370
LNR[2]	0.25 ± 0.24	0.252 ± 0.19	0.170
Number of harvested LNs	18.2 ± 8.5	14.8 ± 7.1	< 0.001
< 12 LNs harvested	153 (21.1)	96 (39.5)	< 0.001
Number of positive LNs	3.9 ± 3.7	2.8 ± 2.8	< 0.001
p/yp N category			< 0.001
p/yp N1	445 (61.5)	181 (74.5)	
p/yp N2	279 (38.5)	62 (25.5)	
Follow-up duration, (Interquartile range) (mo)	60 (39-80)	56 (43-68)	0.540

[1]From the anal verge; [2]Lymph node ratio. PCRT: Preoperative chemoradiotherapy; LNR: Lymph node ratio; LNs: Lymph nodes.

cross-sectional imaging every 6 to 12 mo for 5 years.

Statistical analysis

To investigate the association of the metastatic LNR with oncologic outcome, categorization of LNRs was performed. A cut-off value of 0.25 was chosen to facilitate patient assignment to subgroups because 0.25 represents the number of metastatic lymph node of pN1 category based on 12 LNs harvested, which are recommend by the current TNM staging system for proper staging.

Patients were then assigned to two groups based on their LNR: LNR1, less than or equal to 0.25; LNR2, greater than 0.25. Pathologic N category of TNM staging system was chosen for comparison of function as a prognostic predictor with the two LNR subgroups of patients.

Categorical data were summarized by frequency within each cohort, and comparisons were performed using the χ^2 test for proportions. A test for binary correlation was used to assess associations between selected polynomial categorical variables. For recurrence-free survival (RFS) analysis, cases were identified as failures at the time of disease recurrence. RFS rates were determined for each LNR-based group using pT category and current TNM stage-based group. Cox proportional hazards regression analysis was performed for multivariate comparisons. *P* values less than 0.05 were considered statistically significant.

RESULTS

Patient population and tumor characteristics

A total of 256 patients who were treated with PCRT and 724 who were treated with upfront surgery for rectal cancer during the study period and had pathologically proven cancer with metastatic LNs (ypN+) were included. The median age was 55 years [interquartile range (IQR): 48-62 years)]. The median distance of the tumor from the anal verge was 5 cm (IQR: 4-7 cm). All patients underwent total mesorectal excision. A sphincter-saving procedure was performed in 777 patients (80.3%). Age, gender, and sphincter preservation rates did not differ between patients who underwent PCRT and those who did not. The number of harvested and metastatic LNs was significantly lower among patients treated with PCRT (Table 1).

There were 96 patients (39.5%) in the PCRT group and 153 patients (21.1%) in the -No PCRT group who had less than 12 LNs resected. Of the 724 patients in the No PCRT group, 445 (61.5%) were N1 and 279 (38.5%) were N2. In the PCRT group, 181 (74.5%) were N1 and 62 (25.5%) were N2 (Table 1). The mean LNR was not different between the two groups.

Recurrence-free survival and prognostic factors for recurrence-free survival

The median follow-up duration was 40 mo (IQR: 32-58 mo) for the entire cohort. Within the same ypN category, the 5-year RFS rate differed significantly according to the LNR group. By contrast, significant differences in ypN were not found within LNR groups (Table 2). RFS for each group according to the pN category and the LNR category was analyzed. Both pN category and LNR category showed stratification of RFS in the No PCRT group (Figure 1). In the PCRT group, however, RFS did not differ by the pN category. Only the LNR category showed stratification of RFS in the PCRT group (Figure 1).

Influence of the pN and the LNR category on RFS was evaluated according to the number of harvested LNs. In the No PCRT group, RFS differed according to both the pN and the LNR category regardless of whether 12 LNs were examined. For the PCRT group, RFS differed according to LNR when < 12 and ⩾ 12 LNs were harvested; in contrast, the pN category did not statistically significantly impact RFS irrespective of the number of harvested lymph node (Table 3).

Risk factors of recurrence-free survival: Prognostic implication of pN and LNR category

In univariate analysis, LNR category was associated with RFS in both the No PCRT and the PCRT group. pN category, however, was not associated with RFS in the PCRT group. Other factors related with RFS in the No PCRT group were location of tumor, lymphovascular

Table 2 Five-year recurrence-free survival for T-stage subgroups stratified by lymph node ratio and pN category

	No PCRT			*P* value[2]	PCRT			*P* value[2]
	pN1	pN2	Overall		pN1	pN2	Overall	
LNR1	78.6%	60.1%	76.2%	< 0.001	58.7%	42.0%	59.2%	< 0.001
LNR2	59.5%	51.2%	52.9%		27.6%	38.1%	33.8%	
Overall	76.3%	53.4%			54.3%	44.3%		
P value[1]		< 0.001				< 0.262		

[1]RFS according to the LNR; [2]RFS according to pN category. PCRT: Preoperative chemoradiotherapy; LNR: Lymph node ratio; RFS: Recurrence-free survival.

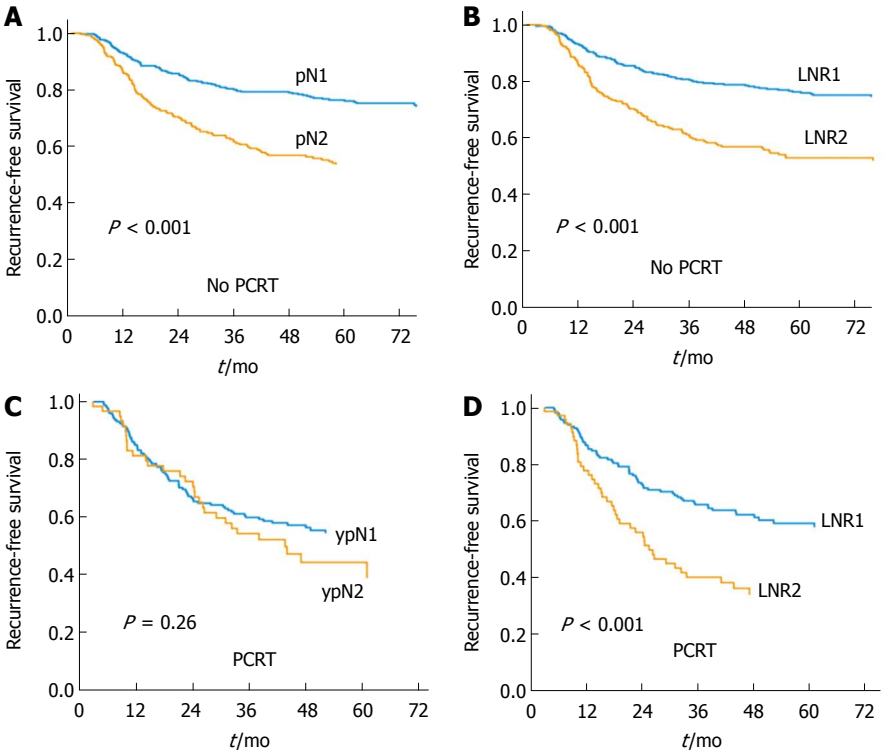


Figure 1 Recurrence-free survival. A: pN category in the no PCRT group; B: LNR category in the No PCRT group; C: ypN category in the PCRT group; D: LNR category in the PCRT group. LNR represents prognostic groups in both the No PCRT and the PCRT group. Current ypN status failed to show stratification with advancement of ypN status. PCRT: Preoperative chemoradiotherapy; LNR: Lymph node ratio.

Table 3 Five-year recurrence-free survival stratified by lymph node ratio and pN-category according to the number of harvested lymph nodes

	No PCRT				PCRT			
	< 12	*P* value	⩾ 12	*P* value	< 12	*P* value	⩾ 12	*P* value
p/yp N1	67.8%	0.01	79.2%	< 0.001	43.8%	0.90	62.4%	0.080
p/yp N2	43.3%		55.0%		36.5%		47.2%	
LNR1	74.2%	0.01	76.6%	< 0.001	50.2%	0.05	63.0%	0.007
LNR2	51.3%		53.6%		31.9%		38.1%	

PCRT: Preoperative chemoradiotherapy; LNR: Lymph node ratio.

invasion, perineural invasion, and increased preoperative serum CEA (sCEA). In the PCRT group, perineural invasion was the only factor associated with RFS. In multivariate analysis, both the pN and the LNR category were confirmed as independent prognostic factors of RFS in the No PCRT group. However, in the PCRT group, only the LNR category was an independent prognostic factor showing stratification for RFS (Table 4).

Prognostic groups combined with p/ypT category and LNR

We compared the 5-year RFS according to the current 7th TNM stage (Figure 2). The current TNM stage could

Table 4 Factors associated with recurrence-free survival: Multivariate analysis

Factor	No PCRT			PCRT		
	HR	95%CI	*P* value	HR	95%CI	*P* value
p/yp N category			< 0.001			0.380
N1	1.00			1.00		
N2	1.90	1.43-2.53		1.21	0.79-1.85	
LNR category			< 0.001			0.001
LNR1	1.00			1.00		
LNR2	1.97	1.48-2.63		1.94	1.31-2.88	
Lymphovascular invasion			0.34			
None	1.00			1.00		0.110
Present	1.24	0.93-1.67		1.42	0.89-2.27	
Perineural invasion			0.04			0.030
None	1.00			1.00		
Undetermined	1.65	1.12-2.43		1.86	1.18-2.93	
Location			0.02			0.040
6-10 cm	1.00			1.00		
≤ 5 cm	1.34	1.05-1.85		0.62	0.40-0.98	
Preoperative CEA			0.02			
Normal	1.00					
Increased	1.46	1.07-1.98				

PCRT: Preoperative chemoradiotherapy; LNR: Lymph node ratio; CEA: Carcinoembryoinc antigen.

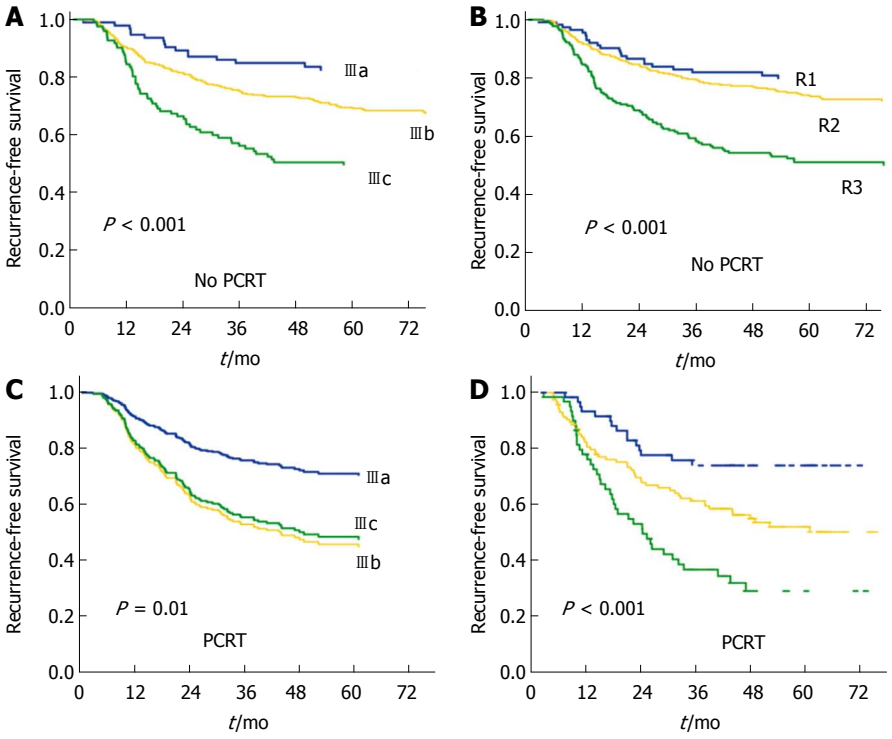


Figure 2 Recurrence-free survival. A: TNM stage by the 7th AJCC cancer staging system in the No PCRT group; B: R stage combined with pT/LNR in the No PCRT group; C: TNM stage in the PCRT group; D: R stage using yp T/LNR in the PCRT group. ypT/LNR combined groups showed significant categorization of prognostic groups. PCRT: Preoperative chemoradiotherapy; LNR: Lymph node ratio.

not effectively represent prognostic groups among patients of the PCRT group. We further analyzed the 5-year RFS considering the ypT and the LNR category and divided patients into three groups that showed statistical differences in RFS, R1 as ypT0-2 LNR1, R2 as ypT3-4 LNR1 and ypT0-2 LNR2, and R3 as ypT3-4 LNR2. These groups, which combined ypT and the LNR showed significant differences in 5-year RFS (Figure 2) and effectively separated patients into prognostic groups. For the No PCRT group, the R group, which is based on LNR and pT category, also showed stratification of RFS. In contrast, in the PCRT group, the R groups, but not the current TNM subgroups, showed significant differences in RFS.

DISCUSSION

In the PCRT group, LNR was found to be the most

significant prognostic factor for RFS in the present study. However, pN category could not discriminate patients into prognostic groups. Indeed, the LNR was significant both in patients with more than 12 LNs as well as in patients with less than 12 LNs examined. Although the harvesting of more than 12 LNs is recommended for proper staging, the number of LNs that can be harvested decreases in rectal cancer patients who have undergone PCRT[16,18]. Therefore, it is questionable whether the same absolute number-based staging system should be applied to all patients, including those with less than 12 retrieved LNs or patients who have been treated with PCRT. Based on the results of this study, the LNR could be applied to such patients as a prognostic predictor.

Patients within the same ypN category had a diverse distribution of the LNR. When patients within the same ypN category were stratified according to the LNR, there were significant differences in the 5-year RFS between LNR groups. In contrast, patients within the same LNR group did not show significant differences in RFS according to ypN category, except for the LNR1 group. This suggests that patients within the same ypN category could be further divided into different prognostic groups according to the LNR which might be a more proper discriminating category.

The LNR was confirmed as the only independent prognostic factor for RFS in the PCRT group using multivariate analysis. These findings suggest that a ratio-based approach is a better predictor of RFS than absolute number-based LN staging in patients with stage Ⅲ rectal cancer treated with PCRT.

Invasion through the bowel wall is also an independent high risk factor for recurrence and survival. We compared the 5-year RFS according to the current TNM stage and R group combined with the pT/ypT and the LNR category. The 5-year RFS significantly differed according to both TNM stage and the ypT/LNR-based R group in the No PCRT group. However, the current TNM stage could not show corresponding poor outcome according to advanced stage in the PCRT group. R groups, in comparison, showed better stratification for RFS in the PCRT group. The inherent value of any cancer staging system lies in its reproducibility and applicability to current methods of pathological assessment. As the stage Ⅲ patient group is defined by the identification and quantification of mesenteric nodes, accuracy of staging is directly proportional to nodal identification. Whereas examination of at least 12 LNs has been recommended for adequate determination of stage Ⅲ colorectal cancer, the finding of any nodal involvement, regardless of the number of nodes examined, is defined as stage Ⅲ disease. Therefore, a LNR was introduced to complement lymph node retrieval. In addition, the depth of invasion of tumor to bowel wall has to be considered alongside nodal status because nodal status was not the only determinant of pathologic stage. The present study compared RFS based on stage including the p/ypT category as well as the p/ypN category, and the LNR.

The results showed that the LNR-based category may be a useful prognostic factor accompanying p/yp T category. For patients in the PCRT group, LNR-based stage, but not the current TNM stage, was able to stratify patient for RFS.

This study has several limitations. Although the data were collected prospectively, the study was designed retrospectively, which may have introduced a selection bias. In addition, the prognostic significance of the LNR has been previously evaluated using different methodologies yielding varying results, particularly due to differences in the cut-off values used for grouping patients and heterogeneity of collected data[8-10,21-23] for colorectal cancer. For the practical use of the LNR as a prognostic variable, the most effective LNR cut-off values need to be determined. Although many studies, including our study, demonstrated that the LNR was a significant prognostic factor, further larger-scale comprehensive studies are warranted to determine the LNR cut-off values for rectal cancer. However, in the present study, the ratio between the number of positive LNs(4) which is generated by dividing pN2 from pN1 and the number of retrieved LNs(12) recommended for proper staging using the current staging system was used as a cut-off LNR value (0.25). Therefore, this LNR value is likely reasonable to compare the prognostic implication of p/yp N categories of the current TNM staging system with LNR-based categories.

Furthermore, studies regarding the LNR for rectal cancer patients treated with PCRT should be performed independently because PCRT influences LN status and significantly reduces the number of harvested LNs. Persistence of LN metastasis after PCRT may serve as a marker for a more aggressive biologic behavior of a tumor and the consequent need for more intensive postoperative treatment.

In conclusion, we found that the LNR was a more important prognostic factor for RFS in patients with lymph node metastasis after PCRT than those who did not undergo PCRT. Furthermore, absolute number-based nodal staging could not adequately predict prognosis for patients treated with PCRT. In addition, the predictive ability of the LNR was maintained when less than 12 LNs were harvested. Combined with ypT status, the LNR could be used to assign patients to a prognostic group as an alternative to the current TNM staging system. A large-scale comparative study to confirm the prognostic impact of the LNR and to determine the optimal LNR cut-off value is required.

COMMENTS

Background

The number of metastatic lymph node could be various according how many lymph nodes (LNs) were examined. The current staging system using number of metastatic LNs as N category for colorectal cancer, therefore, has a limitation in terms of influence by number of harvested LNs. The metastatic LN ratio (LNR), defined as the ratio of metastatic to examined LNs, has been shown to be

useful in identifying prognostic subgroups for non-irradiated rectal cancer, colon cancer and other type of cancers. Preoperative chemoradiotherapy (PCRT) which is one of the standard treatment for locally advanced rectal cancer has been shown to influence on the number of retrieved lymph node. Therefore, the LNR may more useful for patients treated with PCRT. To evaluate the efficacy of LNR as a prognostic indicator in patient who receive PCRT, the present study compare oncologic outcomes according to current TNM stage and new classification using LNR among patients treated with PCRT and those without PCRT.

Research frontiers

Risk of recurrence was well stratified based on LNR in PCRT patients. In case of patients treated with PCRT, categories based on LNR had stronger association with recurrence-free survival. Future investigation is required to make staging system based on LNR and decide on clinical suitability of new staging system based on LNR in PCRT patients.

Innovations and breakthroughs

The current study shows the effectiveness of LNR to predict prognosis in patients who did not receive PCRT as well as those treated with PCRT. The present study gives importance of LNR on prognostication in PCRT than no-PCRT setting and suggests new staging system based on LNR and ypT category to apply practically.

Applications

The LNR-based category of patients with advanced rectal cancer treated by PCRT can be used to predict oncologic outcome. It is more accurate than LN number based staging system for prognostication. Adjuvant treatment and surveillance need to be given based on prognostic implication based on pathologic stage and LNR may have a role in case under-staging was suspicious based on current pathologic staging system.

Peer-review

This article is very important because it underlines the impact of neoadjuvant radiochemotherapy of rectal cancer and moreover can predict recurrence free survival. It would be useful to check these patients data according to tumor regression grade as well.

REFERENCES

1 **Sobin LH**, Gospodarowicz MK, Wittekind C. TNM Classification of Malignant Tumours (UICC International Union Against Cancer). 7th ed. New York: Wiley-Blackwell, 2009

2 **Goldstein NS**, Sanford W, Coffey M, Layfield LJ. Lymph node recovery from colorectal resection specimens removed for adenocarcinoma. Trends over time and a recommendation for a minimum number of lymph nodes to be recovered. *Am J Clin Pathol* 1996; **106**: 209-216 [PMID: 8712176]

3 **Le Voyer TE**, Sigurdson ER, Hanlon AL, Mayer RJ, Macdonald JS, Catalano PJ, Haller DG. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089. *J Clin Oncol* 2003; **21**: 2912-2919 [PMID: 12885809 DOI: 10.1200/JCO.2003.05.062]

4 **Swanson RS**, Compton CC, Stewart AK, Bland KI. The prognosis of T3N0 colon cancer is dependent on the number of lymph nodes examined. *Ann Surg Oncol* 2003; **10**: 65-71 [PMID: 12513963 DOI: 10.1245/ASO.2003.03.058]

5 **Joseph NE**, Sigurdson ER, Hanlon AL, Wang H, Mayer RJ, MacDonald JS, Catalano PJ, Haller DG. Accuracy of determining nodal negativity in colorectal cancer on the basis of the number of nodes retrieved on resection. *Ann Surg Oncol* 2003; **10**: 213-218 [PMID: 12679304 DOI: 10.1245/ASO.2003.03.059]

6 **Marchet A**, Mocellin S, Ambrosi A, Morgagni P, Garcea D, Marrelli D, Roviello F, de Manzoni G, Minicozzi A, Natalini G, De Santis F, Baiocchi L, Coniglio A, Nitti D. The ratio between metastatic and examined lymph nodes (N ratio) is an independent prognostic factor in gastric cancer regardless of the type of lymphadenectomy: results from an Italian multicentric study in 1853 patients. *Ann Surg* 2007; **245**: 543-552 [PMID: 17414602 DOI: 10.1097/01.sla.0000250423.43436.e1]

7 **Mariette C**, Piessen G, Briez N, Triboulet JP. The number of metastatic lymph nodes and the ratio between metastatic and examined lymph nodes are independent prognostic factors in esophageal cancer regardless of neoadjuvant chemoradiation or lymphadenectomy extent. *Ann Surg* 2008; **247**: 365-371 [PMID: 18216546 DOI: 10.1097/SLA.0b013e31815aaadf]

8 **Rosenberg R**, Friederichs J, Schuster T, Gertler R, Maak M, Becker K, Grebner A, Ulm K, Höfler H, Nekarda H, Siewert JR. Prognosis of patients with colorectal cancer is associated with lymph node ratio: a single-center analysis of 3,026 patients over a 25-year time period. *Ann Surg* 2008; **248**: 968-978 [PMID: 19092341 DOI: 10.1097/SLA.0b013e318190eddc]

9 **Kim YS**, Kim JH, Yoon SM, Choi EK, Ahn SD, Lee SW, Kim JC, Yu CS, Kim HC, Kim TW, Chang HM. lymph node ratio as a prognostic factor in patients with stage III rectal cancer treated with total mesorectal excision followed by chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2009; **74**: 796-802 [PMID: 19289261 DOI: 10.1016/j.ijrobp.2008.08.065]

10 **Peschaud F**, Benoist S, Julié C, Beauchet A, Penna C, Rougier P, Nordlinger B. The ratio of metastatic to examined lymph nodes is a powerful independent prognostic factor in rectal cancer. *Ann Surg* 2008; **248**: 1067-1073 [PMID: 19092352 DOI: 10.1097/SLA.0b013e31818842ec]

11 **Bosset JF**, Collette L, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, Daban A, Bardet E, Beny A, Ollier JC. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* 2006; **355**: 1114-1123 [PMID: 16971718 DOI: 10.1056/NEJMoa060829]

12 **Sauer R**, Becker H, Hohenberger W, Rödel C, Wittekind C, Fietkau R, Martus P, Tschmelitsch J, Hager E, Hess CF, Karstens JH, Liersch T, Schmidberger H, Raab R. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med* 2004; **351**: 1731-1740 [PMID: 15496622 DOI: 10.1056/NEJMoa040694]

13 **Sebag-Montefiore D**, Stephens RJ, Steele R, Monson J, Grieve R, Khanna S, Quirke P, Couture J, de Metz C, Myint AS, Bessell E, Griffiths G, Thompson LC, Parmar M. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. *Lancet* 2009; **373**: 811-820 [PMID: 19269519 DOI: 10.1016/S0140-6736(09)60484-0]

14 **Rullier A**, Laurent C, Capdepont M, Vendrely V, Belleannée G, Bioulac-Sage P, Rullier E. Lymph nodes after preoperative chemoradiotherapy for rectal carcinoma: number, status, and impact on survival. *Am J Surg Pathol* 2008; **32**: 45-50 [PMID: 18162769 DOI: 10.1097/PAS.0b013e3180dc92ab]

15 **Chang GJ**, Rodriguez-Bigas MA, Eng C, Skibber JM. Lymph node status after neoadjuvant radiotherapy for rectal cancer is a biologic predictor of outcome. *Cancer* 2009; **115**: 5432-5440 [PMID: 19673001]

16 **Baxter NN**, Morris AM, Rothenberger DA, Tepper JE. Impact of preoperative radiation for rectal cancer on subsequent lymph node evaluation: a population-based analysis. *Int J Radiat Oncol Biol Phys* 2005; **61**: 426-431 [PMID: 15667963 DOI: 10.1016/j.ijrobp.2004.06.259]

17 **de la Fuente SG**, Manson RJ, Ludwig KA, Mantyh CR. Neoadjuvant chemoradiation for rectal cancer reduces lymph node harvest in proctectomy specimens. *J Gastrointest Surg* 2009; **13**: 269-274 [PMID: 18850250 DOI: 10.1007/s11605-008-0717-2]

18 **Thorn CC**, Woodcock NP, Scott N, Verbeke C, Scott SB, Ambrose NS. What factors affect lymph node yield in surgery for rectal cancer? *Colorectal Dis* 2004; **6**: 356-361 [PMID: 15335370 DOI: 10.1111/j.1463-1318.2004.00670.x]

19 **Wang H**, Safar B, Wexner S, Zhao R, Cruz-Correa M, Berho M. Lymph node harvest after proctectomy for invasive rectal adenocarcinoma following neoadjuvant therapy: does the same standard apply? *Dis Colon Rectum* 2009; **52**: 549-557 [PMID: 19404052 DOI: 10.1007/DCR.0b013e31819eb872]

20 **Morcos B**, Baker B, Al Masri M, Haddad H, Hashem S. Lymph node yield in rectal cancer surgery: effect of preoperative chemoradiotherapy. *Eur J Surg Oncol* 2010; **36**: 345-349 [PMID: 20071133 DOI: 10.1016/j.ejso.2009.12.006]

21 **Derwinger K**, Carlsson G, Gustavsson B. A study of lymph node ratio as a prognostic marker in colon cancer. *Eur J Surg Oncol* 2008; **34**: 771-775 [PMID: 18079086 DOI: 10.1016/

j.ejso.2007.11.002]

22 **Wang J**, Hassett JM, Dayton MT, Kulaylat MN. Lymph node ratio: role in the staging of node-positive colon cancer. *Ann Surg Oncol* 2008; **15**: 1600-1608 [PMID: 18327530 DOI: 10.1245/s10434-007-9716-x]

23 **Berger AC**, Sigurdson ER, LeVoyer T, Hanlon A, Mayer RJ, Macdonald JS, Catalano PJ, Haller DG. Colon cancer survival is associated with decreasing ratio of metastatic to examined lymph nodes. *J Clin Oncol* 2005; **23**: 8706-8712 [PMID: 16314630 DOI: 10.1200/JCO.2005.02.8852]

P- Reviewer: Elpek GO, Furka A, Steele SR
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3282

World J Gastroenterol 2015 March 21; 21(11): 3282-3290
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Real-life outcome of anti-tumor necrosis factor α in the ambulatory treatment of ulcerative colitis

Enayatullah Baki, Philipp Zwickel, Anna Zawierucha, Robert Ehehalt, Daniel Gotthardt, Wolfgang Stremmel, Annika Gauss

Enayatullah Baki, Philipp Zwickel, Anna Zawierucha, Daniel Gotthardt, Wolfgang Stremmel, Annika Gauss, Division of Gastroenterology, University of Heidelberg, 69120 Heidelberg, Germany
Robert Ehehalt, Outpatient Clinic of Gastroenterology, 69121 Heidelberg, Germany
Author contributions: Gauss A and Baki E designed the research; Baki E performed the research; Gauss A wrote the manuscript; Zwickel P, Zawierucha A and Ehehalt R collected data and contributed to analysis and interpretation of data; Gotthardt D and Stremmel W contributed to analysis and interpretation of data; all authors revised the manuscript critically and approved its final version.

Correspondence to: Annika Gauss, MD, Division of Gastroenterology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg,
Germany. annika.gauss@med.uni-heidelberg.de
Telephone: +49-6221-5638865
Fax: +49-6221-565255
Received: September 14, 2014
Peer-review started: September 15, 2014
First decision: October 14, 2014
Revised: October 24, 2014
Accepted: December 1, 2014
Article in press: December 1, 2014
Published online: March 21, 2015

Abstract

AIM: To evaluate the outcome of anti-tumor necrosis factor alpha (anti-TNFα) therapy in outpatients with ulcerative colitis at a tertiary referral center.

METHODS: All patients with a confirmed diagnosis of ulcerative colitis undergoing therapy with infliximab and/or adalimumab at the outpatient clinic for inflammatory bowel diseases at the University Hospital Heidelberg between January 2011 and February 2014 were retrospectively enrolled. Patients with a follow-up period of less than 6 mo from start of anti-TNFα therapy were excluded. Medical records of all eligible individuals were carefully reviewed. Steroid-free clinical remission of a duration of at least 3 mo, colectomy rate, duration of anti-TNFα therapy, need for anti-TNFα dose escalation, and the occurrence of adverse events were evaluated as the main outcome parameters.

RESULTS: Seventy-two patients were included (35 treated with infliximab, 17 with adalimumab, 20 with both consecutively). Median follow-up was 27 mo (range: 6-87 mo). Steroid-free clinical remission was achieved by 22.2% of the patients (median duration: 21 mo until end of follow-up; range: 3-66 mo). Patients attaining steroid-free clinical remission displayed lower hemoglobin and albumin blood levels at the start of treatment than those who did not achieve remission. The overall colectomy rate was 20.8%. Nearly 50% of the patients underwent anti-TNFα dose escalation during the follow-up period. For both the infliximab and the adalimumab treated patients, non-response to anti-TNFα therapy was the major reason for treatment discontinuation. 18.2% of the infliximab-treated patients and 13.5% of the adalimumab-treated patients had to discontinue their therapy due to adverse events.

CONCLUSION: Real-life remission rates of ulcerative colitis under anti-TNFα are overall low, but some patients have a clear long-term benefit.

Key words: Inflammatory bowel disease; Tumor necrosis factor α inhibitors; Ulcerative colitis; Outcome; Real life

Core tip: Tumor necrosis factor alpha inhibitors are a widely accepted therapeutic option for the treatment of ulcerative colitis. Results from different real-life settings on their use in ulcerative colitis are controversial. Weighing anti tumor necrosis factor alpha against other treatment options, it is very important to decide on the best therapy for a patient. This retrospective study from a tertiary referral centre shows a rate of steroid-free clinical remission of 22.2% and a colectomy rate of 20.8% for ambulatory patients with ulcerative colitis under therapy with tumor necrosis factor alpha inhibitors. These rather disappointing outcomes should be thoroughly discussed with the patients before start of therapy.

Baki E, Zwickel P, Zawierucha A, Ehehalt R, Gotthardt D, Stremmel W, Gauss A. Real-life outcome of anti-tumor necrosis factor α in the ambulatory treatment of ulcerative colitis. *World J Gastroenterol* 2015; 21(11): 3282-3290 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3282.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3282

INTRODUCTION

Despite considerable progress over the past decades, treatment options for ulcerative colitis (UC) are still unsatisfactory. Realistically attainable remission rates are far below the goals set, and long-term remission remains elusive. The introduction of tumor necrosis factor alpha (TNFα) inhibitors into the therapy of Crohn's disease revolutionized its treatment[1-5]. Infliximab (IFX) and adalimumab (ADA) were the first anti-TNFα agents to be also approved for induction and maintenance of remission in UC[6,7]. The ACT-1 and ACT-2 studies evaluated the efficacy of IFX for induction and maintenance therapy in adults with UC[6]. Remission rates at week 8 were at 38.8% in ACT-1 and 33.9% in ACT-2[6]. Rates of sustained remission (at week 8 and week 30) were even lower at 26.2% (ACT-1) and 22.5% (ACT-2)[6]. Results of the phase Ⅲ trials of ADA were slightly less promising: at week 8, 18.5% of the patients in the high-dose group were in remission, compared with 9.2% in the placebo group[7]. The recently approved TNFα inhibitor golimumab was also shown to induce and maintain clinical remission in patients with moderate-to-severe UC[8,9].

Post-marketing experience reveals inconsistent results. Armuzzi *et al*[10] found remission rates of 17%, 28.4%, 36.4% and 43.2% at 4, 12, 24 and 54 wk in 88 patients treated with ADA for active UC. Considering that many of their patients had previously been treated with IFX, these results compare favorably to the phase Ⅲ data cited above[7]. In their retrospective study, Zhou *et al*[11] described a remarkably high remission rate of 61.5% by week 30 of IFX treatment, but only 24 patients were included in their study. There are no head-to-head clinical trials that compare IFX with ADA in the treatment of UC. However, a recent meta-analysis suggests that IFX, ADA and golimumab are equally effective[12].

Although randomized controlled trials (RCTs) are considered the gold standard for the evaluation of the efficacy of a drug, real-life data provide more insight into factors that might influence therapy outcomes. Therefore, this study was aimed at evaluating firm practical end points of therapy outcomes with TNFα antagonists in a real-life setting, and to identify factors influencing the efficacy of the treatment. It was not the objective of this study to compare outcomes of IFX therapy with those of ADA therapy.

MATERIALS AND METHODS

Study design and data collection

This is an uncontrolled, open-label retrospective study of outpatients with moderate or severe UC at a German university hospital serving as a tertiary referral center for the treatment of inflammatory bowel diseases (IBD). The study was approved by the Ethics Committee of the University of Heidelberg. Inclusion criteria were: a confirmed diagnosis of UC (based on the European Crohn's and Colitis Organisation criteria[13]); treatment with IFX or ADA or both consecutively; and a documented follow-up after start of treatment of at least 6 mo.

Patients who changed to the outpatient IBD clinic of the University of Heidelberg after they had started anti-TNFα treatment, patients who had previously undergone intestinal surgery, and patients with acute severe colitis were excluded. All data were retrieved from entirely computerized medical records. To identify eligible individuals, electronically available daily lists of all patients treated in the outpatient IBD clinic between January 1, 2011 and February 28, 2014 were screened. Patients who had started anti-TNFα treatment at our outpatient clinic prior to January 1, 2011, but were treated within the time frame of the study, were also included. Demographic and clinical parameters of all eligible individuals were entered into a Microsoft Excel spreadsheet. At the IBD outpatient unit, patients under anti-TNFα therapy are routinely examined by a gastroenterologist 6 and 12 wk after the start of therapy, and every 3 mo thereafter. IFX was delivered *via* intravenous (IV) infusions (5 mg/kg body weight) at weeks 0, 2 and 6. After that, patients received scheduled infusions (5 mg/kg body weight) every 8 wk, if no dose intensification was deemed necessary. ADA was delivered by subcutaneous injections of 80 mg at days 1, 2 and 14, and then 40 mg every other week as long as no dose escalation was required. In this study, blood concentrations of IFX and ADA and anti drug antibodies were not measured, so that decisions on dose escalation were mainly based on the patients' symptoms.

Definitions

The Montréal classification for UC was applied to categorize disease extent[14]. Steroid-free clinical remission was defined by the absence of diarrhea (≥ 4 bowel movements per day), bloody stools and abdominal pain without intake of steroids for at least 3 mo, as evaluated by the treating physician. In our study, response was not employed as an outcome parameter, as variables for the calculation of reliable disease activity scores had not been documented precisely enough in our sample of patients. The decision to discontinue therapy due to inadequate response was in all cases made by a senior gastroenterologist. Dose escalation of anti-TNFα therapy involved a reduction of the IFX dosing interval to at least 4 wk and/or an increase of the dose to at most 10 mg/kg body weight. For ADA, dose escalation meant shortening of the dosing interval to at least 7 d. The decision on dose intensification was left to the treating physician's judgment. Primary non-response was defined as absence of amelioration of UC symptoms up to 3 mo of treatment. Concomitant immunosuppressive treatment was considered if a patient was on immunomodulators for at least 3 mo after start of anti-TNFα therapy.

End points

The primary end point was the induction of steroid-free clinical remission under anti-TNFα therapy. Secondary end points were the need for colectomy within the follow-up period, discontinuation of therapy due to insufficient efficacy, discontinuation of treatment due to adverse events, and need for dose escalation according to the treating physician's judgment. Patients were not followed up if they left the outpatient clinic to change to a different treatment center or practice. Therefore, colectomy rates could only be calculated for the time that the patients stayed at our outpatient clinic.

Further information retrieved from the electronic patient charts comprised gender, age, disease duration, body mass index (BMI), family history of IBD, presence of extraintestinal manifestations, smoking habits, prior and concomitant medications, side effects under anti-TNFα therapy, and laboratory markers before and after start of therapy, including blood cell counts, plasma ferritin, C-reactive protein (CRP) and serum albumin levels.

Evaluation of disease activity

As this is a retrospective study, disease activity scores were not consistently available. As a surrogate, we analyzed single variables which occur in commonly used UC activity scores, and which were routinely asked by the treating physician and documented in the computerized charts. These included numbers of bowel movements per 24 h and the occurrence of bloody stools. They were evaluated at the start of treatment and 3 mo after the start of treatment.

Statistical analysis

The objective of the study was to assess anti-TNFα in the treatment of UC, not to compare IFX with ADA. Analyses were performed per patient for outcome parameters like remission and need for colectomy, because one individual cannot appear twice in outcome analysis. Other parameters - like the occurrence of adverse events - were analyzed per therapy. Patients who had received IFX and ADA in either order were additionally evaluated in a separate section. All statistical analyses were performed using IBM SPSS Statistics 21.0. Descriptive statistics were calculated as percentages for discrete variables, and presented as medians with ranges, as appropriate. To identify potential predictors of steroid-free clinical remission, the Mann-Whitney-test was used for ordinal variables, and χ^2 tests for categorical variables. Wilcoxon matched pairs signed rank test was used to compare disease activity markers (except for the occurrence of bloody stools) before and after the start of anti-TNFα therapy. McNemar's test was used to compare the number of patients reporting the occurrence of blood in stool before and after the start of anti-TNFα therapy. Statistical significance was set at the 95%CI ($P < 0.05$). To confirm predictors of steroid-free clinical remission, a multivariate, logistic regression analysis was performed. Kaplan-Meier estimator equations were used to compare the survival curves of treatment duration.

RESULTS

Patient demographics and clinical characteristics

In all, 72 patients were enrolled. Thirty-five patients received IFX, 17 underwent treatment with ADA, and 20 patients were on both medications consecutively (15 IFX first, 5 ADA first). The median follow-up time was 27 mo (range, 6-87 mo). All patient demographics and clinical baseline characteristics may be viewed in Table 1.

Prior and concomitant medications

Before starting their first anti-TNFα therapy, 66 (91.7%) of the 72 patients had been on oral 5-aminosalicylic acid (5-ASA), 21 (29.2%) on oral budesonide, 70 (97.2%) on oral cortisone, 55 (76.4%) on azathioprine (AZA), 3 (4.2%) on 6-mercaptopurine (6-MP), 12 (16.7%) on methotrexate (MTX), 4 (5.6%) on tacrolimus, and 4 (5.6%) on cyclosporine. These and the medications concomitant with IFX and ADA treatment are shown in Table 1.

Steroid-free clinical remission under anti-TNFα therapy

Sixteen patients (22.2%) attained steroid-free clinical remission. Seventy-five percent of these patients were on IFX and 25% were on ADA. The median time to steroid-free clinical remission was 3 mo (range: 1-10 mo). The duration of steroid-free remission was

Table 1 Demographic and clinical baseline characteristics of all 72 patients included in the study *n* (%)

Characteristic	
Anti-TNFα therapy	
IFX only	35 (48.6)
ADA only	17 (23.6)
IFX and ADA	15 (20.8)
ADA and IFX	5 (6.9)
Demographic characteristics	
Gender (female:male)	39: 33
Age at start of treatment (median; range) (yr)	33 (15-71)
Disease extent according to Montréal classification, *n* (E1:E2:E3)	5:32:35
Duration of disease at start of anti-TNFα therapy, median (range) (mo)	69.5 (2-480)
Presence of at least one extraintestinal manifestation	30 (41.7)
Smoking status, *n* (active smokers:non-smokers: ex-smokers)	6:54:5 (*n* = 65)
BMI, median (range) (kg/m^2)	24.1 (17.3-61.9) (*n* = 69)
Family history of IBD (*n* positive: *n* negative)	5:18 (*n* = 23)
History of colitis medication *prior to* start of anti-TNFα treatment	
Steroids	70 (97.2)
Oral budesonide	21 (29.2)
5-ASA	66 (91.7)
Azathioprine	55 (76.4)
6-Mercaptopurine	3 (4.2)
Methotrexate	12 (16.7)
Tacrolimus	4 (5.6)
Cyclosporine	4 (5.6)
Medications concomitant with IFX therapy at start of therapy (*n* = 55)	
Steroids	37 (67.3)
Oral budesonide	16 (29.1)
5-ASA	35 (63.6)
Azathioprine	16 (29.1)
6-Mercaptopurine	1 (1.8)
Methotrexate	3 (5.5)
Tacrolimus	1 (1.8)
Cyclosporine	0
Medications concomitant with ADA therapy at start of therapy (*n* = 37)	
Steroids	25 (67.6)
Oral budesonide	10 (27.0)
5-ASA	28 (75.7)
Azathioprine	11 (29.7)
6-Mercaptopurine	0 (0)
Methotrexate	1 (2.7)
Tacrolimus	0 (0)
Cyclosporine	0 (0)

ADA: Adalimumab; 5-ASA: 5-Aminosalicylic acid; BMI: Body mass index; IBD: Inflammatory bowel disease; IFX: Infliximab, TNFα: Tumor necrosis factor alpha.

21 mo (range: 3-66 mo). The median follow-up of the patients attaining remission was 24 mo (range: 6-69 mo). Eleven of the 16 patients (68.8%) were on additional anti-inflammatory or immunosuppressive medications when they reached remission: 68.8% took a 5-ASA preparation and 18.8% were on AZA. Eleven of the 16 patients (68.8%) were still treated at our outpatient clinic and in remission at the end of the follow-up period. In 81.3% of all patients achieving remission, the status of steroid-free clinical remission could be maintained for more than one year, in 56.3% for more than 2 years, and in 37.5% for more than 3 years. The rate of steroid-free clinical remission was not related to: patient age at the start of treatment; gender; disease extent according to the Montréal classification; disease duration; the presence of extraintestinal manifestations; smoking status; BMI; family history of IBD; previous treatment with purines, methotrexate or a calcineurin inhibitor; or concomitant therapy with steroids, purines, or 5-ASA (Table 2). Patients with higher plasma CRP concentrations before treatment tended to achieve steroid-free remission more often than those with lower plasma CRP concentrations (P = 0.104; Table 2). Leukocyte and platelet numbers, and plasma ferritin levels at start of treatment did not differ between the remission and the non-remission group. Patients with lower hemoglobin concentrations at the start of treatment achieved remission more frequently than those with higher hemoglobin concentrations (P = 0.023; Table 2). Steroid-free clinical remission was more often induced in patients with lower serum albumin levels at the start of treatment than in those with higher albumin levels (P = 0.009; Table 2).

Colectomy rate during the follow-up period

During the period that the patients were observed at our clinic, 15 (20.8%) patients underwent colectomy, all due to refractory UC. There were no emergency colectomies. Seven of these patients had been on therapy with IFX alone and eight with subsequent IFX/ADA or ADA/IFX prior to colectomy. The median duration of anti-TNFα therapy before surgery was 9 mo (range: 2-44 mo). Among the patients undergoing colectomy, nine were male and six were female. Their median age was 33 years (range: 19-72 years).

Disease activity under anti-TNFα treatment

While under both IFX and ADA treatment, surrogates of disease activity changed to the expected directions, the changes were in all small. The only significant differences were realized for the number of bowel movements per 24 h as well as leukocyte numbers in the IFX-treated patients, and platelet numbers in the IFX- and the ADA-treated patients (Table 3).

Anti-TNFα treatment duration

At month 6, 67.3% of the patients receiving IFX were still under treatment, at month 12, 50.5% continued their treatment. Of the patients under ADA therapy, 64.9% were still receiving treatment at month 6, and 47.9% at month 12 (Figure 1).

Indications for anti-TNFα treatment discontinuation

The reasons for discontinuation of anti-TNFα treatment were grouped into five categories: non-response; loss of response; adverse events; treatment pause in stable remission; or other. Of the 55 patients receiving

Table 2 Characteristics of patients who achieved steroid-free clinical remission under anti-tumor necrosis factor alpha treatment and of patients who did not achieve remission

Variable	remission (n = 16)	no remission (n = 56)	P value (univariate)	P value (multivariate)
Age, (yr)	32 (15-58)	34 (18-71)	0.755	0.685
Sex (female: male)	11:5	28:28	0.184	0.560
Disease extent according to Montréal (E1:E2:E3)	1:7:8	4:25:27	0.988	
Disease duration, (mo)	69.5 (7-288)	66 (2-480)	0.968	0.873
Patients with extraintestinal manifestations	6 (37.5)	24 (42.9)	0.338	
Smoking (active:non-smokers: ex-smokers)	2:9:2 (n = 13)	4:45:3 (n = 52)	0.318	
BMI, (kg/m^2)	23.9 (18.9-30.8)	24.2 (17.3-61.9) (n = 53)	0.654	0.546
Previous medications				
Purine analogons	14 (87.5)	42 (75.0)	0.289	
Methotrexate	4 (25.0)	8 (14.3)	0.310	
Calcineurin inhibitors	2 (12.5)	5 (8.9)	0.671	
Concomitant medications				
Steroids, (at start of treatment)	12 (75.0)	40 (71.4)	0.778	
5-ASA	12 (75.0)	40 (71.4)	0.778	
Purine analogons	4 (25.0)	20 (35.7)	0.423	
Laboratory parameters before start of treatment with anti-TNFα				
CrP, (mg/L)	7.9 (0-45.3) (n = 14)	3.1 (0-88.9) (n = 55)	0.104	
Leukocyte number, (G/L)	8.8 (3.6-16.6) (n = 14)	8.8 (2.7-22.0) (n = 55)	0.817	
Hemoglobin, (g/dl)	11.3 (8.3-13.9) (n = 14)	12.4 (9.0-17.0) (n = 55)	0.023	0.561
MCV, (fl)	82.5 (65-105) (n = 14)	87 (61-112) (n = 55)	0.374	
Platelet number, (G/L)	408 (233-666) (n = 14)	333 (150-850) (n = 55)	0.114	
Albumin concentration, (g/L)	41.5 (26.6-45.1) (n = 9)	43.9 (36.0-48.8) (n = 44)	0.009	0.034
Ferritin concentration, (μg/L)	17 (2-201) (n = 8)	27.5 (5-489) (n = 34)	0.223	

Data are expressed as n (%) or median (range). 5-ASA: 5-Aminosalicylic acid; BMI: Body mass index, CrP: C-reactive protein (normal: < 5 mg/L); MCV: Mean cell volume.

Table 3 Surrogates of disease activity before and 3 mo after start of infliximab or adalimumab therapy, median (range)

Variable	Before start of therapy		3 mo after start of therapy		P value	
	IFX	ADA	IFX	ADA	IFX	ADA
Number of bowel movements per 24 h	6 (1-30)	6 (1-17)	5 (1-40)	5 (1-20)	0.042	0.229
Occurrence of blood in stool (yes/no) (%)	32/47 (68)	23/36 (63.9)	29/50 (58)	19/35 (54.3)	0.678	0.453
CRP, (mg/L)	4.9 (0-51)	3.6 (0-122)	2.9 (0-312)	3.6 (0-145)	0.310	0.435
Leukocyte number (G/L)	9.4 (2.7-17.6)	7.7 (2.5-22)	7.4 (2.2-22.9)	7.3 (3.9-15.4)	0.037	0.524
Platelet number (G/L)	338 (150-879)	335 (194-850)	307 (166-758)	298 (170-787)	0.005	0.007
Hemoglobin (g/dL)	12.2 (8.3-15.9)	12.4 (8.1-17)	12.7 (8.5-15.9)	12.9 (6.4-16.4)	0.084	0.501

ADA: Adalimumab; IFX: Infliximab; CrP: C-reactive protein (normal: < 5 mg/L).

IFX (including those who had previously been on ADA), 39 (70.9%) discontinued their therapy during the follow-up period after a treatment duration of 8 mo (range: 0.5-44 mo). The reasons were: non-response in 16 patients (41%); loss of response in 12 (30.8%); adverse events in 10 (25.6%); and stable deep remission in one patient (2.6%). Among the 37 patients on ADA (including those who had previously been on IFX), 25 (67.6%) discontinued their therapy after a median treatment duration of 6 mo (range: 1-29 mo). Reasons for discontinuation of treatment with ADA were: non-response in 14 patients (56%), loss of response in 5 patients (20%), adverse events in 5 patients (20%), and pregnancy in one patient (4%).

Steroid withdrawal during anti-TNFα treatment

Among 53 patients who were on steroids at the start of treatment with at least one of the anti-TNFα agents, 23 (43.4%) were able to taper off their steroids completely during anti-TNFα treatment. Of these 23 patients, 14 (60.9%) were successful in tapering under IFX treatment, and 9 (39.1%) under ADA treatment.

Anti-TNFα dose escalation (therapy intensification)

Under the assessment of the treating physician, the doses of 26 of all 54 IFX therapies (48.1%; no sufficient information on dose escalation in one patient) were escalated after a median treatment duration of 5 mo (range: 1-26 mo), and in 17 of all 37 ADA therapies (45.9%) after a median treatment duration of 4 mo (range: 1-12 mo).

Safety and tolerability of anti-TNFα treatment

Among 55 patients who underwent therapy with IFX, 39 patients (70.9%) reported at least one side effect during the treatment. The most frequently observed

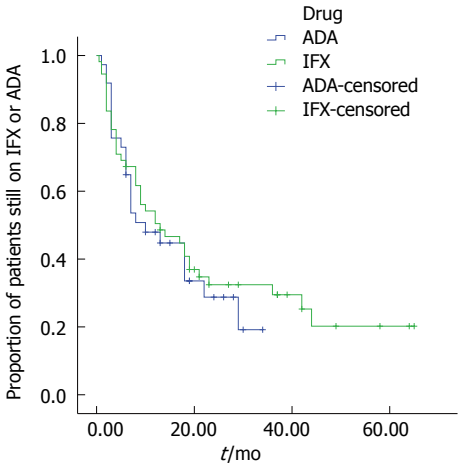


Figure 1 Cumulative probability of continuing infliximab or adalimumab therapy during follow-up. The dashes represent patients who remained on therapy during the follow-up and mark their individual end of follow-up. IFX: Infliximab; ADA: Adalimumab.

side effects were fatigue (reported in 23.6%), a rash (reported in 23.6%), pruritus (20%), and arthralgias which had not been present before start of therapy (20%). One patient developed acute bullous dermatosis (linear IgA dermatosis) after his third IFX infusion, and one patient without previous neurological disease suffered an epileptic seizure after his first IFX infusion. Finally, one patient experienced Varizella Zoster Virus (VZV) reactivation under IFX therapy. Side effects under IFX treatment may be viewed in Figure 2A. Ten patients (18.2%) had to discontinue IFX treatment under the advice of the treating physician due to severe side effects (Table 4 for individual reasons). Among 37 ADA-treated patients (partly overlapping with the IFX-treated group), 25 (67.6%) developed at least one relevant adverse event (Figure 2B). The most frequently reported side effect was a rash (21.6%). All side effects may be viewed in Figure 2B. Five patients (13.5%) had to discontinue ADA therapy due to adverse events (Table 4 for individual reasons).

Patients switching between TNFα inhibitors

Among the 72 patients in this study, 20 received both IFX and ADA consecutively; 15 were on IFX first, and five were on ADA first. Nine patients changed the anti-TNFα agent due to primary non-response, while four switched agents after a secondary loss of response and six due to side effects induced by the first TNFα inhibitor they used. One patient stopped IFX therapy as he had attained remission, and started ADA treatment after an interval of no anti-TNFα therapy. Three of six patients who switched due to side effects experienced allergic reactions to IFX, two switched due to IFX-induced arthralgia, and one switched from ADA to IFX due to ADA-induced hepatitis. Two of the six patients who switched therapy due to side effects achieved remission on the second anti-TNFα antibody, while none of the 13 patients who had switched the

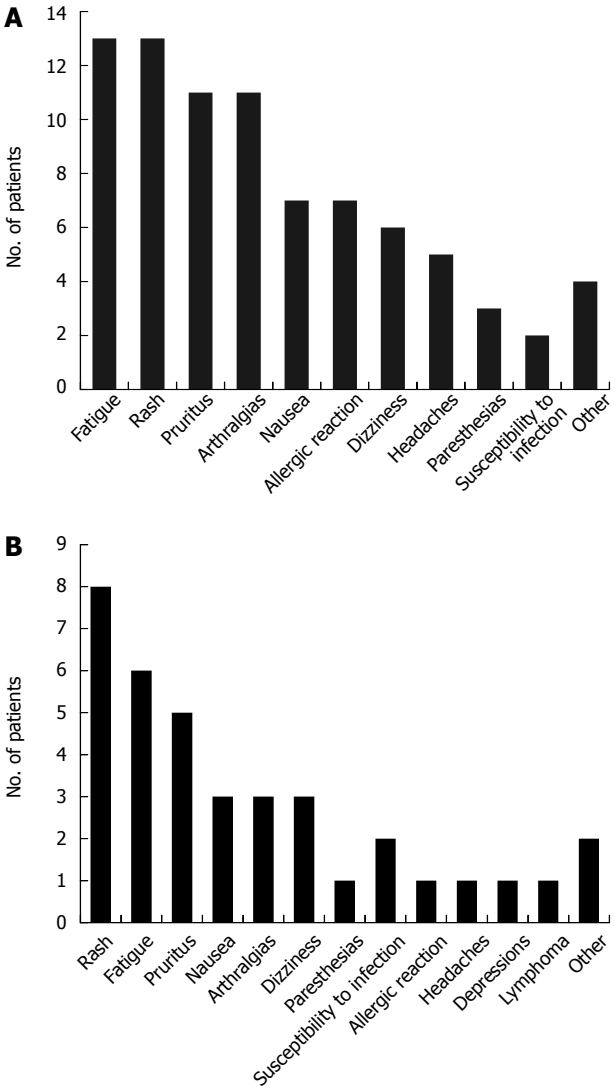


Figure 2 Side effects of infliximab treatment (A) and adalimumab treatment (B).

TNFα antibody for non-response or secondary loss of response achieved remission on the second antibody. This means that the group switching due to side effects reached remission significantly more often than the group switching due to non-response or secondary loss of response (P = 0.028).

DISCUSSION

Although a considerable number of UC patients are currently treated with TNFα inhibitors, the number of published reports on real-life experience using anti-TNFα agents to treat UC is relatively low[10,11,15-19].

Our study suggests that the clinical outcome of anti-TNFα treatment for UC in a real-life setting at a tertiary referral center is rather disappointing. However, interpretation of our results should take into consideration that patients transferred to a tertiary referral center usually suffer from refractory colitis that was not manageable using corticosteroid or immunomodulator treatment. As a matter of course,

Table 4 Adverse events necessitating discontinuation of infliximab therapy and adalimumab therapy

Adverse events necessitating discontinuation of infliximab therapy
Severe non-preexisting arthralgia with high anti-IFX antibody titer
IFX-induced linear IgA dermatosis after 3rd infusion
Epileptic seizure (first event) directly after start of 1st infusion
IFX-induced hepatitis
Severe non-preexisting arthralgia
Severe anaphylactic reaction
Severe anaphylactic reaction
Allergic reaction after 19 mo of therapy
Severe non-preexistent myalgia and arthralgia
Severe anaphylactic reaction
Adverse events necessitating discontinuation of adalimumab therapy
Generalized pruritus and exanthema, classified as allergic reaction
ADA-induced hepatitis
Acute absceding pyelonephritis
EBV-associated B-cell Hodgkin lymphoma, nodular-sclerosing type, stadium IIIB[1]
Frequent infections, especially of the upper respiratory tract

[1]The patient had been under therapy with azathioprine for one year, subsequently with IFX for 19 mo, and then after a pause with ADA for 6 mo. IgA: Immunoglobulin A; ADA: Adalimumab; EBV: Epstein-Barr virus; IFX: Infliximab.

clinical trials also include individuals with refractory disease, but they carry the bias of strict inclusion criteria and select patients who are more likely to be motivated and compliant.

The rate of steroid-free clinical remission under anti-TNFα treatment in this real-life study was only 22.2%. Comparing our results with those of RCTs is difficult, as definitions and time points vary greatly in different studies. In the ACT study by Rutgeerts *et al*[6] the clinical remission rate at week 54 of IFX treatment was 34.7%, and in the ULTRA-2 study, the one year remission rate of ADA-treated patients was 22%[20]. Our rate of overall steroid-free clinical remission under anti-TNFα therapy is comparable to the latter.

Our data stand in sharp contrast to data from an otherwise comparable real-life observational study from Canada that included 53 UC patients[17]. Responses to induction therapy were 96.4% for IFX and 80% for ADA (P = 0.089). However, these data cannot be readily compared with ours, as our end point of steroid-free clinical remission was more rigorous than an end point of response. Yet, in our study, the overall reduction of the number of bowel movements, bloody stools and biochemical parameters of inflammation under anti-TNFα treatment was remarkably poor. However, our results should not be interpreted too negatively, as most of the patients who achieved remission had a clear benefit from the treatment with remission times up to 69 mo.

Another objective of our study was to examine whether there are potential factors influencing the response to anti-TNFα therapy in UC patients. It has been shown previously that outcomes under anti-TNFα therapy may be more favorable in patients with no prior use of immunosuppressants[10]. In our study, with steroid-free clinical remission as the primary end point, we could not confirm this relationship. Surprisingly, we found that patients with lower hemoglobin and serum albumin concentrations at the start of treatment had a greater chance of achieving steroid-free clinical remission than those with higher concentrations. These results are in contrast to those of Oussalah *et al*[18] who found that one of the predictors for primary non-response to anti-TNFα in UC was a hemoglobin level of less than 9.4 g/dL before treatment. It seems logical that anti-TNFα therapy might be more successful in patients with more severe inflammation, thus expressing more anti-TNFα in their intestinal mucosa, but patients with very low hemoglobin levels might have too severe disease to respond to therapy. It is also known that very low levels of plasma albumin can impair the efficacy of anti-TNFα treatment[21], yet our ambulatory patients did not display very low albumin concentrations.

In the period of this study, 20.8% of our patients required colectomy because of non-response to therapy. In the literature, colectomy rates for UC vary between 9% and 35% and thus correspond to our numbers[6]. However, the colectomy rate might not be a reliable outcome parameter in our study, as patients whose therapies failed sometimes changed to a different center or doctor or alternative treatment to avoid surgery. These patients were lost to our follow-up. This is why real colectomy rates may be higher than the ones found in this study.

Another important result of this study is that, in our cohort, no patient who switched from one TNFα antagonist to the other for primary non-response or loss of efficacy had a therapeutic benefit from the second TNFα inhibitor.

In our cohort, adverse events under anti-TNFα therapy were frequent (in 70.9% of IFX-treated patients and 67.6% of ADA-treated patients). Comparable numbers have been reported in large RCTs. In the ACT study, 87.6% of the patients treated with an IFX dose of 5 mg/kg body weight and 91% of the patients treated with a dose of 10 mg/kg body weight developed side effects[6]. In the ULTRA-1 study, about 50% of the patients treated with ADA experienced side effects[7]. As expected, due to the higher immunogenicity of IFX as compared with ADA, allergic reactions were observed more often in IFX-treated patients than in ADA-treated patients.

In our study, 18.2% of the IFX-treated patients and 13.5% of the ADA-treated patients had to discontinue their therapy due to severe side effects. This difference between the two medications may be explained by the relatively high number of severe allergic reactions - often manifesting as anaphylaxis - in the IFX-treated group.

There are several interesting individual findings in this study concerning potential anti-TNFα side effects. One of our male patients developed drug-induced linear IgA dermatosis with a clear temporal relationship

to IFX therapy. After discontinuation of treatment, the disease only healed using high doses of steroids plus diamino-diphenyl sulfone (dapsone) for a long time period. To our knowledge, this is the first report on such a case in the literature. In addition, there was one case of a very young male who developed EBV-positive B cell Hodgkin lymphoma while under ADA treatment after consecutive therapies with AZA and IFX. The effect of immunosuppressive drugs on the risk of lymphoma remains a matter of debate[22]. Data from patients with rheumatoid arthritis suggest an increased incidence of malignancy in patients with rheumatoid arthritis treated with TNFα antibodies, with a disproportionate representation by lymphoma, but mainly Non-Hodgkin lymphoma[23]. Some authors suggest that AZA therapy is more detrimental than anti-TNFα therapy regarding the development of malignancies and especially lymphomas[24,25]. In our case, it cannot be proven whether anti-TNFα therapy was responsible for the development of lymphoma, or whether AZA, or the subsequent immunosuppressive therapy - or none of the treatments - were. Yet this case should remind us of this potential life-threatening complication when starting a patient on anti-TNFα therapy, especially if he or she is EBV positive.

Overall, IFX and ADA can be considered as effective treatment options for UC, but only in about a fifth of all patients. Those patients who achieve clinical remission mostly have a long-term benefit from the therapy. However, as remission rates are still low, patients should be made aware of the expected success rates and be offered alternative therapies, such as proctocolectomy. Furthermore, our data suggest that switching anti-TNFα agents for UC for lack of response is no viable option to improve response to therapy.

ACKNOWLEDGMENTS

We would like to thank C. W. Gauss for the proof-reading. Parts of this manuscript appear in the doctoral thesis of E. Baki.

COMMENTS

Background

One of the therapeutic options for moderate to severe ulcerative colitis is anti-tumor necrosis factor alpha (anti-TNFα).

Research frontiers

Therapeutic options for the treatment of patients with refractory ulcerative colitis are very limited, and in many cases do not meet the treatment goals set for the patients. Also, they often come along with partly severe side effects restricting their use even in cases of good efficacy. Yet efficacy data are also still overall disappointing.

Innovations and breakthroughs

Many novel medications to treat ulcerative colitis are recently released or still in the pipeline, and it is very important for the treating doctors to know as much as possible about expected response and discontinuation rates, especially in real-life settings, for the best of their patients.

Terminology

Ulcerative colitis is a chronic inflammatory condition of the colon with yet unknown etiology. TNFα inhibitors are monoclonal antibodies directed against tumor necrosis factor alpha, which is a cytokine involved in systemic inflammation, also playing a role in the pathogenesis of ulcerative colitis. TNFα inhibitors approved for the treatment of ulcerative colitis are infliximab, adalimumab, and golimumab.

Peer-review

The present article deals with an important health problem: How to treat patients with ulcerative colitis? The purpose of the study was to determine to what extent treatment with anti-TNFα agents (infliximab and adalimumab) may lead to clinical remission of ulcerative colitis in patients (in a real-life setting). Real life remission rates were overall low. The results show that about one fifth of the 72 patients achieved remission. Although the data presented indicate that the real life remission rates of ulcerative colitis after treatment with anti-TNFα were low, the report gives, nevertheless, information that is very useful for further work in the field.

REFERENCES

1 **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549 [PMID: 12047962 DOI: 10.1016/S0140-6736(02)08512-4]

2 **Hanauer SB**, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, Panaccione R, Wolf D, Pollack P. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006; **130**: 323-333; quiz 591 [PMID: 16472588 DOI: 10.1053/j.gastro.2005.11.030]

3 **Sandborn WJ**, Hanauer SB, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh DG, Panaccione R, Wolf D, Kent JD, Bittle B, Li J, Pollack PF. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. *Gut* 2007; **56**: 1232-1239 [PMID: 17299059 DOI: 10.1136/gut.2006.106781]

4 **Colombel JF**, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R, Schreiber S, Byczkowski D, Li J, Kent JD, Pollack PF. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology* 2007; **132**: 52-65 [PMID: 17241859 DOI: 10.1053/j.gastro.2006.11.041]

5 **Sands BE**, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, Kamm MA, Korzenik JR, Lashner BA, Onken JE, Rachmilewitz D, Rutgeerts P, Wild G, Wolf DC, Marsters PA, Travers SB, Blank MA, van Deventer SJ. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004; **350**: 876-885 [PMID: 14985485 DOI: 10.1056/NEJMoa030815]

6 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]

7 **Reinisch W**, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, Panaccione R, Fedorak RN, Tighe MB, Huang B, Kampman W, Lazar A, Thakkar R. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011; **60**: 780-787 [PMID: 21209123 DOI: 10.1136/gut.2010.221127]

8 **Sandborn WJ**, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, Adedokun OJ, Guzzo C, Colombel JF, Reinisch W, Gibson PR, Collins J, Järnerot G, Hibi T, Rutgeerts P. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2014; **146**: 85-95; quiz e14-e15 [PMID: 23735746 DOI: 10.1053/j.gastro.2013.05.048]

9 **Sandborn WJ**, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, Adedokun OJ, Guzzo C, Colombel JF, Reinisch W, Gibson PR, Collins J, Järnerot G, Rutgeerts P. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2014; **146**: 96-109.e1 [PMID: 23770005 DOI: 10.1053/j.gastro.2013.06.010]

10 **Armuzzi A**, Biancone L, Daperno M, Coli A, Pugliese D, Annese V, Aratari A, Ardizzone S, Balestrieri P, Bossa F, Cappello M, Castiglione F, Cicala M, Danese S, D'Incà R, Dulbecco P, Feliciangeli G, Fries W, Genise S, Gionchetti P, Gozzi S, Kohn A, Lorenzetti R, Milla M, Onali S, Orlando A, Papparella LG, Renna S, Ricci C, Rizzello F, Sostegni R, Guidi L, Papi C. Adalimumab in active ulcerative colitis: a "real-life" observational study. *Dig Liver Dis* 2013; **45**: 738-743 [PMID: 23683530 DOI: 10.1016/j.dld.2013.03.018]

11 **Zhou YL**, Xie S, Wang P, Zhang T, Lin MY, Tan JS, Zhi FC, Jiang B, Chen Y. Efficacy and safety of infliximab in treating patients with ulcerative colitis: experiences from a single medical center in southern China. *J Dig Dis* 2014; **15**: 483-490 [PMID: 24828856 DOI: 10.1111/1751-2980.12161]

12 **Stidham RW**, Lee TC, Higgins PD, Deshpande AR, Sussman DA, Singal AG, Elmunzer BJ, Saini SD, Vijan S, Waljee AK. Systematic review with network meta-analysis: the efficacy of anti-TNF agents for the treatment of Crohn's disease. *Aliment Pharmacol Ther* 2014; **39**: 1349-1362 [PMID: 24749763 DOI: 10.1111/apt.12644]

13 **Stange EF**, Travis SP, Vermeire S, Reinisch W, Geboes K, Barakauskiene A, Feakins R, Fléjou JF, Herfarth H, Hommes DW, Kupcinskas L, Lakatos PL, Mantzaris GJ, Schreiber S, Villanacci V, Warren BF. European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. *J Crohns Colitis* 2008; **2**: 1-23 [PMID: 21172194 DOI: 10.1016/j.crohns.2007.11.001]

14 **Satsangi J**, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut* 2006; **55**: 749-753 [PMID: 16698746 DOI: 10.1136/gut.2005.082909]

15 **Ferrante M**, Vermeire S, Fidder H, Schnitzler F, Noman M, Van Assche G, De Hertogh G, Hoffman I, D'Hoore A, Van Steen K, Geboes K, Penninckx F, Rutgeerts P. Long-term outcome after infliximab for refractory ulcerative colitis. *J Crohns Colitis* 2008; **2**: 219-225 [PMID: 21172214 DOI: 10.1016/j.crohns.2008.03.004]

16 **Afif W**, Leighton JA, Hanauer SB, Loftus EV, Faubion WA, Pardi DS, Tremaine WJ, Kane SV, Bruining DH, Cohen RD, Rubin DT, Hanson KA, Sandborn WJ. Open-label study of adalimumab in patients with ulcerative colitis including those with prior loss of response or intolerance to infliximab. *Inflamm Bowel Dis* 2009; **15**: 1302-1307 [PMID: 19408340 DOI: 10.1002/ibd.20924]

17 **Gies N**, Kroeker KI, Wong K, Fedorak RN. Treatment of ulcerative colitis with adalimumab or infliximab: long-term follow-up of a single-centre cohort. *Aliment Pharmacol Ther* 2010; **32**: 522-528 [PMID: 20500733 DOI: 10.1111/j.1365-2036.2010.04380.x]

18 **Oussalah A**, Laclotte C, Chevaux JB, Bensenane M, Babouri A, Serre AA, Boucekkine T, Roblin X, Bigard MA, Peyrin-Biroulet L. Long-term outcome of adalimumab therapy for ulcerative colitis with intolerance or lost response to infliximab: a single-centre experience. *Aliment Pharmacol Ther* 2008; **28**: 966-972 [PMID: 18652603 DOI: 10.1111/j.1365-2036.2008.03811.x]

19 **Halpin SJ**, Hamlin PJ, Greer DP, Warren L, Ford AC. Efficacy of infliximab in acute severe ulcerative colitis: a single-centre experience. *World J Gastroenterol* 2013; **19**: 1091-1097 [PMID: 23467174 DOI: 10.3748/wjg.v19.i7.1091]

20 **Sandborn WJ**, van Assche G, Reinisch W, Colombel JF, D' Haens G, Wolf DC, Kron M, Tighe MB, Lazar A, Thakkar RB. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2012; **142**: 257-265.e1-e3 [PMID: 22062358]

21 **Fasanmade AA**, Adedokun OJ, Olson A, Strauss R, Davis HM. Serum albumin concentration: a predictive factor of infliximab pharmacokinetics and clinical response in patients with ulcerative colitis. *Int J Clin Pharmacol Ther* 2010; **48**: 297-308 [PMID: 20420786 DOI: 10.5414/CPP48297]

22 **Mariette X**, Tubach F, Bagheri H, Bardet M, Berthelot JM, Gaudin P, Heresbach D, Martin A, Schaeverbeke T, Salmon D, Lemann M, Hermine O, Raphael M, Ravaud P. Lymphoma in patients treated with anti-TNF: results of the 3-year prospective French RATIO registry. *Ann Rheum Dis* 2010; **69**: 400-408 [PMID: 19828563 DOI: 10.1136/ard.2009.117762]

23 **Bongartz T**, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* 2006; **295**: 2275-2285 [PMID: 16705109 DOI: 10.1001/jama.295.19.2275]

24 **Kotlyar DS**, Lewis JD, Beaugerie L, Tierney A, Brensinger CM, Gisbert JP, Loftus EV Jr, Peyrin-Biroulet L, Blonski WC, Van Domselaar M, Chaparro M, Sandilya S, Bewtra M, Beigel F, Biancone L, Lichtenstein GR. Risk of Lymphoma in Patients With Inflammatory Bowel Disease Treated With Azathioprine and 6-Mercaptopurine: A Meta-analysis. *Clin Gastroenterol Hepatol* 2014; Epub ahead of print [PMID: 24879926 DOI: 10.1053/j.gastro.2013.07.015]

25 **Khan N**, Abbas AM, Lichtenstein GR, Loftus EV, Bazzano LA. Risk of lymphoma in patients with ulcerative colitis treated with thiopurines: a nationwide retrospective cohort study. *Gastroenterology* 2013; **145**: 1007-1015.e3 [PMID: 23891975 DOI: 10.1053/j.gastro.2013.07.035]

P- Reviewer: Berg T, Wu WJ **S- Editor**: Qi Y **L- Editor**: A **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3291

World J Gastroenterol 2015 March 21; 21(11): 3291-3299
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Hepcidin/ferroportin expression levels involve efficacy of pegylated-interferon plus ribavirin in hepatitis C virus-infected liver

Motoyuki Kohjima, Tsuyoshi Yoshimoto, Munechika Enjoji, Nobuyoshi Fukushima, Kunitaka Fukuizumi, Tsukasa Nakamura, Miho Kurokawa, Nao Fujimori, Yusuke Sasaki, Yasushi Shimonaka, Yusuke Murata, Susumu Koyama, Ken Kawabe, Kazuhiro Haraguchi, Yorinobu Sumida, Naohiko Harada, Masaki Kato, Kazuhiro Kotoh, Makoto Nakamuta

Motoyuki Kohjima, Tsuyoshi Yoshimoto, Nobuyoshi Fukushima, Kunitaka Fukuizumi, Tsukasa Nakamura, Miho Kurokawa, Nao Fujimori, Ken Kawabe, Kazuhiro Haraguchi, Yorinobu Sumida, Naohiko Harada, Makoto Nakamuta, Department of Gastroenterology and Clinical Research Center, National Hospital Organization Kyushu Medical Center, Fukuoka 810-8563, Japan
Munechika Enjoji, Yusuke Murata, Susumu Koyama, Health Care Center, Fukuoka University, Fukuoka 814-0180, Japan
Yusuke Sasaki, Yasushi Shimonaka, Product Research Department, Chugai Pharmaceutical Co., Ltd., Tokyo 103-8324, Japan
Masaki Kato, Kazuhiro Kotoh, Department of Medicine and Bioregulatory Science, Kyushu University, Fukuoka 812-8582, Japan
Author contributions: Kohjima M, Nakamuta M and Enjoji M designed the research; and all of the authors performed the research; Kohjima M and Enjoji M wrote the paper.
Supported by grants from Research Program of Intractable Disease provided by the Ministry of Health, Labor and Welfare of Japan, and a Grant-in-Aid for Clinical Research from the National Hospital Organization of Japan.

Correspondence to: Munechika Enjoji, MD, PhD, Health Care Center, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. enjoji@adm.fukuoka-u.ac.jp
Telephone: +81-92-8716631
Fax: +81-92-8630389
Received: September 3, 2014
Peer-review started: September 4, 2014
First decision: October 14, 2014
Revised: October 22, 2014
Accepted: December 8, 2014
Article in press: December 8, 2014
Published online: March 21, 2015

Abstract

AIM: To investigate the relationship between the iron-metabolism-related gene expression profiles and efficacy of antiviral therapy in chronic hepatitis C patients.

METHODS: The hepatic expression profile of iron-metabolism-related genes was analyzed and its association with virological response to pegylated-interferon plus ribavirin combination therapy was evaluated. A hundred patients with chronic hepatitis C (genotype1b, *n* = 50; genotype 2, *n* = 50) were enrolled and retrospectively analyzed. Liver biopsy samples were subjected to quantitative polymerase chain reaction for iron-metabolism-related genes and protein expression (Western blotting analysis) for ferroportin. As a control, normal liver tissue was obtained from 18 living donors of liver transplantation. Serum hepcidin level was measured by sensitive liquid chromatography/electrospray ionization tandem mass spectrometry.

RESULTS: Iron overload is associated with liver damage by increasing oxidative stress and hepatitis C virus (HCV) is reported to induce iron accumulation in hepatocytes *in vivo*. Conversely, iron administration suppresses HCV replication *in vitro*. Therefore, the association between HCV infection and iron metabolism remains unclear. Compared with controls, patients had significantly higher gene expression for transferrin, iron-regulatory

proteins 1 and 2, divalent metal transporter 1, and ferroportin, but similar for transferrin receptors 1 and 2, and hepcidin. When the expression profiles were compared between sustained virological response (SVR) and non-SVR patients, the former showed significantly lower transcription and protein expression of hepcidin and ferroportin. Expression of hepcidin-regulating genes, BMPR1, BMPR2, and hemojuvelin, was significantly increased, whereas BMP2 was decreased in HCV-infected liver. BMPR2 and hemojuvelin expression was significantly lower in the SVR than non-SVR group. HCV infection affects the expression of iron-metabolism-related genes, leading to iron accumulation in hepatocytes.

CONCLUSION: Decreased expression of hepcidin and ferroportin in SVR patients indicates the importance of hepatocytic iron retention for viral response during pegylated-interferon plus ribavirin treatment.

Key words: Chronic hepatitis C; Iron-metabolism; Hepcidin; Ferroportin; Interferon

Core tip: The first showing the relationship between expression of iron-metabolism-related genes and response to pegylated-interferon (PEG-IFN) and ribavirin (RBV) therapy in patients with chronic hepatitis C. The expression of hepcidin and ferroportin in the liver before therapy was significantly lower in sustained virological response (SVR) patients than non-SVR patients. The expression of hepcidin was positively correlated with that of ferroportin. The variation in hepatic expression profile in iron-metabolism-related genes is important for the response to PEG-IFN + RBV treatment.

Kohjima M, Yoshimoto T, Enjoji M, Fukushima N, Fukuizumi K, Nakamura T, Kurokawa M, Fujimori N, Sasaki Y, Shimonaka Y, Murata Y, Koyama S, Kawabe K, Haraguchi K, Sumida Y, Harada N, Kato M, Kotoh K, Nakamuta M. Hepcidin/ferroportin expression levels involve efficacy of pegylated-interferon plus ribavirin in hepatitis C virus-infected liver. *World J Gastroenterol* 2015; 21(11): 3291-3299 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3291.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3291

INTRODUCTION

Hepatitis C virus (HCV) is a major pathogen of chronic hepatitis and subsequent liver cirrhosis and hepatocellular carcinoma. Approximately 170 million people are infected with HCV worldwide and, according to natural history studies, 5%-20% of patients develop cirrhosis after about 20 years of infection[1]. Pegylated-interferon (PEG-IFN) plus ribavirin (RBV) combination therapy has developed as a basic antiviral treatment for chronic hepatitis C because it can bring patients into sustained virological response (SVR) at a high rate. Nowadays, an inhibitor of HCV NS3/4A protease, telaprevir or simeprevir, is added to PEG-IFN and RBV to achieve higher SVR rates[2,3]. However, some relevant adverse events such as severe anemia still hinder the effect of the treatment by leading to dose reduction and cessation of treatment.

Iron is an essential biometal, and mammalian cells require sufficient amounts of iron to satisfy metabolic needs and accomplish some specialized functions. In humans, the vast majority of iron (> 2 g) is distributed to hemoglobin and is involved in oxygen transport. A significant amount of iron is also present in macrophages (≤ 600 mg) and in the myoglobin of muscles (≤ 300 mg), whereas excess body iron (about 1 g) is stored in the liver. Mammals lose iron by sloughing off their mucosal and skin cells, but do not possess any regulated mechanism for iron excretion from the body. Therefore, the iron balance needs to be regulated tightly, although the amount of iron uptake from nutrition and iron excretion is relatively low (1-2 mg)[4]. Liver iron overload is a well-described but not fully understood feature of HCV infection, which can induce liver damage by increasing oxidative stress. More than 30% of patients with chronic HCV infection have shown increased serum and hepatic iron concentrations[5-9]. Elevated iron index is correlated with progression of liver disease, while iron administration *in vitro* suppresses HCV replication[10]. Although the mechanism of disordered iron metabolism has not been fully elucidated, the recent discovery of hepcidin, a liver-derived iron-regulatory protein (IRP), has changed the philosophy of iron metabolism[1,11,12]. Iron is absorbed by intestinal villous cells through divalent metal transporter (DMT) 1 and transported into blood through ferroportin expressed on the basal membrane of villous cells. Serum iron is bound to transferrin and imported into hepatocytes *via* the function of transferrin receptor (TFR) 1 and 2, and DMT1 expressed on hepatocytes. Absorbed iron is stored with ferritin and ferroportin excretes iron into blood. Hepcidin, a hepatic peptide hormone, is a primary regulator of systemic iron status by blocking iron release from villous cells into the blood through binding to and driving degradation of ferroportin[1]. Recent studies have shown that the function of hepcidin is reduced in patients with chronic hepatitis C, leading to the pathogenesis of hepatic iron overload[13,14].

In some studies, hepatic iron accumulation was associated with resistance to IFN-based antiviral therapy and iron depletion before therapy improved SVR rates in patients with chronic hepatitis C[15-24]. Conversely, another study showed that hepatic iron storage was predominant in treatment responders and useful as a predictive marker for efficacy of IFN-based therapy[25]. Some studies have shown an association between iron overload and virological response to IFN

Table 1 Demographic and clinical characteristics of the patients

Number of patients	100
SVR/non-SVR	63/37
Age (yr)	56.3 ± 7.4
Male/female	39/61
Genotype (1b/2a/2b)	50/17/33
HCV RNA (log IU/mL)	5.87 ± 0.95
Aspartate aminotransferase (< 30 IU/L)	51.1 ± 35.9
Alanine aminotransferase (< 30 IU/L)	61.8 ± 53.0
γ-glutamyl transpeptidase (< 50 IU/L)	50.7 ± 58.9
Fe (male, 55-200 μg/dL; female, 45-180 μg/dL)	130.3 ± 64.2
Unsaturated iron binding capacity (105-300 μg/dL)	215.1 ± 84.2
WBC (4000-9000/μL)	4987 ± 1303
Hemoglobin (male, 13-17; female, 11-15 g/dL)	13.6 ± 1.5
Platelet (11×10^4-35×10^4/μL)	19.6 ± 6.7
Dose reduction of ribavirin (%)	47

Normal range is presented in parenthesis. Measured values are shown in mean ± SD. SVR: Sustained virological response; HCV: Hepatitis C virus; WBC: White blood cell.

Table 2 Primer sets used for real-time polymerase chain reaction

Gene	Forward primer		Reverse primer	
	5′	3′	5′	3′
Ferritin	CAGGTGCGCCAGAACTAC-CA		CCACATCATCGCG-GTCAAAG	
Transferrin	CGAAGACTGCATCGCCAA-GA		ACACTTGCCCGCTAT-GTAGACAAAC	
Hepcidin	AGCCTGACCAGTGGCTCTGT		TTCGCCTCTGGAA-CATGG	
Ferroportin	AAGGGCAAGAATCCCAATT-TAACTC		TGCCAGGCT-GAAGGCTTACAC	
TFR1	GCATGTGGCATGTTCATCG-TATAA		TCTCAAGACCAG-GAGCTTGTCACTA	
TFR2	GCGACTGACACGCATGTA-CAAC		CCATGAAGATGTG-GCGGAAC	
DMT1	CTTGCGAGGCAATCTCAG-GA		CTGAGACAGT-GAACTTTGCAACCA	
IRP1	GAAACAGTCCTGCTGCTC-GCTAC		GAGCCATAGGAGTT-GAATTCTCGTG	
IRP2	TTTATCTCCAGGCAGT-GGGATG		CTGCGTCTGATA-AGGGTGCTGTA	
BMPR1	TGGGAGTTGCTGCATTGCT-GACC		ATGTAGCGTTTGGT-GCCCACCC	
BMPR2	GCCACAAATGTCCTGGATG-GCA		GAGGGGCGCCACC-GCTTAAG	
BMP2	TTGCGCCAGGTCCTTTGAC-CAG		ACCTGGGGAAGCAG-CAACGCTA	
Hemojuvelin	TGCCAGACGGCTGTG-CAAGG		CGGGCATCCTC-CAGTGCTGC	
RBBP6	GCGACCTGCAGATCACCAA		TGCCATCGCTG-GTTCAGTTC	

TFR: Transferrin receptor; DMT: Divalent metal transporter; IRP: Iron-regulatory protein; BMP: Bone morphogenetic protein; BMPR: BMP receptor; RBBP: Retinoblastoma binding protein.

+ RBV therapy[26-28], but others have shown that iron content is not correlated with response to antiviral therapy[29-36]. The relationship between iron metabolism and response to antiviral therapy is still confused. In this study, we analyzed the hepatic expression of iron-metabolism-related genes and evaluated its association with virological response to PEG-IFN+RBV therapy.

MATERIALS AND METHODS

Study population

In Kyushu Medical Center, a standard protocol in Japan [subcutaneous PEG-IFNα2a (180 μg) or PEG-IFNα2b (median dose of 1.5 μg/kg, range 1.3-1.7) weekly, along with oral RBV daily for 48 wk] was adopted for chronic hepatitis C. The dose of RBV was adjusted according to body weight: 600 mg for patients weighing < 60 kg; 800 mg for patients weighing 60-80 kg; and 800 mg for patients weighing > 80 kg. In these protocols, 48-wk and 24-wk regimens were applied to patients infected with HCV genotype 1b (HCV-1b) and those infected with HCV genotype 2 (HCV-2), respectively[37]. The study protocol was approved by the Ethics Committee of the National Hospital Organization, and written informed consent was obtained from all patients. HCV-1b patients (n = 50) and HCV-2 patients (n = 50) were enrolled and retrospectively analyzed. The background of the patients is shown in Table 1. Blood biochemistry was examined 1 or 2 d before biopsy. For real-time reverse transcription polymerase chain reaction (RT-PCR), tissue samples were obtained by liver biopsy. As a control, normal liver tissue was obtained from 18 living donors of liver transplantation whose liver function and histological findings were normal. Written informed consent was obtained from these donors for this investigation.

Laboratory data

Hematological, biochemical and virological parameters were determined by the clinical laboratory at Kyushu Medical Center. Serum HCV RNA concentrations were measured by the COBAS TaqMan PCR HCV test (Roche Diagnostics, Tokyo, Japan). SVR was defined as undetectable HCV RNA at 24 wk after therapy completion.

Real-time RT-PCR

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, United States) and cDNA was synthesized from 1.0 μg RNA using GeneAmp RNA PCR (Applied Biosystems, Branchburg, NJ, United States) with random hexamers. Real-time RT-PCR was performed using LightCycler-FastStart DNA Master SYBR Green 1 (Roche, Basel, Switzerland) according to the manufacturer's instructions[38]. The reaction mixture (20 μL) contained LightCycler-FastStart DNA Master SYBR Green 1, 4 mmol/L $MgCl_2$, 0.5 μmol/L upstream and downstream PCR primers, and 2 μL first-strand cDNA as a template. To control for reaction variations, all PCR data were normalized against the expression of retinoblastoma binding protein 6, which was also selected in previous studies[37,39]. Primer sets used for real-time PCR are shown in Table 2.

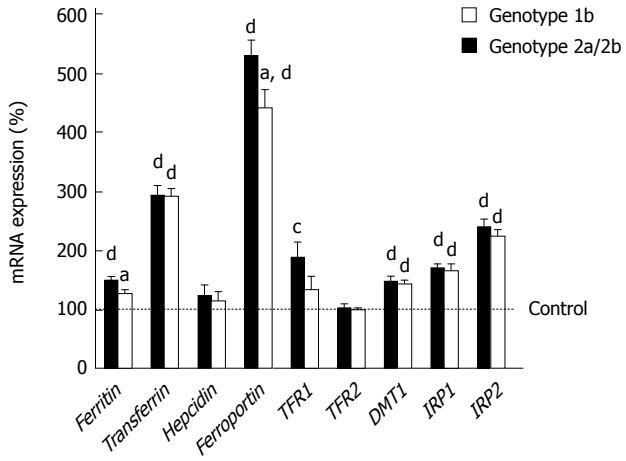


Figure 1 Expression levels of iron-metabolism-related genes in hepatitis C virus-infected liver in each genotype. The levels were measured by real-time reverse transcription polymerase chain reaction. $^{a}P < 0.05$ *vs* genotype1b, $^{b}P < 0.01$ *vs* genotype1b, $^{c}P < 0.05$ *vs* control, $^{d}P < 0.01$ *vs* control.

Protein expression

Liver biopsy samples were lysed in phosphate-buffered saline containing 1% Triton X-100. Forty-microgram aliquots of total tissue lysate were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and blotted onto Immobilon-P polyvinylidene fluoride membranes (Millipore, Billerica, MA, United States). The membranes were incubated with primary antibodies to ferroportin (ab85370: rabbit polyclonal to SLC40A1, Abcam, Tokyo, Japan) and β-actin (ab3280: mouse monoclonal to actin, Abcam), followed by incubation with peroxidase-labeled anti-rabbit and anti-mouse IgG antibodies (170-5046 and 170-5047, Bio-Rad, Tokyo, Japan), respectively. The bands were visualized by chemiluminescence using the ECL Western blotting analysis system (Amersham Biosciences, Little Chalfont, Bucks, United Kingdom).

Measurement of hepcidin

Serum hepcidin level was measured by sensitive liquid chromatography/electrospray ionization tandem mass spectrometry using an AB Sciex Triple Quad 5500 system (AB Sciex, Foster City, CA, United States) equipped with a Prominence UFLCXR system (Shimadzu Corporation, Kyoto, Japan), as reported previously[40,41]. Hepcidin exists in three isoforms, the iron bioactive 25-amino acid peptide (Hep-25) and its two amino-terminal truncated isoforms (Hep-20 and -22). In mass spectrometry-based studies, Hep-25 and Hep-20 can be measured in serum, while Hep-22 is found only in urine[42].

Statistical analysis

Statistical analysis was performed using JMP software (SAS Institute, Cary, NC, United States). Mann-Whitney *U* test was used for continuous variables including the difference in gene expression. A value of $P \leqslant 0.05$ was considered to be statistically significant.

RESULTS

Expression of iron-metabolism-related genes in hepatitis C patients

We examined the expression profile of the genes associated with iron metabolism by quantitative real-time RT-PCR to investigate disorders of iron metabolism in the liver of HCV-infected patients (HCV liver) (Figure 1). mRNA levels of transferrin, ferroportin, DMT1, IRP1 and IRP2 were significantly increased in HCV-1b or HCV-2 liver compared with normal controls. The levels of ferritin and TFR1 were significantly increased in HCV-1b liver, but not in HCV-2 liver. Expression levels of hepcidin and TFR2 in HCV-1b and HCV-2 liver were similar to the control level. The expression profile was consistent regardless of HCV genotype, except for ferritin, ferroportin and TFR1.

Expression of iron-metabolism-related genes and treatment outcome

We studied the involvement of iron metabolism in outcomes of PEG-IFN + RBV combination therapy. SVR patients showed significantly lower expression of hepcidin and ferroportin than non-SVR patients (Figure 2A), but no significant difference was found in other iron-metabolism-related genes (data not shown). Anemia is one of the critical adverse events during therapy and often compels a reduction in total RBV dose. It is possible that the changes in expression of hepcidin and ferroportin might affect iron metabolism and anemia. Furthermore, HCV genotype might be involved in the expression of the genes and treatment response. We compared the expression of hepcidin and ferroportin between the treatment outcomes for each HCV genotype, and between patients with and without RBV dose reduction. The expression of hepcidin and ferroportin was still lower in the SVR group both in HCV-1b and HCV-2 liver, although the difference was significant only in HCV-2 (Figure 2B). SVR patients, who did not need RBV reduction, showed significantly lower hepcidin and ferroportin expression than non-SVR patients showed, while expression did not differ significantly between SVR and non-SVR patients in the group with RBV dose reduction (Figure 2C). Serum hepcidin levels were also lower in SVR patients, although the difference was not significant (Figure 3A). Hepcidin is the protein regulating ferroportin expression through binding and degrading ferroportin, and might influence the protein level of ferroportin in hepatocytes as well as villous cells. We examined the expression of ferroportin protein in the liver. Hepatic levels of ferroportin protein, as well as RNA, were significantly lower in SVR patients than non-SVR patients (Figure 3B).

Relationship between hepcidin-expression-associated gene expression and treatment outcome

We examined the gene expression of known regulators

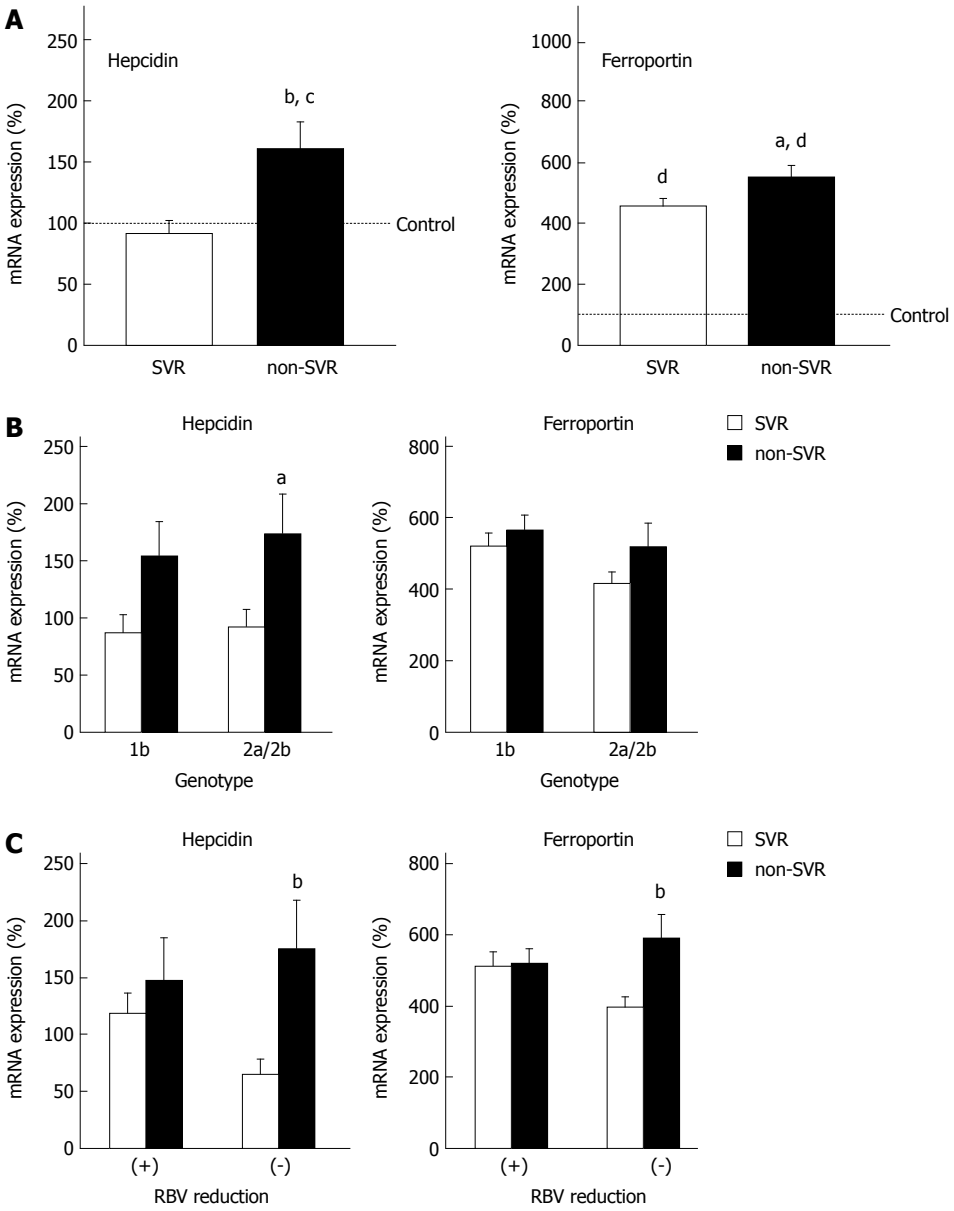


Figure 2 Expression levels of hepcidin and ferroportin genes. A: Sustained virological response (SVR) *vs* non-SVR; B: Genotype 1b *vs* 2a/2b; C: RBV dose reduction (+) *vs* (−). The levels were measured by real-time reverse transcription polymerase chain reaction. [a]*P*< 0.05 *vs* SVR, [b]*P* < 0.01 *vs* SVR, [c]*P* < 0.05 *vs* control, [d]*P* < 0.01 *vs* control.

of hepcidin expression. Although the regulation of hepcidin expression is not completely clear, a few pathways are known to control hepcidin expression: (1) TFR2 for sensing serum iron and saturated transferring; (2) IL-6 receptor (IL-6R) and signal transducer and activator of transcription (STAT) pathway for reflecting infection and inflammation; and (3) bone morphogenetic protein receptor (BMPR) and hemojuvelin, which are receptor and co-receptor for BMP2 and 6, respectively[43-45]. Hepatic expression of TFR2 and IL-6R was similar in SVR and non-SVR patients, and control subjects (data not shown). The levels of BMPR2 and hemojuvelin were significantly lower in SVR liver, and the same trend was found for BMPR1 and BMP2 expression, although the difference was not significant (Figure 4).

DISCUSSION

In this study, we examined the hepatic expression of the genes involved in iron metabolism and compared the expression levels between SVR and non-SVR patients. In HCV liver, expression levels of transferrin, TFR1, DMT1, ferritin, ferroportin, IRP1 and IRP2 were upregulated, while those of hepcidin and TFR2 were similar to the control levels. Transcription of ferritin and ferroportin was higher in patients with HCV-1b than HCV-2a/2b. It is possible that the change of expression might have affected iron deposition in the liver, although the mechanism was unclear. HCV genotype 2a/2b patients are known to achieve higher viral clearance during IFN treatment than patients with

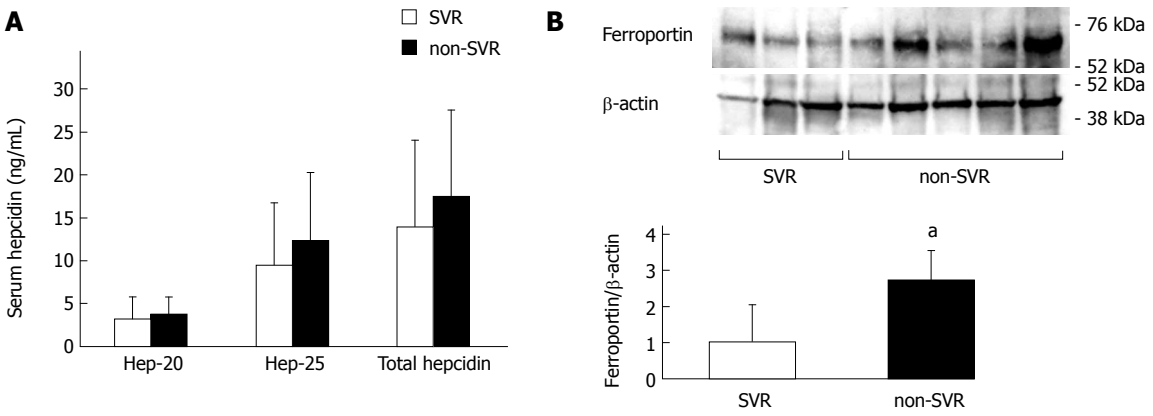


Figure 3 Protein expression of hepcidin and ferroportin. A: Serum hepcidin levels measured by sensitive liquid chromatography/electrospray ionization tandem mass spectrometry (total hepcidin and its two isoforms, Hep-20 and Hep 25); B: Ferroportin levels in the liver measured by Western blotting. [a]*P* < 0.05 *vs* sustained virological response (SVR).

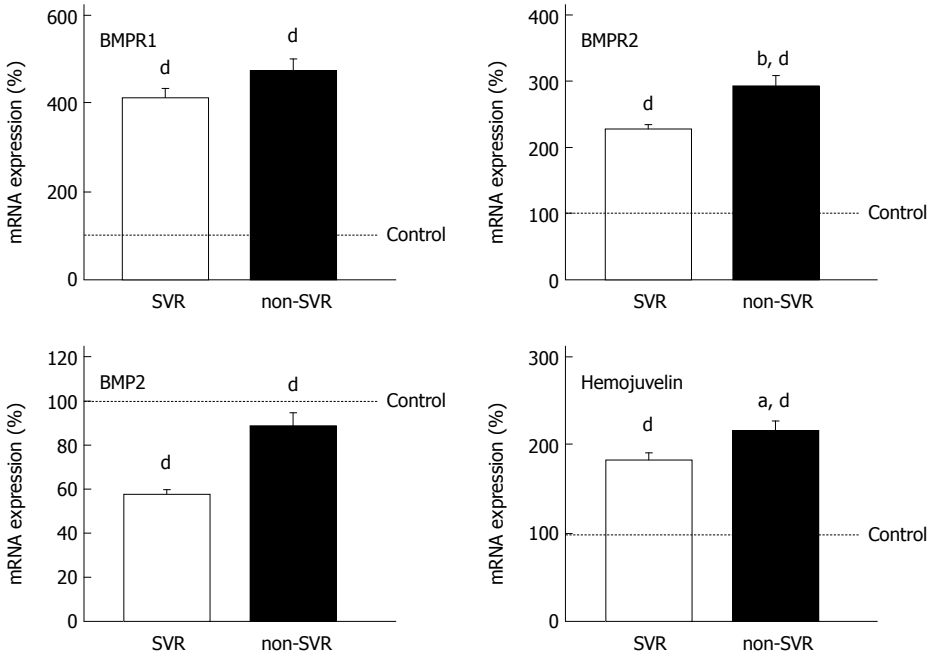


Figure 4 Expression levels of genes regulating hepcidin expression. The levels were measured by real-time reverse transcription polymerase chain reaction. [a]*P* < 0.05 *vs* sustained virological response (SVR), [b]*P* < 0.01 *vs* SVR, [d]*P* < 0.01 *vs* control.

genotype 1b. The difference in gene expression might be affected by innate immunity reaction or influence viral response through iron metabolism.

It is reported that iron administration *in vitro* suppresses HCV replication[10]. Furthermore, hepatic iron storage is reported to be predominant in treatment responders and useful as a predictive marker for efficacy of IFN-based antiviral therapy[25]. Conversely, iron overload and HFE gene mutations are associated with resistance to IFN therapy, and iron depletion before therapy is effective in patients with chronic hepatitis C[15-24]. In other studies, no association was found between iron overload and the response to IFN and RBV therapy[29-36]. Therefore, the relationship between iron metabolism and response to antiviral therapy is still unclear and controversial. The present study is believed to be the first showing the relationship between expression of iron-metabolism-related genes and response to PEG-IFN and RBV therapy. The expression of hepcidin and ferroportin in the liver before therapy was significantly lower in SVR patients than non-SVR patients regardless of HCV genotype and RBV dose reduction. Serum hepcidin and hepatic ferroportin protein were also lower in SVR patients. Jaroszewicz *et al*[27] have also reported that a decrease in serum prohepcidin level was associated with successful treatment using PEG-IFN and RBV. When we checked iron storage in the liver by staining biopsy tissue, we could not detect much difference between the liver of SVR and non-SVR patients (data not shown). These findings indicate the following: (1) Patients with enough capacity for accumulating iron in

the liver achieve viral clearance during PEG-IFN+RBV therapy; (2) The amount of hepatic iron deposition at the beginning of treatment might not influence the therapeutic response to PEG-IFN and RBV; (3) Patients who have higher hepcidin and ferroportin expression in the liver could store more hepatic iron released from red blood cells *via* RBV-induced hemolysis; and (4) Oxidative stress from accumulated iron might inhibit viral replication and help completion of viral clearance.

Transcriptional regulation of hepcidin might be mediated by the BMP-BMPR pathway but not the TFR2 or IL-6 pathway. It is possible that decreased hepcidin expression in SVR patients is affected by decreased iron in serum or liver tissue. However, serum iron concentration, as well as iron deposition in the liver, was similar between SVR and non-SVR patients. In addition, we could not detect any major difference in the expression of TFR2 that could play a role in tracking iron concentration between SVR and non-SVR patients. These findings indicate that downregulation of hepcidin in the liver of SVR patients might not reflect iron insufficiency, and that patients with decreased hepatic hepcidin *via* expression change in the BMP-BMPR pathway have greater capacity to absorb iron into the body and liver, and higher capacity for viral clearance. Moreover, the expression of hepcidin showed parallel change with the expression of ferroportin, and we found that these expressions were positively correlated with each other. It is possible that these expressions are affected by HCV infection or iron demand *via* a shared mechanism.

The variation in hepatic expression profile in iron-metabolism-related genes in patients with chronic hepatitis C is important for the response to PEG-IFN + RBV treatment. As an adverse event, anemia is more serious during triple therapy with telaprevir or boceprevir in combination with PEG-IFN and RBV. Therefore, characterization of iron metabolism during triple therapy has become more important. Further studies for controlling iron balance and metabolism could not only prevent dose reduction during therapy, but also enhance the therapeutic effect.

COMMENTS

Background

Iron overload is associated with liver damage by increasing oxidative stress and hepatitis C virus (HCV) is reported to induce iron accumulation in hepatocytes *in vivo*. Conversely, iron administration suppresses HCV replication *in vitro*. Therefore, the association between HCV infection and iron metabolism remains unclear.

Research frontiers

The association between iron metabolism/accumulation in the liver and viral response to antiviral treatments has been scarcely investigated.

Innovations and breakthroughs

The authors investigated the iron-metabolism-related gene expression profiles in HCV-infected liver, and the relationship between the profiles and therapeutic efficacy of pegylated-interferon (PEG-IFN) and ribavirin (RBV) treatment.

Applications

A decrease in hepcidin and ferroportin levels was associated with successful treatment using PEG-IFN and RBV. Patients with enough capacity for accumulating iron in the liver may achieve viral clearance during PEG-IFN + RBV therapy.

Terminology

HCV infection affects the expression of iron-metabolism-related genes, leading to iron accumulation in hepatocytes. Decreased expression of hepcidin and ferroportin in SVR patients, which can be regulated *via* the BMP-BMPR pathway, indicates the importance of hepatocytic iron retention for viral response during PEG-IFN + RBV treatment.

Peer-review

The authors present novel data and have presented a good context for the findings. The results indicating the importance of hepatic iron retention for viral response were interesting for readers.

REFERENCES

1 **Alter HJ**. HCV natural history: the retrospective and prospective in perspective. *J Hepatol* 2005; **43**: 550-552 [PMID: 16099527 DOI: 10.1016/jhep.2005.07.002]

2 **Aghemo A**, Rumi MG, Colombo M. Pegylated IFN-alpha2a and ribavirin in the treatment of hepatitis C. *Expert Rev Anti Infect Ther* 2009; **7**: 925-935 [PMID: 19803700 DOI: 10.1586/eri.09.70]

3 **Kumada T**, Toyoda H, Honda T, Kuzuya T, Katano Y, Nakano I, Goto H. Treatment of chronic hepatitis C with interferon alone or combined with ribavirin in Japan. *Intervirology* 2006; **49**: 112-118 [PMID: 16166799 DOI: 10.1159/000087273]

4 **Wang J**, Pantopoulos K. Regulation of cellular iron metabolism. *Biochem J* 2011; **434**: 365-381 [PMID: 21348856 DOI: 10.1042/BJ20101825]

5 **Di Bisceglie AM**, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. *Gastroenterology* 1992; **102**: 2108-2113 [PMID: 1587431]

6 **Bonkovsky HL**, Banner BF, Rothman AL. Iron and chronic viral hepatitis. *Hepatology* 1997; **25**: 759-768 [PMID: 9049232 DOI: 10.1002/hep.510250345]

7 **Bonkovsky HL**. Iron as a comorbid factor in chronic viral hepatitis. *Am J Gastroenterol* 2002; **97**: 1-4 [PMID: 11808931 DOI: 10.1111/j.1572-0241.2002.05390.x]

8 **Price L**, Kowdley KV. The role of iron in the pathophysiology and treatment of chronic hepatitis C. *Can J Gastroenterol* 2009; **23**: 822-828 [PMID: 20011735 DOI: 10.1093/ndt/gft467]

9 **Hörl WH**, Schmidt A. Low hepcidin triggers hepatic iron accumulation in patients with hepatitis C. *Nephrol Dial Transplant* 2014; **29**: 1141-1144 [PMID: 24286977]

10 **Yano M**, Ikeda M, Abe K, Dansako H, Ohkoshi S, Aoyagi Y, Kato N. Comprehensive analysis of the effects of ordinary nutrients on hepatitis C virus RNA replication in cell culture. *Antimicrob Agents Chemother* 2007; **51**: 2016-2027 [PMID: 17420205 DOI: 10.1128/AAC.01426-06]

11 **Park CH**, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem* 2001; **276**: 7806-7810 [PMID: 11113131 DOI: 10.1074/jbc.M008922200]

12 **Aoki CA**, Rossaro L, Ramsamooj R, Brandhagen D, Burritt MF, Bowlus CL. Liver hepcidin mRNA correlates with iron stores, but not inflammation, in patients with chronic hepatitis C. *J Clin Gastroenterol* 2005; **39**: 71-74 [PMID: 15599216]

13 **Fujita N**, Sugimoto R, Urawa N, Tanaka H, Konishi M, Kobayashi Y, Iwasa M, Watanabe S, Kaito M. Influence of phlebotomy on iron-related gene expression levels in the livers of patients with chronic hepatitis C. *J Gastroenterol* 2007; **42**: 326-327 [PMID: 17464464 DOI: 10.1007/s00535-007-2004-5]

14 **Fujita N**, Sugimoto R, Takeo M, Urawa N, Mifuji R, Tanaka H, Kobayashi Y, Iwasa M, Watanabe S, Adachi Y, Kaito M. Hepcidin expression in the liver: relatively low level in patients with chronic hepatitis C. *Mol Med* 2007; **13**: 97-104 [PMID: 17515961 DOI: 10.2119/2006-00057.Fujita]

15 **Fargion S**, Fracanzani AL, Rossini A, Borzio M, Riggio O, Belloni G, Bissoli F, Ceriani R, Ballarè M, Massari M, Trischitta C, Fiore P, Orlandi A, Morini L, Mattioli M, Oldani S, Cesana B, Fiorelli G.

Iron reduction and sustained response to interferon-alpha therapy in patients with chronic hepatitis C: results of an Italian multicenter randomized study. *Am J Gastroenterol* 2002; **97**: 1204-1210 [PMID: 12014729 DOI: 10.1111/j.1572-0241.2002.05705.x]

16 **Carlo C**, Daniela P, Giancarlo C. Iron depletion and response to interferon in chronic hepatitis C. *Hepatogastroenterology* 2003; **50**: 1467-1471 [PMID: 14571765]

17 **Van Thiel DH**, Friedlander L, Molloy PJ, Kania RJ, Fagiuoli S, Wright HI, Gasbarrini A, Caraceni P. Retreatment of hepatitis C interferon non-responders with larger doses of interferon with and without phlebotomy. *Hepatogastroenterology* 1996; **43**: 1557-1561 [PMID: 8975965]

18 **Fong TL**, Han SH, Tsai NC, Morgan TR, Mizokami M, Qian D, Phan C, Goad K, Redeker AG. A pilot randomized, controlled trial of the effect of iron depletion on long-term response to alpha-interferon in patients with chronic hepatitis C. *J Hepatol* 1998; **28**: 369-374 [PMID: 9551672 DOI: 10.1016/S0168-8278(98)80308-5]

19 **Fontana RJ**, Israel J, LeClair P, Banner BF, Tortorelli K, Grace N, Levine RA, Fiarman G, Thiim M, Tavill AS, Bonkovsky HL. Iron reduction before and during interferon therapy of chronic hepatitis C: results of a multicenter, randomized, controlled trial. *Hepatology* 2000; **31**: 730-736 [PMID: 10706565]

20 **Fujita N**, Sugimoto R, Urawa N, Araki J, Mifuji R, Yamamoto M, Horiike S, Tanaka H, Iwasa M, Kobayashi Y, Adachi Y, Kaito M. Hepatic iron accumulation is associated with disease progression and resistance to interferon/ribavirin combination therapy in chronic hepatitis C. *J Gastroenterol Hepatol* 2007; **22**: 1886-1893 [PMID: 17914965 DOI: 10.1111/j.1440-1746.2006.04759.x]

21 **Franchini M**, Targher G, Capra F, Montagnana M, Lippi G. The effect of iron depletion on chronic hepatitis C virus infection. *Hepatol Int* 2008; **2**: 335-340 [PMID: 19669262 DOI: 10.1007/s12072-008-9076-z]

22 **Lin TJ**, Liao LY, Lin CL, Chang TA, Liu SO. Hepatic iron influences responses to combination therapy with peginterferon alfa and ribavirin in chronic hepatitis C. *Hepatogastroenterology* 2008; **55**: 1412-1415 [PMID: 18795701]

23 **Gentile I**, Viola C, Paesano L, D'Onofrio M, D'Agostino E, Cerini R, Borrelli F, Piazza M, Borgia G. Iron depletion before HCV antiviral therapy: a pilot, randomized, controlled trial. *J Clin Apher* 2009; **24**: 190-196 [PMID: 19760753 DOI: 10.1002/jca.20210]

24 **Sikorska K**, Stalke P, Izycka-Swieszewska E, Romanowski T, Bielawski KP. The role of iron overload and HFE gene mutations in the era of pegylated interferon and ribavirin treatment of chronic hepatitis C. *Med Sci Monit* 2010; **16**: CR137-CR143 [PMID: 20190684]

25 **Akiyoshi F**, Sata M, Uchimura Y, Suzuki H, Tanikawa K. Hepatic iron stainings in chronic hepatitis C patients with low HCV RNA levels: a predictive marker for IFN therapy. *Am J Gastroenterol* 1997; **92**: 1463-1466 [PMID: 9317063]

26 **Fargion S**, Fracanzani AL, Sampietro M, Molteni V, Boldorini R, Mattioli M, Cesana B, Lunghi G, Piperno A, Valsecchi C, Fiorelli G. Liver iron influences the response to interferon alpha therapy in chronic hepatitis C. *Eur J Gastroenterol Hepatol* 1997; **9**: 497-503 [PMID: 9187884]

27 **Jaroszewicz J**, Rogalska M, Flisiak I, Flisiak R. Successful antiviral therapy is associated with a decrease of serum prohepcidin in chronic hepatitis C. *World J Gastroenterol* 2010; **16**: 1747-1752 [PMID: 20380007 DOI: 10.3748/wjg.v16.i14.1747]

28 **Carneiro MV**, Souza FF, Teixeira AC, Figueiredo JF, Villanova MG, Secaf M, Passos AD, Ramalho LN, Carneiro FP, Zucoloto S, Candolo Martinelli AL. The H63D genetic variant of the HFE gene is independently associated with the virological response to interferon and ribavirin therapy in chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2010; **22**: 1204-1210 [PMID: 20555268 DOI: 10.1097/MEG.0b013e32833bec1e]

29 **Herrera JL**. Iron depletion is not effective in inducing a virologic response in patients with chronic hepatitis C who failed to respond to interferon therapy. *Am J Gastroenterol* 1999; **94**: 3571-3575 [PMID: 10606321 DOI: 10.1111/j.1572-0241.1999.01648.x]

30 **Sievert W**, Pianko S, Warner S, Bowden S, Simpson I, Bowden D, Locarnini S. Hepatic iron overload does not prevent a sustained virological response to interferon-alpha therapy: a long term follow-up study in hepatitis C-infected patients with beta thalassemia major. *Am J Gastroenterol* 2002; **97**: 982-987 [PMID: 12003436 DOI: 10.1111/j.1572-0241.2002.05550.x]

31 **Pianko S**, McHutchison JG, Gordon SC, Heaton S, Goodman ZD, Patel K, Cortese CM, Brunt EM, Bacon BR, Blatt LM. Hepatic iron concentration does not influence response to therapy with interferon plus ribavirin in chronic HCV infection. *J Interferon Cytokine Res* 2002; **22**: 483-489 [PMID: 12034031 DOI: 10.1089/10799900252952271]

32 **Hofer H**, Osterreicher C, Jessner W, Penz M, Steindl-Munda P, Wrba F, Ferenci P. Hepatic iron concentration does not predict response to standard and pegylated-IFN/ribavirin therapy in patients with chronic hepatitis C. *J Hepatol* 2004; **40**: 1018-1022 [PMID: 15158344 DOI: 10.1016/j.jhep.2004.02.030]

33 **Rulyak SJ**, Eng SC, Patel K, McHutchison JG, Gordon SC, Kowdley KV. Relationships between hepatic iron content and virologic response in chronic hepatitis C patients treated with interferon and ribavirin. *Am J Gastroenterol* 2005; **100**: 332-337 [PMID: 15667490 DOI: 10.1111/j.1572-0241.2005.41112.x]

34 **Souza RM**, Freitas LA, Lyra AC, Moraes CF, Braga EL, Lyra LG. Effect of iron overload on the severity of liver histologic alterations and on the response to interferon and ribavirin therapy of patients with hepatitis C infection. *Braz J Med Biol Res* 2006; **39**: 79-83 [PMID: 16400467 DOI: 10.1590/S0100-879X2006000100009]

35 **Jurczyk K**, Karpińska E, Wawrzynowicz-Syczewska M, Morańska I, Noceń I, Chlubek D, Boroń-Kaczmarska A. State of the iron metabolism in patients with chronic hepatitis C type C does not influence antiviral treatment with interferon and ribavirin. *Hepatogastroenterology* 2008; **55**: 557-561 [PMID: 18613407]

36 **Pereira Pda S**, Silva IS, Uehara SN, Emori CT, Lanzoni VP, Silva AE, Ferraz ML. Chronic hepatitis C: hepatic iron content does not correlate with response to antiviral therapy. *Rev Inst Med Trop Sao Paulo* 2009; **51**: 331-336 [PMID: 20209268 DOI: 10.1590/S0036-46652009000600004]

37 **Kohjima M**, Enjoji M, Yoshimoto T, Yada R, Fujino T, Aoyagi Y, Fukushima N, Fukuizumi K, Harada N, Yada M, Kato M, Kotoh K, Nakashima M, Sakamoto N, Tanaka Y, Nakamuta M. Add-on therapy of pitavastatin and eicosapentaenoic acid improves outcome of peginterferon plus ribavirin treatment for chronic hepatitis C. *J Med Virol* 2013; **85**: 250-260 [PMID: 23161429 DOI: 10.1002/jmv.23464]

38 **Kohjima M**, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Fujino T, Yada M, Yada R, Harada N, Enjoji M, Takayanagi R, Nakamuta M. SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease. *Int J Mol Med* 2008; **21**: 507-511 [PMID: 18360697 DOI: 10.3892/ijmm.21.4.507]

39 **Nakamuta M**, Fujino T, Yada R, Aoyagi Y, Yasutake K, Kohjima M, Fukuizumi K, Yoshimoto T, Harada N, Yada M, Kato M, Kotoh K, Taketomi A, Maehara Y, Nakashima M, Enjoji M. Expression profiles of genes associated with viral entry in HCV-infected human liver. *J Med Virol* 2011; **83**: 921-927 [PMID: 21412800 DOI: 10.1002/jmv.22042]

40 **Murao N**, Ishigai M, Yasuno H, Shimonaka Y, Aso Y. Simple and sensitive quantification of bioactive peptides in biological matrices using liquid chromatography/selected reaction monitoring mass spectrometry coupled with trichloroacetic acid clean-up. *Rapid Commun Mass Spectrom* 2007; **21**: 4033-4038 [PMID: 18000836 DOI: 10.1002/rcm.3319]

41 **Hosoki T**, Ikuta K, Shimonaka Y, Sasaki Y, Yasuno H, Sato K, Ohtake T, Sasaki K, Torimoto Y, Saito K, Kohgo Y. Heterogeneous expressions of hepcidin isoforms in hepatoma-derived cells detected using simultaneous LC-MS/MS. *Proteomics Clin Appl* 2009; **3**: 1256-1264 [PMID: 21136948 DOI: 10.1002/prca.200900112]

42 **Kemna EH**, Tjalsma H, Podust VN, Swinkels DW. Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. *Clin Chem* 2007; **53**: 620-628 [PMID: 17272487 DOI: 10.1373/clinchem.2006.079186]

43 **Deicher R**, Hörl WH. New insights into the regulation of iron

homeostasis. *Eur J Clin Invest* 2006; **36**: 301-309 [PMID: 16634833 DOI: 10.1111/j.1365-2362.2006.01633.x]

44 **Anderson GJ**, Darshan D, Wilkins SJ, Frazer DM. Regulation of systemic iron homeostasis: how the body responds to changes in iron demand. *Biometals* 2007; **20**: 665-674 [PMID: 17273818 DOI: 10.1007/s10534-006-9030-2]

45 **Core AB**, Canali S, Babitt JL. Hemojuvelin and bone morphogenetic protein (BMP) signaling in iron homeostasis. *Front Pharmacol* 2014; **5**: 104 [PMID: 24860505 DOI: 10.3389/fphar.2014.00104]

P- Reviewer: Georgopoulou U, Goll R, Kakizaki S **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3300

World J Gastroenterol 2015 March 21; 21(11): 3300-3307
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Hepatectomy *vs* radiofrequency ablation for colorectal liver metastasis: A propensity score analysis

Huisong Lee, Jin Seok Heo, Yong Beom Cho, Seong Hyeon Yun, Hee Cheol Kim, Woo Yong Lee, Seong Ho Choi, Dong Wook Choi

Huisong Lee, Jin Seok Heo, Yong Beom Cho, Seong Hyeon Yun, Hee Cheol Kim, Woo Yong Lee, Seong Ho Choi, Dong Wook Choi, Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, South Korea

Author contributions: Heo JS is the guarantor of this entire study; all authors participated in the study design and data analysis, participated in drafting the article and gave final approval; Lee H, Kim HC, and Choi DW researched the literature; Heo JS, Lee H, Lee WY, Choi SH, and Choi DW performed the clinical studies; Lee H, Heo JS and Yun SH conducted statistical analyses; manuscript editing was carried out by all authors.

Correspondence to: Jin Seok Heo, MD, PhD, Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 135-710, South Korea. jsheo.md@gmail.com
Telephone: +82-10-99330262
Fax: +82-2-34106980

Received: August 19, 2014
Peer-review started: August 20, 2014
First decision: September 27, 2014
Revised: November 3, 2014
Accepted: December 8, 2014
Article in press: December 8, 2014
Published online: March 21, 2015

Abstract

AIM: To compare outcomes from radiofrequency ablation (RFA) and hepatectomy for treatment of colorectal liver metastasis (CRLM).

METHODS: From January 2000 to December 2009, 408 patients underwent curative intent treatment for CRLM. We excluded patients using the criteria: size of CRLM > 3 cm, number of CRLM ⩾ 5, percutaneous RFA, follow-up period < 12 mo, double primary cancer, or treatment with both RFA and hepatectomy. We matched 51 patients who underwent RFA with 102 patients who underwent hepatectomy by propensity scores.

RESULTS: The median follow-up period was 45 mo (range, 12 mo to 158 mo). Hepatic recurrence was more frequent in the RFA than the hepatectomy group (P = 0.021) although extrahepatic recurrence curves were similar (P = 0.716). Survival curves of hepatectomy group were better than that of RFA for multiple, large (> 2 cm) CRLM (P = 0.034). However, survival curves were similar for single or small (⩽ 2 cm) CRLM (P = 0.714, P = 0.740).

CONCLUSION: Hepatectomy is better than RFA for the treatment of CRLM. However, RFA might be suitable for selected patients with single, small (⩽ 2 cm) CRLM.

Key words: Colorectal neoplasm; Metastasis; Catheter ablation; Hepatectomy; Liver

Core tip: Previous studies reported that hepatectomy is better than radiofrequency ablation (RFA) to improve survival outcomes in the patients with colorectal liver metastasis (CRLM). However, there is still controversy that RFA is beneficial in selected patients. In this study, hepatectomy was better than RFA for the treatment of CRLM. However, RFA might be suitable for selected patients with single, small CRLM (⩽ 2 cm).

Lee H, Heo JS, Cho YB, Yun SH, Kim HC, Lee WY, Choi SH, Choi DW. Hepatectomy *vs* radiofrequency ablation for colorectal liver metastasis: A propensity score analysis. *World J Gastroenterol* 2015; 21(11): 3300-3307 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3300.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3300

INTRODUCTION

Hepatectomy is the standard treatment for colorectal liver metastasis (CRLM). Estimated 5-year overall survival (OS) rates are between 27% and 58%[1]. However, hepatectomy is difficult to perform and has a high complication rate. To reduce morbidity, radiofrequency ablation (RFA) has been used for decades to treat patients with CRLM[2,3].

Numerous studies have reported that RFA is a safe and feasible treatment option for a limited population of patients with CRLM[4]. RFA has a low risk of complications and is an effective treatment[3,5-8]. Recent studies, however, demonstrated that hepatectomy is superior to RFA and that RFA should be used only in patients unsuitable for hepatectomy[9-11]. However, outcomes after RFA to treat CRLM have rarely been evaluated according to location, number, and synchronicity of metastases even though these are important considerations in a treatment plan. Moreover, a recent study reported that survival was comparable for CRLM patients who underwent RFA or hepatectomy, despite the high local recurrence rate observed after RFA[2].

A randomized controlled trial to compare the outcomes of RFA and hepatectomy would be difficult. Instead, propensity score matching analysis has been used to minimize bias in evaluating the effectiveness of RFA in patients with hepatocellular carcinoma[12-14]. However, no propensity score analysis for patients with CRLM has been published.

Our aim in this study was to use propensity score matching to determine if survival outcomes were different between patients who underwent RFA and patients who underwent hepatectomy.

MATERIALS AND METHODS

We reviewed 1189 colorectal cancer patients with liver metastasis between January 2000 and December 2009. We identified 408 patients who underwent curative intent hepatectomy or intraoperative RFA to treat CRLM. Exclusion criteria were CRLM size > 3 cm, number ⩾ 5, percutaneous RFA, follow-up period < 12 mo, double primary cancer, hereditary nonpolyposis colorectal cancer (HNPCC), or treatment by both RFA and hepatectomy. Based on these criteria, 174 patients were excluded: 100 for CRLM size > 3 cm, 6 for CRLM number ⩾ 5, 4 for percutaneous RFA, 11 for follow-up < 12 mo, 11 for double primary cancer, 3 for HNPCC, and 39 for both RFA and hepatectomy.

We calculated propensity scores using a multivariable logistic model considering the variables sex; age; preoperative carcinoembryonic antigen (CEA) level; location of the primary colon cancer; number, location and maximal size of CRLM; tumor node metastasis (TNM) stage; lymphatic invasion; vascular invasion; neoadjuvant chemotherapy; adjuvant chemotherapy; and cell differentiation. Using the logit of the estimated propensity score, one case from the RFA group was matched to two cases from the hepatectomy group using a caliper of 0.2. Covariate balance and surgical outcomes between the matched groups were evaluated after matching. We could not take indocyanine green (ICG) clearance test for calculating propensity score because we did not performed ICG retention test in every patients with colorectal liver metastasis. We routinely checked ICG retention rate in selected patients with chronic liver disease.

We matched 51 patients who underwent RFA with 102 patients who underwent hepatectomy using propensity scores. From RFA group, 5 patients were not matched and from the hepatectomy group, 76 were not matched (Figure 1).

We investigated extrahepatic metastases preoperatively using computed tomography (CT) and positron emission tomography images. All patients underwent prior surgical excision of a primary colorectal cancer. In the RFA group, an interventional radiologist performed RFA using open surgical or laparoscopic approaches with a 460 KHz generator expendable needle radiofrequency system (model 500 or 1500; RITA Medical Systems, Mountain View, CA; Cool Tip, Radionics Corporation, Burlington, MA). For lesions at the liver surface or adjacent to the intestine, patients were treated by laparoscopic or intraoperative RFA. For all patients who underwent RFA, complete necrosis of liver metastases was confirmed by intraoperative ultrasonography and CT or magnetic resonance imaging within 1 week of the procedure.

To prevent recurrence, we recommended adjuvant chemotherapy based on fluorouracil (5-FU) for all patients. Postoperative surveillance for recurrence was performed every 3 to 6 mo for the first 3 years and annually thereafter; this included physical examination, chest X-ray, and abdominal CT scanning. Local recurrence was defined as recurrence at the RFA-ablated area or resection margin of the hepatectomy. In addition to medical records, Roentgen images were reviewed retrospectively to identify recurrence patterns. Endpoints were time to tumor recurrence and time to death.

Sex, age, preoperative CEA level, location of the primary colon cancer, number, location and size of CRLM, TNM stage, lymphatic invasion, vascular invasion, cell differentiation, comorbidity, postoperative complication, recurrence, site of recurrence, death, disease-free survival (DFS), and OS were recorded for each patient. TNM staging, lymphatic invasion, vascular invasion, and cell differentiation were determined from

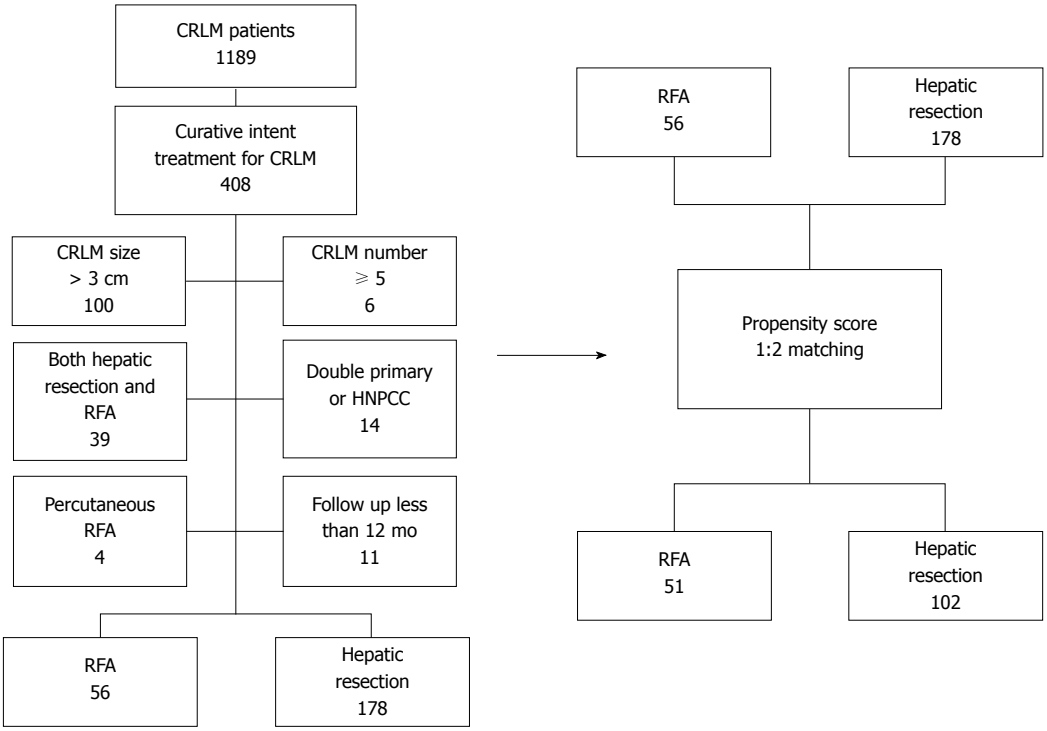


Figure 1 Flow diagram of patients identified and selected. CRLM: Colorectal liver metastasis; RFA: Radiofrequency ablation; HNPCC: Hereditary nonpolyposis colorectal cancer.

the primary colorectal cancer. However, postoperative complications, recurrence, and site of recurrence were associated with the CRLM. Complication severity was classified using the Clavien-Dindo (CD) grading system[15]. CD scores of two or higher were regarded as a significant morbidity case. American Joint Committee on Cancer (AJCC) 7th TNM classification was used to define disease stage[16].

Statistical analysis

Statistical analyses were used the statistical software SPSS (SPSS for Windows version 20.0, Chicago, IL). Categorical variables are reported as numbers (percentages). Continuous variables are reported as medians (ranges). Categorical variables were compared using a χ^2 or Fisher's exact test. Continuous variables were compared using the Mann-Whitney *U* test. Survival was calculated by Kaplan-Meier survival analysis and Cox proportional hazard model. Differences in survival curves were assessed by multivariate analyses that included clinically important factors such as sex, age, T stage, lymph node involvement, and primary tumor location. We calculated hazard ratio (HR) and 95%CI. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Demographics

The hepatectomy group comprised 102 patients and the RFA group comprised 51. Median age was 60 years (range, 30 years to 79 years) and median follow-up duration was 45 mo (range, 12 mo to 158 mo). In the hepatectomy group, 29 patients (28%) were women; in the RFA group, 16 (31%) were women. CRLM had a colon cancer origin in 53 patients (52%) in the hepatectomy group and 29 (57%) in the RFA group.

Analysis of patient characteristics revealed no significant differences between groups in sex; age; preoperative CEA level; primary tumor location (colon or rectum); neoadjuvant chemotherapy; adjuvant chemotherapy; number, location, or maximal diameter of CRLM; TNM stage; lymphatic invasion; vascular invasion; histological differentiation or comorbidities. Median diameter of CRLM was 1.7 cm (range, 0.2 cm to 3.0 cm) in the hepatectomy group and 1.8 cm (range, 1.0 cm to 3.0 cm) in the RFA group. A single CRLM was seen in 63 patients (62%) in the hepatectomy group and 29 (57%) in the RFA group. The CRLM was located in the unilateral hemiliver in 80 patients (78%) in the hepatectomy group and 38 (75%) in the RFA group. All characteristics were appropriately distributed between the two groups (Table 1).

Perioperative outcomes

Postoperative complications (CD score ⩾ 2) developed in 28 patients (27%) after hepatectomy and 5 (10%) after RFA (P = 0.012). However, no significant differences were noted in complication rates for treatment of CRLM. Perihepatic fluid collection or hepatic abscess were seen in 5 patients (5%) in the hepatic resection group and 2 (4%) in the RFA group (P = 0.99) with no postoperative

Table 1 Patient characteristics *n* (%)

	Hepatectomy *n* = 102	RFA *n* = 51	*P*
Sex (M/F)	73/29	35/16	0.71
Age (years) median (range)	60 (3-79)	58.5 (35-79)	0.99
Preoperative CEA (ng/mL) median (range)	4.4 (0.1-202)	6.5 (0.9-144)	0.19
Size of liver metastases, median (range)	1.7 (0.2-3.0)	1.8 (1.0-3.0)	0.26
Number of liver metastasis			
Single	63 (62)	29 (57)	0.56
Multiple	39 (38)	22 (43)	
Metastasis type			
Synchronous	76 (75)	33 (65)	0.21
Metachronous	26 (25)	18 (35)	
Location of liver metastasis			
Unilobal	80 (78)	38 (75)	0.59
Bilobal	22 (22)	13 (25)	
N stage			
N0	30 (29)	13 (25)	0.61
N1, N2	72 (71)	38 (75)	
Location of primary colorectal cancer			
Colon	53 (52)	29 (57)	0.57
Rectum	49 (48)	22 (43)	
Histological differentiation[1]			
High grade	88 (86)	45 (88)	0.73
Low grade	14 (14)	6 (12)	
Comorbidity			
Liver cirrhosis[2]	5 (5)	6 (12)	0.18
Diabetes mellitus	20 (20)	13 (25)	0.40
Hypertension	29 (28)	12 (24)	0.52
Cardiovascular disease	3 (3)	1 (2)	0.72
Pulmonary disease	6 (6)	2 (4)	0.99
Neoadjuvant chemotherapy			
Yes	6 (6)	5 (10)	0.30
No	70 (94)	28 (90)	
Adjuvant chemotherapy			
Yes	95 (93)	46 (90)	0.54
No	7 (7)	5 (10)	

[1]High grade: well or moderately differentiated, Low grade: poorly differentiated or mucinous carcinoma; [2]The grade of liver cirrhosis was Child-Pugh Class A in all 11 patients. RFA: Radiofrequency ablation; CEA: Carcinoembryonic antigen.

mortality.

Recurrences

During follow-up, 98 of 152 patients (64%) experienced recurrence after hepatectomy or RFA. Hepatic recurrences were more common in the RFA than in the hepatectomy group (P = 0.021). Extrahepatic recurrences were not significantly different between the two groups (P = 0.716) (Figure 2).

Survival

DFS was 68.4% at 1 year, 45.2% at 3 years, and 39.7% at 5 years after hepatectomy and 52.9%, 30.4%, and 23.9% after RFA (P = 0.056). OS rates were 93.1% at 1 year, 73.9% at 3 years, and 55.2% at 5 years after hepatectomy and 92.2%, 62.4%, and 48.2% after RFA, respectively. Differences in OS curves were significant between the hepatectomy and RFA groups (P = 0.194) (Figure 3).

We performed subgroup analysis according to CRLM number, size, and location. Survival curves were similar between the two groups for single or small (≤ 2 cm) CRLM (P = 0.714 and P = 0.740). However, the trend was that survival curves for the hepatectomy group were better than for the RFA group for multiple, large (> 2 cm) CRLM (P = 0.034) (Figure 4). No significant differences were seen by tumor distribution.

Associations between RFA, sex, age, CRLM size or number, synchronicity, CRLM location, T stage, N stage, adjuvant chemotherapy, histologic grade and DFS were evaluated using a Cox proportional hazard model. In multivariate analysis, RFA, lymph node metastasis and poorly differentiated grade were significant risk factors for recurrence (HR = 1.57, P = 0.040 for RFA; HR = 1.94, P = 0.015 for lymph node metastasis; and HR = 1.79, P = 0.049 for histologic grade) (Table 2).

DISCUSSION

Survival of colorectal cancer patients has improved over the last decades, largely due to newly developed surgical techniques and chemotherapeutic agents. In addition, techniques such as RFA have also been developed. We found that, by Cox regression multivariate analysis, RFA for CRLM was associated with a high recurrence rate. The hepatic recurrence rate was significantly higher after RFA than after hepatectomy by recurrence pattern analysis. However, oncologic outcomes were similar after RFA and hepatectomy for single or small (≤ 2 cm) CRLM.

We found significant differences in complication rates between the hepatectomy and RFA groups although the overall complication rate was low in both RFA and hepatectomy groups. Other studies also reported that RFA is less invasive than hepatectomy[5-8]. However, RFA should be considered as only an alternative treatment for patients not indicated for hepatectomy[2,9-11,17-21].

Hepatectomy is not always possible, however. A sufficient volume of remnant liver must be present before hepatectomy to reduce surgical risk. Portal blood pressure and biliary drainage are also important factors. If the estimated volume of the remnant liver is too small, portal vein embolization is useful to decrease surgical risk[22].

In our study, survival outcomes were similar in the RFA and hepatectomy groups, in particular for patients with single or small CRLM. However, the location of CRLM was not a significant factor for survival outcomes. Therefore, RFA might be substitute treatment for selected patients with single, small CRLM. The optimum diameter of CRLM for RFA has been controversial. Some studies suggest that RFA is acceptable if the CRLM are less than 3 cm in diameter[18,20,23,24]. However, other studies showed that RFA is associated with higher local recurrence rates, even for CRLMs less than 3 cm[19,25,26]. In this study, survival curves for the hepatic resection

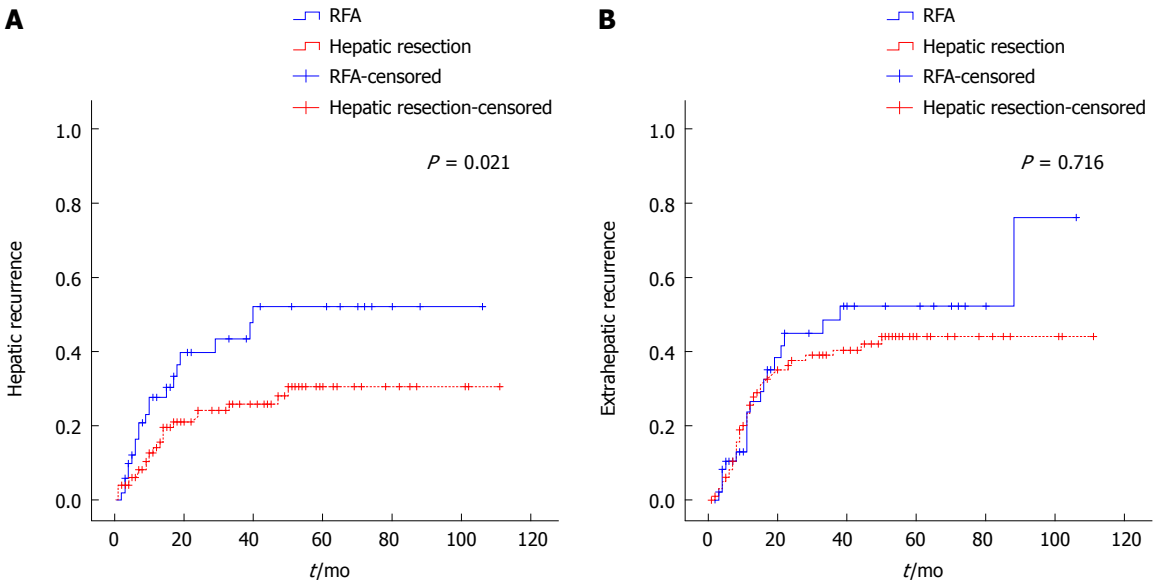


Figure 2 Recurrence patterns after hepatectomy or radiofrequency ablation for colorectal liver metastasis. A: Hepatic recurrence; B: Extrahepatic recurrence. RFA: Radiofrequency ablation; CRLM: Colorectal liver metastasis.

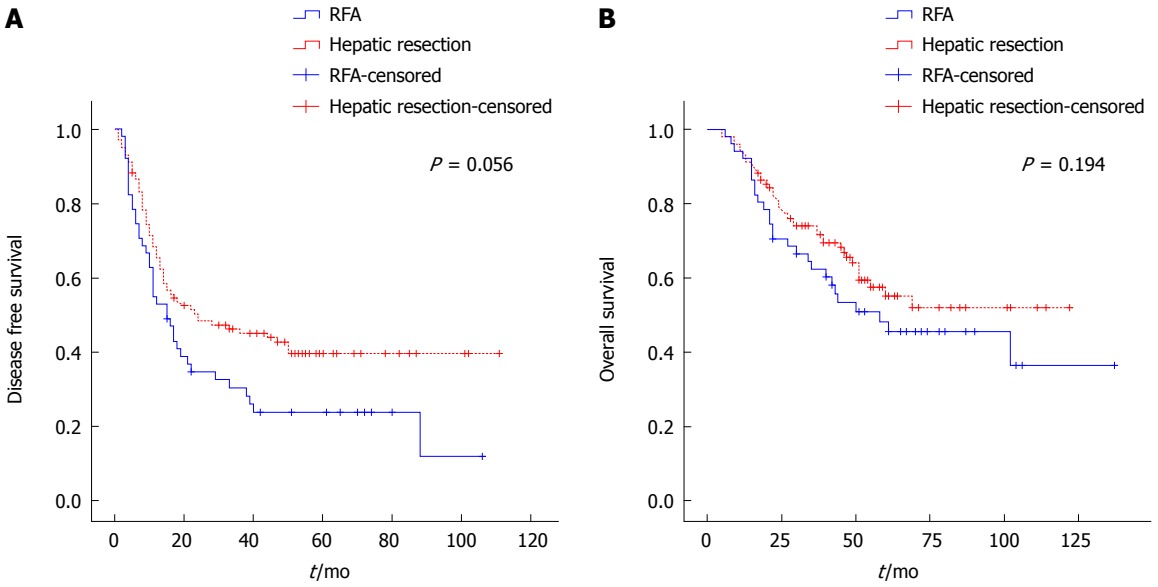


Figure 3 Kaplan-Meier survival analysis for disease-free survival and overall survival after hepatectomy or radiofrequency ablation for colorectal liver metastasis. A: Disease-free survival; B: Overall survival. RFA: Radiofrequency ablation; CRLM: Colorectal liver metastasis.

group were higher than for the RFA group for tumor size > 2 cm, although the difference was not significant.

A study reported that OS of an RFA group was comparable to OS for a hepatectomy group despite a high rate of local recurrence after RFA. This finding might be due to similar rates of extrahepatic recurrence, a crucial survival factor[2]. In our study, we verified RFA using a variety of criteria. RFA was associated not only with high local recurrence, but also poor OS. Cluster of differentiation 95 (CD95) is thought to affect the recurrence of cancer after RFA because of the potential of RFA to cause hypoxic damage. CD95 can induce apoptosis, but can also promote tumorigenesis in apoptosis-resistant tumor cells[27].

We used propensity score matching to minimize possible bias from stratification. We also used a Cox proportional hazard model to analyze multiple variables of RFA, sex, age, CEA, size, number of CRLM, metachronicity, bilobal distribution, TN stage, adjuvant chemotherapy, and cell differentiation. We found that RFA, lymph node metastasis and poorly differentiated cell type were associated with poor prognosis. Lymph node metastasis is a well-known prognostic factor for colorectal cancer treatment[28]. In this study, the lymph node metastasis and poorly differentiated type were poor prognostic factors.

To overcome the shortcomings of RFA, microwave ablation was developed to treat hepatic neoplasms[29-32].

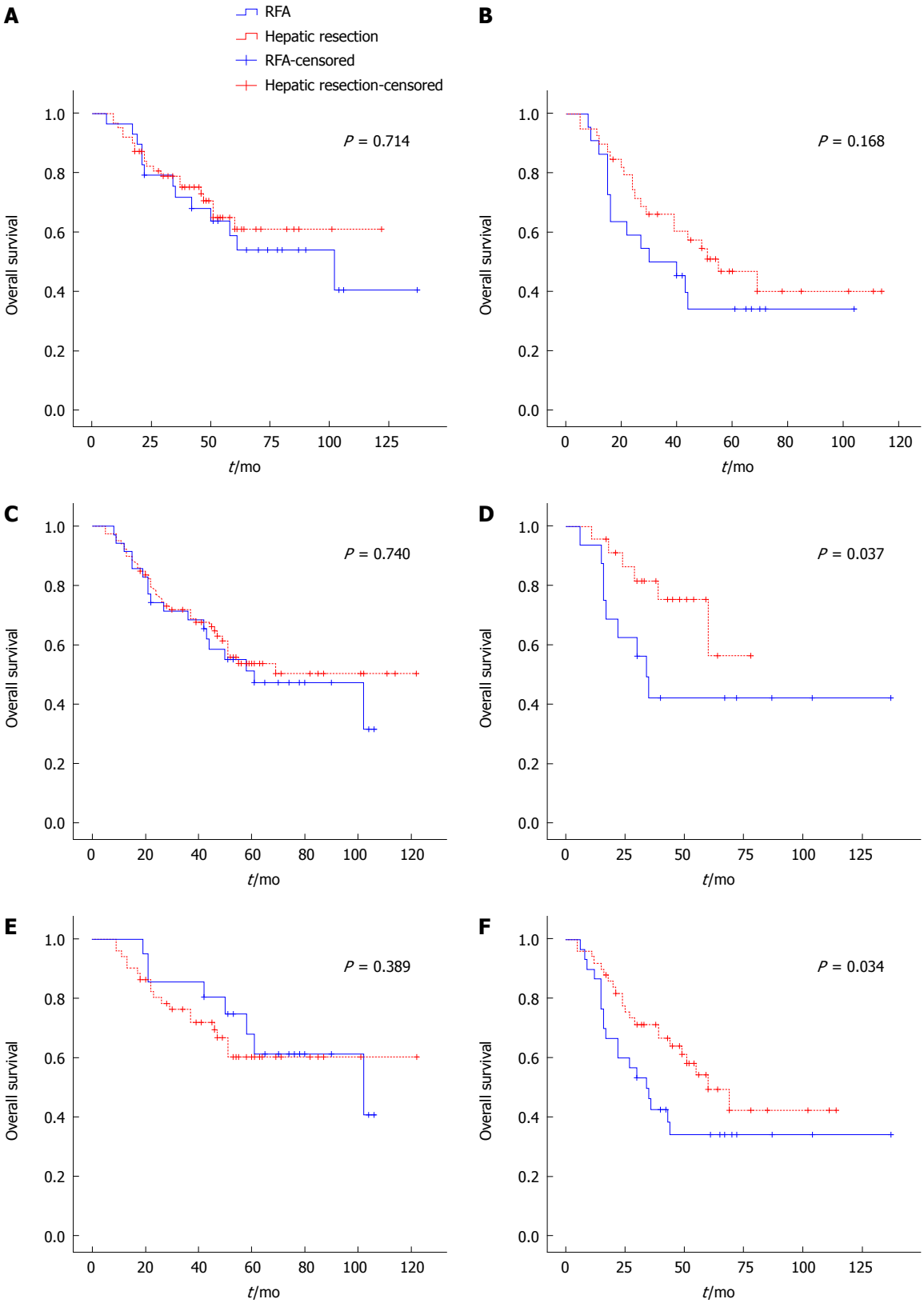


Figure 4 Kaplan-Meier survival analysis for disease-free survival after hepatectomy or radiofrequency ablation for colorectal liver metastasis according to tumor number and size. A: Single CRLM; B: Multiple CRLM; C: Size of CRLM (≤ 2 cm); D: Size of CRLM (2 cm < CRLM ≤ 3 cm); E: Single and size of CRLM (≤ 2 cm); F: Multiple or size of CRLM (2 cm < CRLM ≤ 3 cm). RFA: Radiofrequency ablation; CRLM: Colorectal liver metastasis.

Table 2 Disease-free survival after surgical treatment with curative intent for colorectal liver metastasis by multivariate analysis by Cox regression proportional hazard model (n = 153)

Factors	Univariate analysis of DFS	Multivariate analysis of DFS		
	P	HR	95%CI	*P*[1]
Radiofrequency ablation	0.033	1.57	1.02-2.41	0.040
Female sex	0.890			
Age	0.270			
CEA (ng/mL)	0.130	1.03	0.99-1.01	0.480
Size (cm)	0.210			
Multiple	0.140	1.01	0.65-1.57	0.970
Metachronous	0.089	0.75	0.45-1.24	0.260
Bilobal	0.300			
T2[2]	0.130	0.55	0.18-1.65	0.290
T3[2]	0.090	0.78	0.41-1.49	0.450
Lymph node metastasis	0.003	1.94	1.14-3.31	0.015
Adjuvant chemotherapy	0.810			
Poorly differentiated grade	0.033	1.79	1.01-3.18	0.049

[1]Cox regression proportional hazard model; [2]Compared with T4 (T stage of primary colorectal tumor). CEA: Carcinoembryonic antigen; DFS: Disease-free survival; CRLM: Colorectal liver metastasis.

Recent studies reported that microwave ablation is safe and has a low complication rate, even for tumors located near major vessels. Patients who underwent microwave ablation showed enhanced survival relative to a conventional hepatectomy group based on short-term follow-up results[32,33]. However, few published studies have examined a large number of patients followed for a long period of time.

This study has several limitations that should be considered. The major drawback was that it was neither randomized nor prospective. However, conducting randomization for RFA is difficult so we reduced bias using propensity score matching. Although we could not evaluate ICG clearance test and comorbidity rate to calculate propensity score, it does not seem an essential component to evaluate oncologic outcomes. Moreover, the comorbidity rate was not significantly different between the two groups.

In conclusion, our long-term follow-up survival analysis revealed that hepatectomy was superior to RFA for treating CRLM. RFA was associated with hepatic recurrence and was a poor prognostic factor. However, RFA might be an option for selected patients with single, small (≤ 2 cm) CRLM.

COMMENTS

Background

Numerous studies have reported that radiofrequency ablation (RFA) is a safe and feasible treatment option for a limited population of patients with colorectal liver metastasis (CRLM). Recent studies, however, demonstrated that hepatectomy is superior to RFA and that RFA should be used only in patients unsuitable for hepatectomy. However, outcomes after RFA to treat CRLM have rarely been evaluated according to location, number, and synchronicity of metastases even though these are important considerations in a treatment plan. Moreover, randomized controlled trial is difficult to do to compare the outcomes after hepatectomy and RFA.

Research frontiers

Propensity score matching is widely used in recent studies when randomized controlled trial is difficult to apply. The aim in this study was to use propensity score matching to determine if survival outcomes were different between patients who underwent RFA and patients who underwent hepatectomy.

Innovations and breakthroughs

A randomized controlled trial to compare the outcomes of radiofrequency ablation (RFA) and hepatectomy would be difficult. Instead, propensity score matching analysis has been used to minimize bias in evaluating the effectiveness of RFA in patients with hepatocellular carcinoma. However, no propensity score analysis for patients with CRLM has been published.

Applications

The long-term follow-up survival analysis revealed that hepatectomy was superior to RFA for treating CRLM. RFA was associated with hepatic recurrence and was a poor prognostic factor. However, RFA might be an option for selected patients with single, small (≤ 2 cm) CRLM.

Peer-review

This is a good descriptive study and the methodology was so good. The particular point of this article is "propensity score analysis". The authors used this method to match patients between these two groups and tried to overcome bias resulting from retrospective study.

REFERENCES

1 **White RR**, Avital I, Sofocleous CT, Brown KT, Brody LA, Covey A, Getrajdman GI, Jarnagin WR, Dematteo RP, Fong Y, Blumgart LH, D'Angelica M. Rates and patterns of recurrence for percutaneous radiofrequency ablation and open wedge resection for solitary colorectal liver metastasis. *J Gastrointest Surg* 2007; **11**: 256-263 [PMID: 17458595 DOI: 10.1007/s11605-007-0100-8]

2 **Otto G**, Düber C, Hoppe-Lotichius M, König J, Heise M, Pitton MB. Radiofrequency ablation as first-line treatment in patients with early colorectal liver metastases amenable to surgery. *Ann Surg* 2010; **251**: 796-803 [PMID: 19858704 DOI: 10.1097/SLA.0b013e3181bc9fae]

3 **Pathak S**, Jones R, Tang JM, Parmar C, Fenwick S, Malik H, Poston G. Ablative therapies for colorectal liver metastases: a systematic review. *Colorectal Dis* 2011; **13**: e252-e265 [PMID: 21689362 DOI: 10.1111/j.1463-1318.2011.02695.x]

4 **Kingham TP**, Tanoue M, Eaton A, Rocha FG, Do R, Allen P, De Matteo RP, D'Angelica M, Fong Y, Jarnagin WR. Patterns of recurrence after ablation of colorectal cancer liver metastases. *Ann Surg Oncol* 2012; **19**: 834-841 [PMID: 21879262 DOI: 10.1245/s10434-011-2048-x]

5 **Yoon HM**, Kim JH, Shin YM, Won HJ, Kim PN. Percutaneous radiofrequency ablation using internally cooled wet electrodes for treatment of colorectal liver metastases. *Clin Radiol* 2012; **67**: 122-127 [PMID: 21906730 DOI: 10.1016/j.crad.2011.08.009]

6 **Livraghi T**, Meloni F, Solbiati L, Zanus G. Complications of microwave ablation for liver tumors: results of a multicenter study. *Cardiovasc Intervent Radiol* 2012; **35**: 868-874 [PMID: 21833809 DOI: 10.1007/s00270-011-0241-8]

7 **Van Tilborg AA**, Meijerink MR, Sietses C, Van Waesberghe JH, Mackintosh MO, Meijer S, Van Kuijk C, Van Den Tol P. Long-term results of radiofrequency ablation for unresectable colorectal liver metastases: a potentially curative intervention. *Br J Radiol* 2011; **84**: 556-565 [PMID: 21159807 DOI: 10.1259/bjr/78268814]

8 **Cirimbei C**, Prunoiu V, Marincaş M, Daha C, Cirimbei S, Stefan I, Man C, Brătucu E, Pantiş C, Rădoi S, Romoşan M, Diaconu C, Zamfir C, Nechita D, Coman L. [Radiofrequency ablation for liver metastases--mini invasive therapeutic option for patients with unresecable tumors]. *Chirurgia* (Bucur) 2011; **106**: 465-473 [PMID: 21991871]

9 **Wu YZ**, Li B, Wang T, Wang SJ, Zhou YM. Radiofrequency ablation vs hepatic resection for solitary colorectal liver metastasis: a meta-analysis. *World J Gastroenterol* 2011; **17**: 4143-4148 [PMID: 22039331 DOI: 10.3748/wjg.v17.i36.4143]

10 **Lee KH**, Kim HO, Yoo CH, Son BH, Park YL, Cho YK, Kim H, Han WK. Comparison of radiofrequency ablation and resection for

hepatic metastasis from colorectal cancer. *Korean J Gastroenterol* 2012; **59**: 218-223 [PMID: 22460570]
11 **Mayo SC**, Pawlik TM. Thermal ablative therapies for secondary hepatic malignancies. *Cancer J* 2010; **16**: 111-117 [PMID: 20404607 DOI: 10.1097/PPO.0b013e3181d7ea07]
12 **Lee YH**, Hsu CY, Chu CW, Liu PH, Hsia CY, Huang YH, Su CW, Chiou YY, Lin HC, Huo TI. Radiofrequency Ablation is Better Than Surgical Resection in Patients With Hepatocellular Carcinoma Within the Milan Criteria and Preserved Liver Function: A Retrospective Study Using Propensity Score Analyses. *J Clin Gastroenterol* 2015; **49**: 242-249 [PMID: 24714185 DOI: 10.1097/mcg.0000000000000133]
13 **Takuma Y**, Takabatake H, Morimoto Y, Toshikuni N, Kayahara T, Makino Y, Yamamoto H. Comparison of combined transcatheter arterial chemoembolization and radiofrequency ablation with surgical resection by using propensity score matching in patients with hepatocellular carcinoma within Milan criteria. *Radiology* 2013; **269**: 927-937 [PMID: 24086071 DOI: 10.1148/radiol.13130387]
14 **Liu PH**, Lee YH, Hsu CY, Huang YH, Chiou YY, Lin HC, Huo TI. Survival advantage of radiofrequency ablation over transarterial chemoembolization for patients with hepatocellular carcinoma and good performance status within the Milan criteria. *Ann Surg Oncol* 2014; **21**: 3835-3843 [PMID: 24903236 DOI: 10.1245/s10434-014-3831-2]
15 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542]
16 **Edge SB**, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17**: 1471-1474 [PMID: 20180029 DOI: 10.1245/s10434-010-0985-4]
17 **Reuter NP**, Woodall CE, Scoggins CR, McMasters KM, Martin RC. Radiofrequency ablation vs. resection for hepatic colorectal metastasis: therapeutically equivalent? *J Gastrointest Surg* 2009; **13**: 486-491 [PMID: 18972167 DOI: 10.1007/s11605-008-0727-0]
18 **Hur H**, Ko YT, Min BS, Kim KS, Choi JS, Sohn SK, Cho CH, Ko HK, Lee JT, Kim NK. Comparative study of resection and radiofrequency ablation in the treatment of solitary colorectal liver metastases. *Am J Surg* 2009; **197**: 728-736 [PMID: 18789428 DOI: 10.1016/j.amjsurg.2008.04.013]
19 **Park IJ**, Kim HC, Yu CS, Kim PN, Won HJ, Kim JC. Radiofrequency ablation for metachronous liver metastasis from colorectal cancer after curative surgery. *Ann Surg Oncol* 2008; **15**: 227-232 [PMID: 17882491 DOI: 10.1245/s10434-007-9625-z]
20 **Mulier S**, Ruers T, Jamart J, Michel L, Marchal G, Ni Y. Radiofrequency ablation versus resection for resectable colorectal liver metastases: time for a randomized trial? An update. *Dig Surg* 2008; **25**: 445-460 [PMID: 19212117 DOI: 10.1159/000184736]
21 **Lee WS**, Yun SH, Chun HK, Lee WY, Kim SJ, Choi SH, Heo JS, Joh JW, Choi D, Kim SH, Rhim H, Lim HK. Clinical outcomes of hepatic resection and radiofrequency ablation in patients with solitary colorectal liver metastasis. *J Clin Gastroenterol* 2008; **42**: 945-949 [PMID: 18438208 DOI: 10.1097/MCG.0b013e318064e752]
22 **Adams RB**, Aloia TA, Loyer E, Pawlik TM, Taouli B, Vauthey JN. Selection for hepatic resection of colorectal liver metastases: expert consensus statement. *HPB* (Oxford) 2013; **15**: 91-103 [PMID: 23297719 DOI: 10.1111/j.1477-2574.2012.00557.x]
23 **Abitabile P**, Hartl U, Lange J, Maurer CA. Radiofrequency ablation permits an effective treatment for colorectal liver metastasis. *Eur J Surg Oncol* 2007; **33**: 67-71 [PMID: 17174059 DOI: 10.1016/j.ejso.2006.10.040]
24 **Valls C**, Ramos E, Leiva D, Ruiz S, Martinez L, Rafecas A. Safety and Efficacy of Ultrasound-Guided Radiofrequency Ablation of Recurrent Colorectal Cancer Liver Metastases after Hepatectomy. *Scand J Surg* 2014; Epub ahead of print [PMID: 25332220 DOI: 10.1177/1457496914553147]
25 **Ko S**, Jo H, Yun S, Park E, Kim S, Seo HI. Comparative analysis of radiofrequency ablation and resection for resectable colorectal liver metastases. *World J Gastroenterol* 2014; **20**: 525-531 [PMID: 24574721 DOI: 10.3748/wjg.v20.i2.525]
26 **Nishiwada S**, Ko S, Mukogawa T, Ishikawa H, Matsusaka M, Nakatani T, Kikuchi E, Watanabe A. Comparison between percutaneous radiofrequency ablation and surgical hepatectomy focusing on local disease control rate for colorectal liver metastases. *Hepatogastroenterology* 2014; **61**: 436-441 [PMID: 24901157]
27 **Nijkamp MW**, Hoogwater FJ, Steller EJ, Westendorp BF, van der Meulen TA, Leenders MW, Borel Rinkes IH, Kranenburg O. CD95 is a key mediator of invasion and accelerated outgrowth of mouse colorectal liver metastases following radiofrequency ablation. *J Hepatol* 2010; **53**: 1069-1077 [PMID: 20832890 DOI: 10.1016/j.jhep.2010.04.040]
28 **Ryuk JP**, Choi GS, Park JS, Kim HJ, Park SY, Yoon GS, Jun SH, Kwon YC. Predictive factors and the prognosis of recurrence of colorectal cancer within 2 years after curative resection. *Ann Surg Treat Res* 2014; **86**: 143-151 [PMID: 24761423 DOI: 10.4174/astr.2014.86.3.143]
29 **Stättner S**, Jones RP, Yip VS, Buchanan K, Poston GJ, Malik HZ, Fenwick SW. Microwave ablation with or without resection for colorectal liver metastases. *Eur J Surg Oncol* 2013; **39**: 844-849 [PMID: 23769976 DOI: 10.1016/j.ejso.2013.04.005]
30 **Groeschl RT**, Pilgrim CH, Hanna EM, Simo KA, Swan RZ, Sindram D, Martinie JB, Iannitti DA, Bloomston M, Schmidt C, Khabiri H, Shirley LA, Martin RC, Tsai S, Turaga KK, Christians KK, Rilling WS, Gamblin TC. Microwave ablation for hepatic malignancies: a multiinstitutional analysis. *Ann Surg* 2014; **259**: 1195-1200 [PMID: 24096760 DOI: 10.1097/sla.0000000000000234]
31 **Bala MM**, Riemsma RP, Wolff R, Kleijnen J. Microwave coagulation for liver metastases. *Cochrane Database Syst Rev* 2013; **10**: CD010163 [PMID: 24122576 DOI: 10.1002/14651858.CD010163.pub2]
32 **Correa-Gallego C**, Fong Y, Gonen M, D'Angelica MI, Allen PJ, DeMatteo RP, Jarnagin WR, Kingham TP. A retrospective comparison of microwave ablation vs. radiofrequency ablation for colorectal cancer hepatic metastases. *Ann Surg Oncol* 2014; **21**: 4278-4283 [PMID: 24889486 DOI: 10.1245/s10434-014-3817-0]
33 **Ierardi AM**, Floridi C, Fontana F, Chini C, Giorlando F, Piacentino F, Brunese L, Pinotti G, Bacuzzi A, Carrafiello G. Microwave ablation of liver metastases to overcome the limitations of radiofrequency ablation. *Radiol Med* 2013; **118**: 949-961 [PMID: 23892957 DOI: 10.1007/s11547-013-0968-1]

P- Reviewer: Boin IFSF, Hsu WH **S- Editor**: Yu J
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3308

World J Gastroenterol 2015 March 21; 21(11): 3308-3316
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Significant risk and associated factors of active tuberculosis infection in Korean patients with inflammatory bowel disease using anti-TNF agents

Eun Soo Kim, Geun Am Song, Kwang Bum Cho, Kyung Sik Park, Kyeong Ok Kim, Byung Ik Jang, Eun Young Kim, Seong Woo Jeon, Hyun Seok Lee, Chang Heon Yang, Yong Kook Lee, Dong Wook Lee, Sung Kook Kim, Tae Oh Kim, Jonghun Lee, Hyung Wook Kim, Sam Ryong Jee, Seun Ja Park, Hyun Jin Kim

Eun Soo Kim, Kwang Bum Cho, Kyung Sik Park, Division of Gastroenterology, Department of Internal Medicine, Keimyung University School of Medicine, Daegu 700-721, South Korea
Geun Am Song, Hyung Wook Kim, Division of Gastroenterology, Department of Internal Medicine, Pusan National University School of Medicine, Busan 602-739, South Korea
Kyeong Ok Kim, Byung Ik Jang, Division of Gastroenterology, Department of Internal Medicine, Yeungnam University College of Medicine, Daegu 700-721, South Korea
Eun Young Kim, Dong Wook Lee, Division of Gastroenterology, Department of Internal Medicine, Catholic University of Daegu School of Medicine, Daegu 700-721, South Korea
Seong Woo Jeon, Hyun Seok Lee, Sung Kook Kim, Division of Gastroenterology, Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu 700-721, South Korea
Chang Heon Yang, Yong Kook Lee, Division of Gastroenterology, Department of Internal Medicine, Dongguk University School of Medicine, Gyeongju 780-350, South Korea
Tae Oh Kim, Sam Ryong Jee, Division of Gastroenterology, Department of Internal Medicine, Inje University College of Medicine, Busan 602-739, South Korea
Jonghun Lee, Division of Gastroenterology, Department of Internal Medicine, Dong-A University Medical Center, Busan 602-739, South Korea
Seun Ja Park, Division of Gastroenterology, Department of Internal Medicine, Kosin University College of Medicine, Busan 602-739, South Korea
Hyun Jin Kim, Division of Gastroenterology, Department of Internal Medicine, Gyeongsang National University School of Medicine, Jinju 660-702, South Korea
Author contributions: Kim ES and Song GA contributed equally to this work; Kim SK and Kim ES designed research; Cho KB, Jang BI, Lee HS, Lee YK, Lee DW, Kim TO, Lee J, Kim HW, Jee SR, Park SJ and Kim HJ collected data; Park KS, Kim KO, Jeon SW, Yang CH and Kim EY analyzed data and were involved in editing the manuscript; Kim ES and Song GA wrote the manuscript.

Correspondence to: Sung Kook Kim, MD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu, Korea, 130 Dongdeok-ro, Jung-gu, Daegu 700-721, South Korea. dandy090101@naver.com
Telephone: +82-53-2508096
Fax: +82-53-2507088
Received: September 10, 2014
Peer-review started: September 11, 2014
First decision: October 14, 2014
Revised: October 29, 2014
Accepted: December 1, 2014
Article in press: December 1, 2014
Published online: March 21, 2015

Abstract

AIM: To evaluate the incidence and risk factors of Korean tuberculosis (TB) infection in patients with inflammatory bowel disease (IBD) undergoing anti-TNF treatment.

METHODS: The data of IBD patients treated with anti-TNFs in 13 tertiary referral hospitals located in the southeastern region of Korea were collected retrospectively. They failed to show response or were intolerant to conventional treatments, including steroids or immunomodulators. Screening measures for latent TB infection (LTBI) and the incidence and risk factors of

active TB infection after treatment with anti-TNFs were identified.

RESULTS: Overall, 376 IBD patients treated with anti-TNF agents were recruited (male 255, mean age of anti-TNF therapy 32.5 ± 13.0 years); 277 had Crohn's disease, 99 had ulcerative colitis, 294 used infliximab, and 82 used adalimumab. Before anti-TNF treatment, screening tests for LTBI including an interferon gamma release assay or a tuberculin skin test were performed in 82.2% of patients. Thirty patients (8%) had LTBI. Sixteen cases of active TB infection including one TB-related mortality occurred during 801 person-years (PY) follow-up (1997.4 cases per 100000 PY) after anti-TNF treatment. LTBI (OR = 5.76, 95%CI: 1.57-21.20, P = 0.008) and WBC count < 5000 mm^3 (OR = 4.5, 95%CI: 1.51-13.44, P = 0.007) during follow-up were identified as independently associated risk factors.

CONCLUSION: Anti-TNFs significantly increase the risk of TB infection in Korean patients with IBD. The considerable burden of TB and marked immunosuppression might be attributed to this risk.

Key words: Tuberculosis; Anti-TNF; Korea; Inflammatory bowel disease; Latent tuberculosis infection; Risk factor

Core tip: Anti-TNF antagonist therapy implies significant tuberculosis (TB) risk in inflammatory bowel disease (IBD) patients residing in areas with an intermediate burden of TB infection. The risk of susceptibility to TB under anti-TNF treatment is associated with latent TB infection and considerable immunosuppression. Rigorous and sustained assessment of TB infection should be implemented in Korean IBD patients undergoing anti-TNF therapy.

Kim ES, Song GA, Cho KB, Park KS, Kim KO, Jang BI, Kim EY, Jeon SW, Lee HS, Yang CH, Lee YK, Lee DW, Kim SK, Kim TO, Lee J, Kim HW, Jee SR, Park SJ, Kim HJ. Significant risk and associated factors of active tuberculosis infection in Korean patients with inflammatory bowel disease using anti-TNF agents. *World J Gastroenterol* 2015; 21(11): 3308-3316 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3308.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3308

INTRODUCTION

Anti-TNF agents are able to favorably change the natural course of inflammatory bowel disease (IBD), and these drugs are currently the most effective treatment to achieve sustained clinical remission and mucosal healing[1]. Korea and other East Asian countries, once considered being a region in which IBD was extremely rare, have had a rapidly increasing number of patients with IBD. The population-based Korean data showed that the mean annual incidence rates of Crohn's disease (CD) and ulcerative colitis (UC) increased from 0.05 and 0.34 per 100000 persons, respectively, in 1986-1990 to 1.34 and 3.08 per 100000, respectively, in 2001-2005[2]. Anti-TNF agents such as infliximab and adalimumab have been used with gradually increasing frequency since they were approved for IBD treatment in the mid 2000s in Korea[3-5].

One of the primary concerns regarding anti-TNF agents is an increased risk of tuberculosis (TB) infection. Despite a wide variation in TB rates among different countries, it has been reported that there was an approximately four-fold increased risk of TB in patients with rheumatoid arthritis (RA) treated with anti-TNF agents compared with those not treated with anti-TNF agents[6,7]. For IBD patients, the current incidence of active TB after treatment with an anti-TNF inhibitor is approximately 1%-2%[8,9]. The majority of TB cases occurred within 3-4 mo after anti-TNF therapy, suggesting that TB develops as a result of reactivation of latent disease rather than as a new infection[9,10]. Furthermore, the clinical characteristics of TB infection in anti-TNF treated patients are markedly atypical, presenting a greater chance of disseminated and extra-pulmonary diseases[11,12].

Although the incidence rate of TB infection in South Korea has been declining over recent decades, it remains one of the most common infectious diseases in the country. According to the World Health Organization, the incidence rate of TB in South Korea was 108 per 100000 inhabitants in 2012[13]. Whereas the risk of TB in Korean patients with RA treated with infliximab has been reported[14], there have been no reports on the incidence of TB infection in Korean IBD patients using anti-TNFs. Given that TB risks vary in different countries and with different diseases[15], it would be noteworthy to identify the risk of TB infection due to anti-TNF therapy in Korean patients with IBD. The aim of this study was to evaluate the incidence of active TB infection and associated risk factors in Korean IBD patients treated with anti-TNF agents. Additionally, the clinical characteristics of TB infection in these subjects were estimated.

MATERIALS AND METHODS

This study was conducted in 13 referral hospitals in Gyeongsang province in the southeastern region of Korea. Two anti-TNF agents, infliximab and adalimumab, are currently approved to treat IBD in South Korea. Candidates approved for the use of anti-TNFs by the National Health Insurance Service were patients with a moderate to severe stage of IBD who failed to show response or were intolerant to conventional treatments, including steroids or immunomodulators[4,5]. IBD patients treated with

Table 1 Baseline characteristics of patients treated with anti-TNF agent

	n = 376
Sex (male:female)	255:121
Age of diagnosis (yr)	27.9 ± 12.6
Age at the start of anti-TNFs (yr)	32.5 ± 13.0
Follow-up (mo)	81.6 ± 58.9
Diseases	
Crohn's disease	277 (73.7)
L1/L2/L3/L1+L4/L3+L4	48/60/157/4/8
B1/B2/B3	102/87/88
Perianal disease	160 (42.5)
Ulcerative colitis	99 (26.3)
E1/E2/E3	8/45/46
Previous anti-TB treatment	8 (2.1)
Diabetes Mellitus	12 (3.2)
Anti-TNF agents	
Infliximab	294 (78.2)
Adalimumab	82 (21.8)

Data are espressed as *n* (%) or mean ± SD. TB: Tuberculosis; UC: Ulcerative colitis.

either of these TNF antagonists from June 2003 to January 2014 were included in this study. Information regarding clinical and demographic characteristics such as sex, age of IBD diagnosis, disease duration, anti-TNF drug exposure period, location and behavior of CD, and extent of UC were obtained from medical records. For the risk factors for TB, diabetes mellitus (DM), previous TB infection, latent TB infection (LTBI), concomitant immunosuppressant at the start of anti-TNF therapy, and WBC count measured around the last follow-up day were recorded. When active TB infection developed after anti-TNF therapy, WBC counts at the time of TB diagnosis were counted. However, history regarding contact with active TB patients was not obtained. The study was approved by the ethics review committee of the Institutional Review Board of all of the hospitals participating in the study.

Definition of TB infection and screening modalities

According to the Korean Guidelines for Tuberculosis published in 2011, chest radiography, a tuberculin skin test (TST), and an interferon gamma release assay (IGRA) should be performed for LTBI screening before the initiation of anti-TNF therapy[16]. Abnormal findings on chest radiography included apical densities, pleural scarring, and calcified granulomas. TST was performed according to the Mendel-Mantoux method using purified protein derivative (PPD). Skin induration with a diameter ⩾ 10 mm at 48-72 h after the PPD inoculation on the forearm was considered positive[17]. Two methods for IGRA are available in Korea: QuantiFERON®-TB Gold In-Tube (QFT-GIT; Cellestis, Carnegie, VIC, Australia) and T-SPOT®.*TB* (T-SPOT; Oxford Immunotec, Abingdon, UK). LTBI was defined as (1) cases of an abnormal chest X-ray without previous complete TB treatment or (2) positive results with TST or IGRA[18]. The criteria for active TB infection were as follows: (1) typical symptoms with isolation of *Mycobacterium tuberculosis* from a clinical specimen or (2) typical symptoms with radiological or histological findings of TB without culture or when a culture sample could not be obtained[18]. Although there was no bacterial confirmation, these cases were regarded as active TB when the clinical symptoms and the radiological or histological findings improved with anti-TB therapy[18]. The patients diagnosed with active TB before the initiation of anti-TNF therapy were not counted as the TB cases in the study.

Statistical analysis

The incidence rate of active TB was calculated using person-years (PY) and was expressed as new cases per 100000 PY. Differences in the categorical variables between the groups were assessed with the χ^2 test or Fisher's exact test. For comparisons of continuous variables, the Mann-Whitney test was used. To investigate the independent risk factors associated with active TB infection during anti-TNF therapy, a logistic regression analysis was performed using variables with statistically significant associations identified in a univariate analysis. Age and sex were also included as variables for a multivariate analysis because these are considered important risk factors of TB infection[19,20]. A two-tailed P value < 0.05 was considered significant. The statistical analysis was performed with SPSS version 14.0 (SPSS, Chicago, IL, United States).

RESULTS

In total, 376 IBD patients using anti-TNF agents were included in the study (255 males, mean age at the start of anti-TNF therapy of 32.5 ± 13.0 years, with 277 patients with CD and 99 patients with UC). The ileocolon (157, 56.7%) and non-stricturing non-penetrating type disease (102, 36.8%) were the most common location and behavior of CD, respectively. The majority of the UC patients had extensive disease (46, 46.5%). Eight patients (2.1%) had a previous TB infection history with successful anti-TB treatment. Infliximab and adalimumab were used in 294 (78.2%) and 82 (21.8%) patients, respectively. The baseline characteristics of the patients are described in Table 1.

Screening for latent TB infection before anti-TNF therapy

The screening outcomes prior to anti-TNF therapy are summarized in Table 2. A chest X-ray was taken before anti-TNF therapy in the majority of patients (356, 94.7%); 8 (2.2%) of the chest x-rays showed abnormal appearances, suggesting old pulmonary TB. Among these patients, 4 had a history of a complete course of anti-TB treatment for pulmonary TB infection. IGRA was performed in 276 (73.4%) patients, and the positivity rate was 5.8% (16/276). One hundred and thirty-one patients (34.8%) underwent TST before anti-TNF therapy, and the positivity rate was 9.2% (12/131). Both IGRA and TST were performed in 98 patients

Table 2 Screening outcomes for latent tuberculosis infection before anti-TNF agent

	n = 376, *n* (%)
Chest X-ray	
Done	356 (94.7)
Old tuberculosis	8/356 (2.2)
Negative	348/356 (97.8)
IGRA	
Unknown	9 (2.4)
Done	276 (73.4)
Positive	16/276 (5.8)
Negative	241/276 (87.3)
Indeterminate	19/276 (6.9)
Steroid or thiopurine at IGRA	214/276 (77.5)
QuantiFERON	247 (62.9)
Positive	14/247 (5.7)
Negative	218/247 (88.3)
Indeterminate	15/247 (6)
T-SPOT	29 (12.6)
Positive	2/29 (6.9)
Negative	23/29 (79.3)
Indeterminate	4/29 (13.8)
TST	
Unknown	10 (2.7)
Done	131 (34.8)
Positive	12/131 (9.2)
Negative	117/131 (89.3)
Steroid or thiopurine at TST	104/131 (79.4)
Screening tests for LTBI	
1 test (IGRA or TST)	211 (56.1)
2 tests (IGRA and TST)	98 (26.1)
Neither IGRA or TST	59 (15.7)
Latent tuberculosis infection	30 (8.0)

IGRA: Interferon gamma release assay; TST: Tuberculin skin test; LTBI: Latent tuberculosis infection.

(26.7%). The use of IGRA increased considerably from 34% in 2009 to 90.2% in 2013, whereas there was no significant change in the use of TST during the same period, with TST being performed in 30% and 39.8% of patients in 2009 and 2013, respectively. Using chest X-ray, IGRA and TST as screening measures, LTBI was confirmed in 30 patients (8.0%). Of these LTBI cases, 16 patients received prophylactic anti-TB medications. The patient flow diagram is shown in Figure 1. Immunosuppressants, such as steroids or thiopurine, were being administered at the time of IGRA and TST screening in 77.5% and 79.4% of patients, respectively.

Incidence and risk factors of active TB infection after anti-TNF agents

Sixteen cases of active TB infection occurred during the 801 PY follow-up period after anti-TNF exposure (1997.4 per 100000 PY). The median time from anti-TNF initiation to active TB infection was 28.7 wk (range, 8-142). The clinical characteristics of these patients are summarized in Table 3. Infliximab was used in 15 patients, whereas adalimumab was used in 1 patient. All of the patients except one (who had a chest X-ray only) underwent LTBI screening tests of IGRA (75%, 12/16) or TST (31.2%, 5/16) before initiating anti-TNF treatment. Fifteen patients (93.8%) were taking immunosuppressants, including steroids or azathioprine, within 1 wk of the screening tests, and 4 patients had LTBI. Among the 4 LTBI cases, 2 patients received prophylactic treatment of either isoniazid for 9 mo or isoniazid plus rifampicin for 3 mo, and one patient had a previous complete anti-TB medication history for active pulmonary TB. The majority of the patients (15/16, 93.8%) were taking steroids or azathioprine when the active TB diagnosis was made. We conducted a univariate analysis to identify the risk factors of TB infection during anti-TNF treatment in IBD patients (Table 4). The positive rate of screening for LTBI in the TB infection group was higher than in the non-TB infection group (25% *vs* 7.2%, $P = 0.031$). A WBC count < 5000 mm^3 was more often observed in the TB infection group than in the non-TB infection group (62.5% *vs* 31.9%, $P = 0.015$). There was no significant difference between the groups regarding disease type, DM, anti-TNF agents, and immunomodulator use at the start of anti-TNF treatment. A multivariate analysis using logistic regression after adjustment for age and sex demonstrated that LTBI (OR = 5.76, 95%CI: 1.57-21.20, $P = 0.008$) and white blood cell (WBC) count < 5000 mm^3 during follow-up (OR = 4.5, 95%CI: 1.51-13.44, $P = 0.007$) were significant independent risk factors for active TB infection during anti-TNF agent therapy (Table 5).

One 24-year-old male patient with CD undergoing infliximab treatment died 55 d after miliary TB diagnosis because of devastating acute renal failure. His chest X-ray had been normal, and TST had shown a negative result. He was taking steroids at the time of the TST.

Clinical features of active tuberculosis infection during treatment with anti-TNF agents

Fever (43.8%) was the most common clinical manifestation, followed by cough, dyspnea, abdominal distension, fatigue and chest pain. The most frequent sites of infection were the lung, lymph node and pleura, in that order. Miliary TB was observed in 6 (37.5%) patients. The majority of the patients (81.3%) showed extra-pulmonary TB, indicating an atypical presentation of TB infection. The clinical characteristics of active TB infections are shown in Figures 2A and 2B.

DISCUSSION

In this study, the incidence rate of TB infection was 1997.4 per 100000 PY in IBD patients exposed to anti-TNF inhibitors, and this risk was associated with LTBI confirmed by the screening tests and with leukopenia (WBC < 5000 mm^3) during the follow-up period. These rates are much higher than the rates found in Western studies[7,9,11]. The remarkably high incidence of TB in this study might be related to the large burden of TB in the general population. South Korea has been

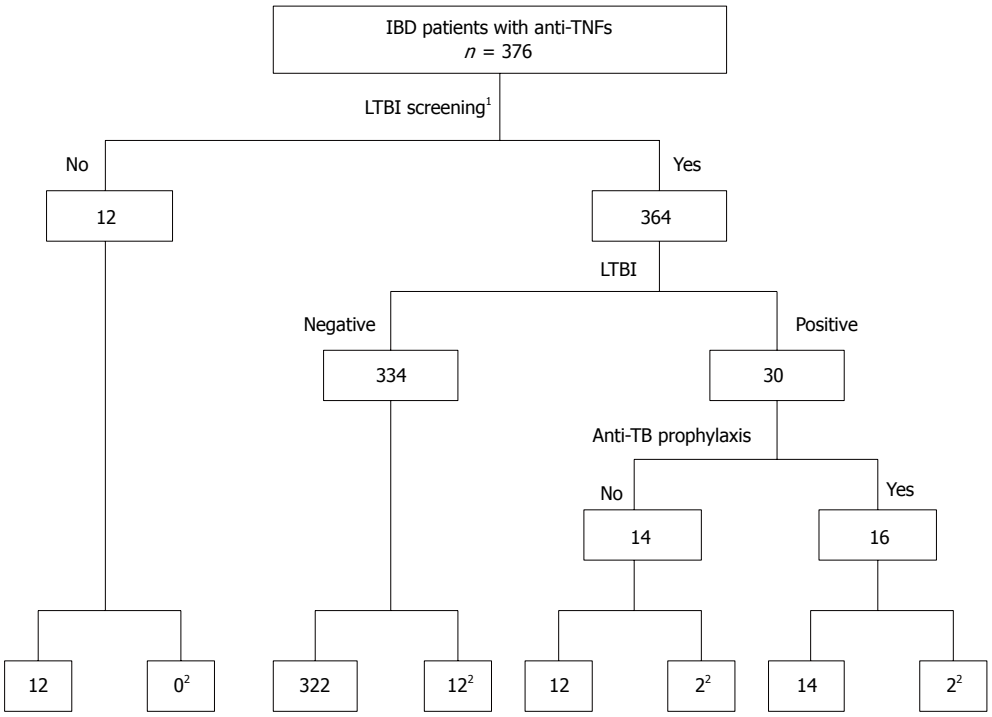


Figure 1 Flow diagram of patients, illustrating screening outcomes. [1]Chest X-ray, tuberculin skin test, or interferon gamma release assay; [2]Development of active tuberculosis infection after anti-TNF therapy. LTBI: Latent tuberculosis infection.

Table 3 Clinical characteristics of patients developing active tuberculosis infection after anti-TNF agent

No.	IBD	Sex	Age of anti-TNFs (yr)	Anti-TNFs	Previous TB treatment	Screening IGRA	Screening TST	IS at screening	Prophylaxis	Interval to TB infection (wk)	IS at TB diagnosis	Extra-pulmonary TB	No. of involvement organ
1	CD	F	21	IFX	No	ND	ND	None	ND	83	CS + AZA	No	1
2	CD	M	24	ADA	No	+	ND	CS	INH	22	None	Yes	1
3	CD	M	24	IFX	No	-	ND	CS +AZA	ND	35	AZA	Yes	3
4	CD	M	42	IFX	Yes	-	ND	CS	ND	46	CS + AZA	Yes	6
5	CD	F	34	IFX	Yes	-	ND	AZA	ND	23	AZA	Yes	1
6	CD	M	29	IFX	No	-	ND	AZA	ND	10	AZA	Yes	5
7	CD	M	41	IFX	No	ND	-	AZA	ND	12	AZA	Yes	1
8[1]	CD	M	24	IFX	No	ND	-	CS	ND	8	CS	Yes	4
9	CD	M	27	IFX	No	ND	-	AZA	ND	22	AZA	No	1
10	CD	M	23	IFX	Yes	+	-	AZA	ND	8	CS + AZA	Yes	1
11	CD	M	21	IFX	No	-	Unknown	CS +AZA	ND	129	CS + AZA	Yes	3
12	UC	M	32	IFX	No	+	ND	AZA	INH + RFP	40	AZA	Yes	4
13	UC	M	70	IFX	No	-	ND	AZA	ND	142	AZA	No	1
14	UC	M	21	IFX	No	-	ND	CS +AZA	ND	52	AZA	Yes	3
15	UC	M	56	IFX	No	-	ND	AZA	ND	47	AZA	Yes	2
16	UC	F	25	IFX	No	+	-	AZA	ND	12	AZA	Yes	2

[1]This patient died 55 d after miliary tuberculosis diagnosis due to devastating acute renal failure. IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; IFX: Infliximab; ADA: Adalimumab; ND: Not done; TB: Tuberculosis; IGRA: Interferon gamma release assay; TST: Tuberculin skin test; IS: Immunosuppressant; CS: Corticosteroid; AZA: Azathioprine; INH: Isoniazid; RFP: Rifampin.

reported to have an intermediate burden of TB with an incidence, mortality and prevalence of 108, 5.4 and 146 per 100000 persons, respectively, in 2012[13]. For reference, the incidence, mortality, and prevalence of TB in the United States were 3.6, 0.14, and 4.7 per 100000 persons, respectively, in the same year[13]. The incidence rates were also considerably high in Korean ankylosing spondylitis (AS) or RA patients using anti-TNF agents, with the rates ranging from 540 to 2558 per 100000 PY, which is similar to the observations in our study[14,18]. To the best of our knowledge, this is the first study to evaluate the risk of TB infection in a large number of Korean IBD patients undergoing anti-TNF therapy.

Although the Korean Guidelines for TB in 2011 recommend TST or IGRA as screening tests before initiating anti-TNF agents, TST and IGRA were used in only 34.8% and 73.4% of patients, respectively, in the present study. This low compliance of screening tests might be partly attributed to the patients who

Table 4 Univariate analysis of risk factors for active tuberculosis infection after anti-TNF therapy *n* (%)

	TB infection + (*n* = 16)	TB infection - (*n* = 360)	*P* value
Age at anti-TNF (yr)	26 (21-70)	30 (11-76)	0.808
Female	3 (18.8)	118 (32.8)	0.287
Diseases			0.772
Crohn's disease	11 (68.8)	266 (73.9)	
Ulcerative colitis	5 (31.3)	94 (26.1)	
Diabetes mellitus	1 (6.3)	11 (3.1)	0.411
Anti-TNF agent			0.212
Infliximab	15 (93.8)	279 (77.5)	
Adalimumab	1 (6.3)	81 (22.5)	
LTBI	4 (25)	26 (7.2)	0.031
IM at anti-TNF agent	13 (81.3)	228 (63.3)	0.187
WBC count < 5000 mm^3	10 (62.5)	115 (31.9)	0.015

LTBI: Latent tuberculosis infection; IM: Immunomodulator.

Table 5 Multivariate analysis of risk factors for active tuberculosis infection after anti-TNF therapy

	OR	95%CI	*P* value
Age at anti-TNF	0.98	0.94-1.02	0.346
Female	1.95	0.53-7.18	0.328
LTBI	5.76	1.57-21.20	0.008
WBC count < 5000 mm^3	4.50	1.51-13.44	0.007

LTBI: Latent tuberculosis infection; WBC: White blood cell.

were taking anti-TNF agents in the early period when LTBI screening before the use of TNF blockers was not strictly performed in Korea. A study from the USA also showed a low rate (65%) of LTBI screening prior to initiating anti-TNF therapy in IBD patients, and the authors reported that the initiation of treatment prior to 2006 was a risk factor for screening failure[21]. In our study, the use of IGRA rose significantly over time from 34% in 2009 to 90.2% in 2013. However, the rate of TST remained low throughout the study period, reflecting the low preference for TST due to insufficient accuracy of TST for LTBI screening, particularly in Korea, where Bacille Calmette-Guerin (BCG) vaccination is mandatory, which might influence TST results. A lack of specificity for pathogenic *Mycobacterium tuberculosis* is a limitation of TST, and this might be due to cross-reactivity with the BCG vaccination and environmental mycobacteria[22,23]. Therefore, IGRA might be more appropriate as a LTBI screening test in countries using routine BCG vaccination, such as Korea[24].

One important finding of the present study was the low positivity rates of each screening test (5.8% for IGRA and 9.2% for TST). There appear to be different positivity rates of LTBI screening tests between patients with different diseases. For example, the TST and IGRA positivity rates of RA patients were relatively high, up to 23% and 31.6%, respectively[25], whereas the rates of IBD patients were 12.5%-16% and 7.2%-9%, respectively[26,27], which are similar to our results. Although the cause of the difference between the studies is unclear, the different patient age ranges and the varying use of concomitant immunosuppressants during screening might be plausible explanations. IBD patients are typically younger than RA patients, and age is strongly associated with the positivity of TST as a result of longer exposure to *Mycobacterium tuberculosis*[26]. Additionally, approximately 80% of patients in the present study were taking steroids or thiopurine during the screening tests, which could lead to low positive results for the tests. Immunosuppression has been known to negatively affect the outcomes of TST and IGRA, resulting in low sensitivity of these screening tests[26]. Therefore, the ideal time for LTBI screening would be prior to the initiation of immunosuppressant therapy.

There has been no study on the risk factors for the development of TB infection during anti-TNF therapy because the number of TB cases is too small for a precise assessment. We identified positive LTBI and a WBC count < 5000 mm^3 as independent risk factors for active TB infection during anti-TNF therapy. Patients with LTBI were more likely to have active TB infection than patients without LTBI (OR = 5.76, 95%CI: 1.57-21.20, *P* = 0.008) (Table 5). This result is not surprising because LTBI is likely to progress to active TB in immunocompromised patients, such as those taking anti-TNF inhibitors[17]. There is clear evidence suggesting that chemoprophylaxis with screening for LTBI considerably reduces the TB reactivation rate[28,29]. However, we should consider that chemoprophylaxis for suspected LTBI prior to anti-TNF therapy does not entirely avoid the development of active disease[30]. It has been reported that chemoprophylaxis is only moderately effective[28,30]. In the present study, we could not find a prophylactic effect of anti-TB medications; 12.5% (2/16) of patients with LTBI who took prophylactic anti-TB medication still developed active TB, whereas 14.3% (2/14) of patients with LTBI who did not take prophylaxis had active TB during anti-TNF therapy (Figure 1). Although the exact reason for the lack of efficacy of prophylaxis in this study is unclear, the emergence of drug-resistant TB in Korea might be a possible explanation[31,32].

Guidelines recommend delaying to begin anti-TNF for at least 3 wk when LTBI is confirmed[16,33]. Anti-TNF agents can be started early in some inevitable cases for disease control. Given that LTBI positivity is a significant risk factor for the development of active TB and there seems to be the lack of efficacy of chemoprophylaxis for that, we should consider seriously undertaking the balance between the risk and the benefit before initiating anti-TNF agents in IBD patients with positive result for LTBI. If anti-TNF is used in these patients, more strict and complete anti-TB prophylaxis measures should be followed by rigorous monitoring for the development of active TB.

Our result showing leukopenia as an independent

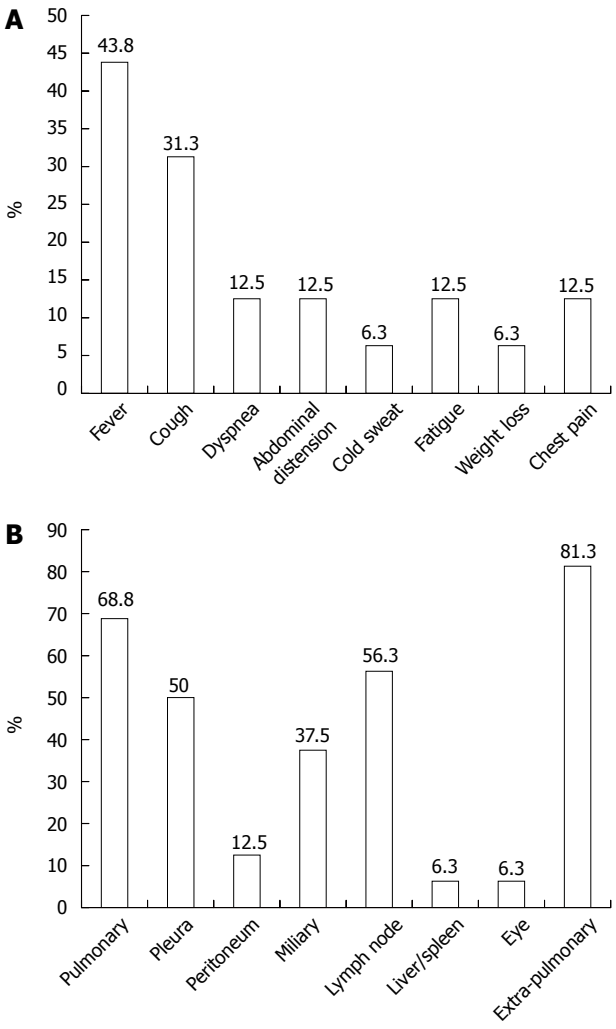


Figure 2 Clinical symptoms (A) and involvement locations (B) of active tuberculosis infection after anti-TNF therapy in patients with inflammatory bowel disease.

factor for active TB infection might indicate the synergistic risk of the substantial level of immunosuppression in anti-TNF users. Anti-TNF blocker plus azathioprine combination therapy has been shown to be the most effective treatment for IBD[34,35]. A significant proportion (87.5%, 14/16) of patients with active TB infection had concomitant azathioprine treatment at the time of TB diagnosis (Table 3). However, we are not certain that the relative leukopenia at the time of TB diagnosis is entirely a result of immunomodulator therapy alone because leukopenia can be observed in some patients with disseminated TB infection, such as miliary TB[36]. Mert *et al*[36] observed leukopenia in 26% of miliary TB patients, and this was considered to be a poor prognostic sign, although the exact cause was not clear[37]. Therefore, the results of our study should be interpreted cautiously.

In contrast to TB in immunocompetent individuals, where pulmonary infection is the main manifestation, patients who received anti-TNF therapy showed a significantly high percentage of extrapulmonary disease, at 57%-75%, and 25% had disseminated disease[7,38]. Similarly, we determined that the rates of extrapulmonary manifestations and miliary TB were considerably high at 81.3% and 37.5%, respectively (Figure 2B). Furthermore, these patients presented a variety of non-specific symptoms, including fever, fatigue, abdominal distension, dyspnea, and chest pain (Figure 2A). These results suggest that the potential diagnosis of active TB infection should be vigilantly evaluated when any patient taking an anti-TNF inhibitor has these constitutional symptoms aside from coughing because the TB manifestations under anti-TNF therapy are remarkably atypical.

Most cases of TB related to anti-TNFs are considered as reactivations of LTBI because it has been reported that TB develops early after the initiation of TNF blockade, usually within 3-4 mo[9,10]. In the present study, however, the median time from the first anti-TNF dose to TB diagnosis was 28.7 wk, which was longer than previous studies, and a quarter of TB cases occurred 1 year after the initiation of anti-TNF agents. We presumed that some TB cases in our study might represent de novo infection from exposure to other TB-infected persons during the course of anti-TNF treatment instead of representing reactivation of LTBI. This finding is in accordance with another Korean study evaluating the TB incidence in AS patients taking anti-TNFs with a long median time of 21.5 mo[18]. These results highlight the recommendation that information regarding close contact with TB-infected individuals should be rigorously and continuously assessed in patients receiving anti-TNF therapy, particularly in countries with a significant TB burden such as South Korea. Further prospective studies are needed to clarify whether periodically repeated screening tests for LTBI are effective in this population.

This study has several limitations. The retrospective design is the major limitation. Important information regarding contact with active TB patients could not be obtained. The WBC count was not systematically collected for the analysis of risk factors. The WBC counts of non-TB patients were obtained at the last follow-up visit, whereas the WBC counts of the TB patients were obtained at TB diagnosis.

In conclusion, anti-TNF inhibitors imply significant TB risk in IBD patients residing in areas with an intermediate burden of TB infection. The risk of susceptibility to TB under anti-TNF therapy is significantly associated with LTBI and considerable immunosuppression. Rigorous and sustained assessment of TB infection should be performed in IBD patients undergoing anti-TNF therapy. Further studies to establish the strategy of effective TB monitoring in this population are required.

COMMENTS

Background

Anti-TNF agents are able to favorably change the natural course of inflammatory bowel disease (IBD), and these drugs are currently the most effective treatment to achieve sustained clinical remission and mucosal healing.

However, Anti-TNF agents considerably increase the risk of tuberculosis (TB) infection.

Research frontiers

Although the use of anti-TNF agents in Korean patients with IBD has recently increased, there have been no reports on the risk of active TB infection in this population.

Innovations and breakthroughs

In this study, the incidence rate of TB infection was 1997.4 per 100000 person-years in IBD patients exposed to anti-TNF inhibitors and these rates are much higher than the rates found in Western studies. The risk was associated with LTBI confirmed by the screening tests and with leukopenia (WBC < 5000 mm^3) during the follow-up period.

Applications

Rigorous and sustained assessment of TB infection should be performed in IBD patients undergoing anti-TNF therapy. Further studies to establish the strategy of effective TB monitoring in patients residing in areas with an intermediate burden of TB infection are required.

Terminology

Latent TB infection was defined as (1) cases of an abnormal chest X-ray without previous complete TB treatment or (2) positive results with tuberculin skin test or interferon gamma release assay.

Peer-review

This article presents important data concerning the safety of anti-TNF therapy in IBD patients who live in countries with a significant burden of TB infection.

REFERENCES

1 **van Assche G**, Vermeire S, Rutgeerts P. Mucosal healing and anti TNFs in IBD. *Curr Drug Targets* 2010; **11**: 227-233 [PMID: 20210770]

2 **Yang SK**, Yun S, Kim JH, Park JY, Kim HY, Kim YH, Chang DK, Kim JS, Song IS, Park JB, Park ER, Kim KJ, Moon G, Yang SH. Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986-2005: a KASID study. *Inflamm Bowel Dis* 2008; **14**: 542-549 [PMID: 17941073 DOI: 10.1002/ibd.20310]

3 **Lee KM**, Jeen YT, Cho JY, Lee CK, Koo JS, Park DI, Im JP, Park SJ, Kim YS, Kim TO, Lee SH, Jang BI, Kim JW, Park YS, Kim ES, Choi CH, Kim HJ. Efficacy, safety, and predictors of response to infliximab therapy for ulcerative colitis: a Korean multicenter retrospective study. *J Gastroenterol Hepatol* 2013; **28**: 1829-1833 [PMID: 23829336 DOI: 10.1111/jgh.12324]

4 **Ye BD**, Yang SK, Shin SJ, Lee KM, Jang BI, Cheon JH, Choi CH, Kim YH, Lee H. [Guidelines for the management of Crohn' s disease]. *Korean J Gastroenterol* 2012; **59**: 141-179 [PMID: 22387837]

5 **Choi CH**, Kim YH, Kim YS, Ye BD, Lee KM, Lee BI, Jung SA, Kim WH, Lee H. [Guidelines for the management of ulcerative colitis]. *Korean J Gastroenterol* 2012; **59**: 118-140 [PMID: 22387836]

6 **Askling J**, Fored CM, Brandt L, Baecklund E, Bertilsson L, Cöster L, Geborek P, Jacobsson LT, Lindblad S, Lysholm J, Rantapää-Dahlqvist S, Saxne T, Romanus V, Klareskog L, Feltelius N. Risk and case characteristics of tuberculosis in rheumatoid arthritis associated with tumor necrosis factor antagonists in Sweden. *Arthritis Rheum* 2005; **52**: 1986-1992 [PMID: 15986370 DOI: 10.1002/art.21137]

7 **Keane J**, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001; **345**: 1098-1104 [PMID: 11596589 DOI: 10.1056/NEJMoa011110]

8 **Mañosa M**, Domènech E, Cabré E. Current incidence of active tuberculosis in IBD patients treated with anti-TNF agents: still room for improvement. *J Crohns Colitis* 2013; **7**: e499-e500 [PMID: 23689076 DOI: 10.1016/j.crohns.2013.04.021]

9 **Jauregui-Amezaga A**, Turon F, Ordás I, Gallego M, Feu F, Ricart E, Panés J. Risk of developing tuberculosis under anti-TNF treatment despite latent infection screening. *J Crohns Colitis* 2013; **7**: 208-212 [PMID: 22677117 DOI: 10.1016/j.crohns.2012.05.012]

10 **Wallis RS**, Broder MS, Wong JY, Hanson ME, Beenhouwer DO. Granulomatous infectious diseases associated with tumor necrosis factor antagonists. *Clin Infect Dis* 2004; **38**: 1261-1265 [PMID: 15127338 DOI: 10.1086/383317]

11 **Gómez-Reino JJ**, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. *Arthritis Rheum* 2003; **48**: 2122-2127 [PMID: 12905464 DOI: 10.1002/art.11137]

12 **Desai SB**, Furst DE. Problems encountered during anti-tumour necrosis factor therapy. *Best Pract Res Clin Rheumatol* 2006; **20**: 757-790 [PMID: 16979537 DOI: 10.1016/j.berh.2006.06.002]

13 **WHO**. Global Tuberculosis Report 2013. [Accessed on Sep 2, 2014]. Available from: http://www.who.int/tb/publications/global_report/ed/

14 **Seong SS**, Choi CB, Woo JH, Bae KW, Joung CL, Uhm WS, Kim TH, Jun JB, Yoo DH, Lee JT, Bae SC. Incidence of tuberculosis in Korean patients with rheumatoid arthritis (RA): effects of RA itself and of tumor necrosis factor blockers. *J Rheumatol* 2007; **34**: 706-711 [PMID: 17309133]

15 **Theis VS**, Rhodes JM. Review article: minimizing tuberculosis during anti-tumour necrosis factor-alpha treatment of inflammatory bowel disease. *Aliment Pharmacol Ther* 2008; **27**: 19-30 [PMID: 17944997 DOI: 10.1111/j.1365-2036.2007.03553.x]

16 Clinical Practice Guidelines for Tuberculosis, Seoul, Korea: Korea Centers for Disease Control and Prevention, 2011. Accessed July 16, 2014. Available from: URL: http://www.lungkorea.org/image/mail/file 11017.pdf/

17 **Solovic I**, Sester M, Gomez-Reino JJ, Rieder HL, Ehlers S, Milburn HJ, Kampmann B, Hellmich B, Groves R, Schreiber S, Wallis RS, Sotgiu G, Schölvinck EH, Goletti D, Zellweger JP, Diel R, Carmona L, Bartalesi F, Ravn P, Bossink A, Duarte R, Erkens C, Clark J, Migliori GB, Lange C. The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement. *Eur Respir J* 2010; **36**: 1185-1206 [PMID: 20530046 DOI: 10.1183/09031936.00028510]

18 **Kim EM**, Uhm WS, Bae SC, Yoo DH, Kim TH. Incidence of tuberculosis among korean patients with ankylosing spondylitis who are taking tumor necrosis factor blockers. *J Rheumatol* 2011; **38**: 2218-2223 [PMID: 21844149 DOI: 10.3899/jrheum.110373]

19 **Li X**, Li T, Tan S. Males, ages ⩾ 45 years, businessperson, floating population, and rural residents may be considered high-risk groups for tuberculosis infection in Guangzhou, China: a review of 136,394 tb confirmed cases. *Rev Inst Med Trop Sao Paulo* 2013; **55**: 366-368 [PMID: 24037294 DOI: 10.1590/S0036-46652013000500013]

20 **Memish ZA**, Bamgboye EA, Abuljadayel N, Smadi H, Abouzeid MS, Al Hakeem RF. Incidence of and risk factors associated with pulmonary and extra-pulmonary tuberculosis in Saudi Arabia (2010-2011). *PLoS One* 2014; **9**: e95654 [PMID: 24824783 DOI: 10.1371/journal.pone.0095654]

21 **Vaughn BP**, Doherty GA, Gautam S, Moss AC, Cheifetz AS. Screening for tuberculosis and hepatitis B prior to the initiation of anti-tumor necrosis therapy. *Inflamm Bowel Dis* 2012; **18**: 1057-1063 [PMID: 21953829 DOI: 10.1002/ibd.21824]

22 **Mow WS**, Abreu-Martin MT, Papadakis KA, Pitchon HE, Targan SR, Vasiliauskas EA. High incidence of anergy in inflammatory bowel disease patients limits the usefulness of PPD screening before infliximab therapy. *Clin Gastroenterol Hepatol* 2004; **2**: 309-313 [PMID: 15067625]

23 **Ponce de León D**, Acevedo-Vásquez E, Sánchez-Torres A, Cucho M, Alfaro J, Perich R, Pastor C, Harrison J, Sánchez-Schwartz C. Attenuated response to purified protein derivative in patients with rheumatoid arthritis: study in a population with a high prevalence of tuberculosis. *Ann Rheum Dis* 2005; **64**: 1360-1361 [PMID: 16100342 DOI: 10.1136/ard.2004.029041]

24 **Mazurek GH**, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K. Updated guidelines for using Interferon Gamma Release Assays to

detect Mycobacterium tuberculosis infection - United States, 2010. *MMWR Recomm Rep* 2010; **59**: 1-25 [PMID: 20577159]
25 **Song GG**, Bae SC, Lee YH. Interferon-gamma release assays versus tuberculin skin testing in patients with rheumatoid arthritis. *Int J Rheum Dis* 2013; **16**: 279-283 [PMID: 23981748 DOI: 10.1111/1756-185X.12098]
26 **Papay P**, Eser A, Winkler S, Frantal S, Primas C, Miehsler W, Novacek G, Vogelsang H, Dejaco C, Reinisch W. Factors impacting the results of interferon-γ release assay and tuberculin skin test in routine screening for latent tuberculosis in patients with inflammatory bowel diseases. *Inflamm Bowel Dis* 2011; **17**: 84-90 [PMID: 20722065 DOI: 10.1002/ibd.21427]
27 **Kim BJ**, Choi YS, Jang BI, Park YS, Kim WH, Kim YS, Jung SA, Han DS, Kim JS, Choi JH, Choi CH, Jeen YT, Cheon JH, Ye BD, Yang SK, Kim YH. Prospective evaluation of the clinical utility of interferon-γ assay in the differential diagnosis of intestinal tuberculosis and Crohn's disease. *Inflamm Bowel Dis* 2011; **17**: 1308-1313 [PMID: 21053248 DOI: 10.1002/ibd.21490]
28 **Carmona L**, Gómez-Reino JJ, Rodríguez-Valverde V, Montero D, Pascual-Gómez E, Mola EM, Carreño L, Figueroa M. Effectiveness of recommendations to prevent reactivation of latent tuberculosis infection in patients treated with tumor necrosis factor antagonists. *Arthritis Rheum* 2005; **52**: 1766-1772 [PMID: 15934089 DOI: 10.1002/art.21043]
29 **Winthrop KL**. Risk and prevention of tuberculosis and other serious opportunistic infections associated with the inhibition of tumor necrosis factor. *Nat Clin Pract Rheumatol* 2006; **2**: 602-610 [PMID: 17075599 DOI: 10.1038/ncprheum0336]
30 **Sichletidis L**, Settas L, Spyratos D, Chloros D, Patakas D. Tuberculosis in patients receiving anti-TNF agents despite chemoprophylaxis. *Int J Tuberc Lung Dis* 2006; **10**: 1127-1132 [PMID: 17044206]
31 **Choi JC**, Lim SY, Suh GY, Chung MP, Kim H, Kwon OJ, Lee NY, Park YK, Bai GH, Koh WJ. Drug resistance rates of Mycobacterium tuberculosis at a private referral center in Korea. *J Korean Med Sci* 2007; **22**: 677-681 [PMID: 17728509]
32 **Lee SW**, Jeon K, Kim KH, Min KH. Multidrug-resistant pulmonary tuberculosis among young Korean soldiers in a communal setting. *J Korean Med Sci* 2009; **24**: 592-595 [PMID: 19654938 DOI: 10.3346/jkms.2009.24.4.592]
33 **Rahier JF**, Ben-Horin S, Chowers Y, Conlon C, De Munter P, D' Haens G, Domènech E, Eliakim R, Eser A, Frater J, Gassull M, Giladi M, Kaser A, Lémann M, Moreels T, Moschen A, Pollok R, Reinisch W, Schunter M, Stange EF, Tilg H, Van Assche G, Viget N, Vucelic B, Walsh A, Weiss G, Yazdanpanah Y, Zabana Y, Travis SP, Colombel JF. European evidence-based Consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2009; **3**: 47-91 [PMID: 21172250 DOI: 10.1016/j.crohns.2009.02.010]
34 **Panaccione R**, Ghosh S, Middleton S, Márquez JR, Scott BB, Flint L, van Hoogstraten HJ, Chen AC, Zheng H, Danese S, Rutgeerts P. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. *Gastroenterology* 2014; **146**: 392-400.e3 [PMID: 24512909 DOI: 10.1053/j.gastro.2013.10.052]
35 **Colombel JF**, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010; **362**: 1383-1395 [PMID: 20393175 DOI: 10.1056/NEJMoa0904492]
36 **Mert A**, Bilir M, Tabak F, Ozaras R, Ozturk R, Senturk H, Aki H, Seyhan N, Karayel T, Aktuglu Y. Miliary tuberculosis: clinical manifestations, diagnosis and outcome in 38 adults. *Respirology* 2001; **6**: 217-224 [PMID: 11555380]
37 **Maartens G**, Willcox PA, Benatar SR. Miliary tuberculosis: rapid diagnosis, hematologic abnormalities, and outcome in 109 treated adults. *Am J Med* 1990; **89**: 291-296 [PMID: 2393033]
38 **Wolfe F**, Michaud K, Anderson J, Urbansky K. Tuberculosis infection in patients with rheumatoid arthritis and the effect of infliximab therapy. *Arthritis Rheum* 2004; **50**: 372-379 [PMID: 14872478 DOI: 10.1002/art.20009]

P- Reviewer: Fujimori S **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3317

World J Gastroenterol 2015 March 21; 21(11): 3317-3324
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

High neutrophil-lymphocyte ratio indicates poor prognosis for acute-on-chronic liver failure after liver transplantation

Bing-Yi Lin, Lin Zhou, Lei Geng, Zhi-Yun Zheng, Jun-Jun Jia, Jing Zhang, Jia Yao, Shu-Sen Zheng

Bing-Yi Lin, Lin Zhou, Zhi-Yun Zheng, Jun-Jun Jia, Jing Zhang, Shu-Sen Zheng, Key Laboratory of Combined Multi-Organ Transplantation, Ministry of Public Health, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang Province, China
Lin Zhou, Lei Geng, Jia Yao, Shu-Sen Zheng, Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang Province, China
Author contributions: All authors contributed equally to this paper.
Supported by National Natural Science Foundation of China for Innovative Research Group, No. 81121002; and grants from Scientific Research Project of Education Department of Zhejiang Province, No. Y201223762.

Correspondence to: Shu-Sen Zheng, PhD, MD, FACS, Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, First Affiliated Hospital, Zhejiang University School of Medicine, No. 866 Yuhangtang Road, Hangzhou 310003, Zhejiang Province, China. zyzss@zju.edu.cn
Telephone: +86-571-87236601
Fax: +86-571-87236628
Received: August 7, 2014
Peer-review started: August 8, 2014
First decision: September 27, 2014
Revised: October 14, 2014
Accepted: December 22, 2014
Article in press: December 22, 2014
Published online: March 21, 2015

Abstract

AIM: To investigate the significance of pre-transplant neutrophil-lymphocyte ratio (NLR) in determining the prognosis of liver transplant (LT) recipients with acute-on-chronic liver failure (ACLF).

METHODS: Data were collected from the liver transplantation data bank. The NLR values and other conventional inflammatory markers were evaluated for their ability to predict the prognosis of 153 patients with ACLF after LT. The NLR cut-off value was based on a receiver operating characteristic curve analysis. A Kaplan-Meier curve analysis and univariate and multivariate Cox regression models were used to define the independent risk factors for poor outcomes.

RESULTS: The optimal NLR cut-off value was 4.6. Out of 153 patients, 83 (54.2%) had an NLR $\geqslant$ 4.6. The 1-, 3-, and 5-year overall survival rates were 94.3%, 92.5% and 92.5%, respectively, in the normal NLR group and 74.7%, 71.8% and 69.8%, respectively, in patients with high NLRs ($P < 0.001$). Furthermore, there was a significant difference in infectious complications after LT between the high and normal NLR groups. There were no significant differences for other complications. In the multivariate Cox regression model, a high NLR was defined as a significant predictor of poor outcomes for LT.

CONCLUSION: A high NLR is a convenient and available predictor for prognosis of LT patients and can potentially optimize the current criteria for LT in ACLF.

Key words: Liver transplantation; Acute-on-chronic liver failure; Neutrophil-lymphocyte ratio; Acute liver failure; Inflammation

Core tip: In China, because of a great many patients with hepatitis B, liver donation is far away from filling in

the need of liver transplantation. Therefore, improving the prognosis of liver transplant (LT) is a hot issue. However, the criteria of LT for acute-on-chronic liver failure (ACLF) are according to acute liver failure, and about 20% of liver recipients are still have poor survival outcomes. The pre-transplant high neutrophil-lymphocyte ratio is a reflection of suboptimal patient conditions and immune response disorder, which could precisely predict the prognosis of LT. This result potentially was applied to select appropriate candidates for LT and even improve the current criteria of LT for ACLF.

Lin BY, Zhou L, Geng L, Zheng ZY, Jia JJ, Zhang J, Yao J, Zheng SS. High neutrophil-lymphocyte ratio indicates poor prognosis for acute-on-chronic liver failure after liver transplantation. *World J Gastroenterol* 2015; 21(11): 3317-3324 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3317.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3317

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a serious condition with a varied etiology that depends on the geographic region and population; in addition, ACLF patients have an inordinately high mortality rate due to rapid disease progression and multiple organ dysfunction resulting from deterioration in liver function[1]. In China, the high prevalence of hepatitis B accounts for more than 80% of chronic liver diseases, which can progress to ACLF[2]. The primary precipitating events responsible for ACLF are quite distinct in the West and the East. Alcohol and drugs are largely responsible for acute insults in the West, whereas infectious incidents are most common in the East[3]. Some patients respond well to appropriate management and can be discharged from hospital quickly; however, a considerable proportion of patients develop complications of cirrhosis and multiple organ dysfunction. These symptoms are often recommended as a suitable indication for liver transplantation (LT). Moreover, several reports have revealed that LT is a convincing choice to improve the prognosis of ACLF patients[4,5]. However, the long-term mortality rate still reaches approximately 20% after LT for ACLF, and a generalizable set of selection criteria for LT remains lacking.

The predictive significance of several scoring systems that address the severity of chronic liver disease with respect to the prognosis of ACLF patients was evaluated[6]. The results revealed that the Model of End Stage Liver Disease (MELD)[7] or the Child-Pugh score[8] was similar to the Acute Physiology, Age and Chronic Health Evaluation (APACHE)[9] and the Sequential Organ Failure Assessment (SOFA)[10]. Thus, liver function is not the main factor affecting the outcomes of cirrhotic patients with ACLF. To our knowledge, the majority of ACLF patients who were currently receiving positive supportive treatments before LT, such as an artificial liver support system, were in relatively stable condition and did not show severe clinical manifestations of extrahepatic organ failure during LT. Predictably, the organ failure scores, such as the APACHE Ⅱ and SOFA, may be less helpful in predicting long-term survival following LT, which is consistent with a report by Binwei *et al*[11]. Therefore, an accurate biomarker to identify the benefits of LT for ACLF that facilitates organ allocation is needed.

Cytokines and inflammatory molecules play significant roles in the rapid development of ACLF[12]. Previous data have revealed that ACLF patients occasionally exhibited clinical manifestations of systemic inflammatory response syndrome (SIRS) and that the SIRS was associated with a pro-inflammatory milieu that exacerbated the previous circulatory disturbance caused by cirrhosis, which led to inadequate tissue perfusion and multiple organ failure[1,12,13]. In addition, SIRS is a significant independent determinant factor for outcomes of liver cirrhosis patients with acute renal failure[14]. SIRS was first defined in 1992; however, this characterization was a conglomeration of very crude and simple clinical and hematological measures using temperature, respiratory and heart rates, and absolute peripheral white cell counts[15]. Neutrophil amplification and lymphocytopenia are physiological responses to adverse stressful events, and a high neutrophil-lymphocyte ratio (NLR) implies the presence of subclinical inflammation. The NLR is normally relatively stable; an increased NLR has recently been suggested to indicate immune disorders and SIRS[16]. An elevated NLR inversely correlates with the overall and cancer-specific survival rates of various malignancies and the prognoses of non-tumorous diseases[17-21]. In addition, researchers explored the feasibility of expanding the LT pool for hepatic carcinoma (HCC) using the NLR[22,23]. Thus, the effect of pre-transplant NLR on ACLF patients after LT is warranted.

The aim of this study was to determine the utility of conventional inflammatory markers and the NLR in predicting the long-term survival outcomes of patients with ACLF following LT. We also determined the association between NLR and complications after LT.

MATERIALS AND METHODS

Ethics statement

The study was performed according to the ethics guidelines of the Declaration of Helsinki in 1975 and was approved by the ethics committee of Zhejiang University. Informed written consent was obtained from all patients.

Study objectives and data collection

The definition of ACLF used in this study conforms

Table 1 Patient characteristics (*P* values indicate differences between two groups)

Variable	High NLR (*n* = 83)	Normal NLR (*n* = 70)	*P* value
Age (yr), mean ± SD	43.9 ± 14.9	48.6 ± 15.3	0.059
Gender			0.585
Male	51	46	
Female	32	24	
WBC (/pL)	(6.87 ± 2.37)	(4.76 ± 2.56)	< 0.001
Neutrophil (/pL)	(5.87 ± 2.43)	(2.80 ± 1.77)	< 0.001
Lymphocyte (/pL)	(0.55 ± 0.28)	(1.21 ± 0.81)	< 0.001
Monocyte (/pL)	(0.53 ± 0.35)	(0.52 ± 0.34)	0.900
PLR	129.33 ± 101.44	60.94 ± 33.53	< 0.001
LMR	1.33 ± 0.97	3.69 ± 4.72	< 0.001
Albumin	32.71 ± 6.92	32.41 ± 6.24	0.779
Serum creatinine > 133	15	9	0.377
AFP > 25	35	15	0.006
Total bilirubin (μmol/L)	419.75 ± 223.78	301.01 ± 182.62	0.001
MELD score	31.01 ± 7.19	28.63 ± 7.26	0.046
INR	2.67 ± 1.33	2.57 ± 1.05	0.605
Diabetes	8	3	0.202
Hypertension	8	0	0.021
HE	36	21	0.088
Child-Pugh scores	11.29 ± 1.16	11.37 ± 1.39	0.690
Total ischemia time (min)	293.91 ± 241.78	255.82 ± 264.47	0.358

WBC: White blood cell; PLR: Platelet-lymphocyte ratio; LMR: Lymphocyte-monocyte ratio; AFP: Alpha feto protein; MELD: Model for end-stage liver disease; INR: International normalized ratio; HE: Hepatic encephalopathy.

to the recommendation provided by the Asian Pacific Association for the study of the liver (APASL). Specifically, ACLF was defined as "acute hepatic insult manifesting as jaundice and coagulopathy, complicated within 4 wk by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease"[3]. Patients who were 18 years of age or older and met the aforementioned diagnostic criteria between January 2004 and December 2012 were enrolled in this study. The operative methods for LT depended on the origin of the donor liver and included both donation after cardiac death liver transplantation (DCDLT) and live donor liver transplantation (LDLT). The eligibility criteria of LT followed the standard King's College Hospital criteria. To exclude the impact of potential infections, patients with high pre-transplant white blood cell counts were withdrawn from the study. To reduce the heterogeneity of patients with ACLF, some patients were precluded from the study because their chronic liver diseases underlying ACLF were not caused by hepatitis B. Additional criteria were used to preclude subjects: (1) HCC confirmed by pathologic examination after LT; (2) loss to follow-up; (3) history of steroid administration that could influence the NLR before LT; and (4) presence of an ABO-incompatible LT with controversial survival outcomes. The primary precipitating events included recurrent hepatitis B, alcohol abuse, infection and upper gastrointestinal hemorrhage.

The demographics of patients, operative variables, preoperative treatments and clinical course were prospectively collected *via* the hospital information collection system of the LT database at the First Affiliated Hospital of Zhejiang University School of Medicine. Venous blood samples were routinely taken the day prior to LT, and the cut-off and predictive values of the NLR, white blood cells, neutrophils, lymphocytes, monocytes, lymphocyte-monocyte ratio (LMR) and platelet-lymphocyte ratio (PLR) were defined using a receiver operating characteristic (ROC) curve analysis. All patients were divided into one of two groups based on either high or normal NLR. The demographics and clinical characteristics of the two groups are presented in Table 1.

Management after LT and subsequent surveillance

The patients were followed closely by the outpatient service or communication system from the date of hospital discharge to the date of death or the last follow-up visit. Graft function was monitored using biochemical tests, ultrasonography, emission computed tomography and liver puncture every 3 mo for 2 years post-transplant and every 6 months thereafter.

The immunosuppression protocol following LT consisted of tacrolimus, basiliximab and mycophenolate mofetil. Antibiotics for trigemini with piperacillin-tazobactam, fluconazole and ganciclovir were administered immediately after the surgery as an anti-infection strategy. To prevent the recurrence of hepatitis B, all liver recipients were treated with low-dose immunoglobulin and oral lamivudine.

Statistical analysis

Overall survival (OS) and graft survival (GS) were calculated from the date of surgery until death or graft dysfunction, respectively. The optimal cut-off values for high NLR, WBC, neutrophil, lymphocyte and monocyte counts, PLR and LMR were evaluated using an ROC curve analysis. Potential predictive factors were assessed using Kaplan-Meier curves and the log-rank test. Preoperative factors that reached significance ($P < 0.10$) for OS or GS in the univariate analysis were entered into a multivariate analysis model using the Cox proportional hazards model (backward selection likelihood function) to determine their independent effects. Fisher's exact test, independent sample *t*-test and Pearson's χ^2 test were performed to assess the differences in the clinicopathologic factors of ACLF patients with high and normal NLRs. The confidence interval (CI) was set at 95%, and the cut-off value for statistical significance was $P < 0.05$. All data analyses were conducted with SPSS ver. 19.0 for Windows (SPSS Company, Chicago, Illinois, United States).

RESULTS

Patient demographics and outcomes

Of the 153 adult patients who underwent LT for ACLF during the study period, 97 (63.4%) were men and 56 (36.6%) were women. The mean age of patients was 46.1 years (range: 24-72 years) at transplant.

Table 2 Univariate analysis of variables affecting overall survival after liver transplant

Variable (cut-off value/median/*n*)	*P* value	HR (95%CI)
Gender		
Male (*n* = 97)	0.370	1.429 (0.655-3.121)
Female (*n* = 56)	0.503	0.774 (0.366-1.638)
Age (46 yr)	< 0.001	4.860 (1.857-12.719)
NLR (4.6)	0.288	0.631 (0.270-1.475)
Albumin (35 g/L)	0.163	1.683 (0.809-3.500)
Total bilirubin (337 μmol/L)	0.010	3.251 (1.328-7.958)
MELD score (28)	0.824	0.912 (0.406-2.050)
Child-Pugh score (11)	0.360	1.371 (0.697-2.695)
INR (2.5)	< 0.001	3.823 (1.813-8.062)
Serum creatinine (133 μmol/L)	0.475	1.545 (0.468-5.100)
Pretransplant diabetes (11)	0.686	0.662 (0.090-4.873)
Hypertension before LT (8)	0.629	1.198 (0.576-2.491)
HE (57)	0.286	0.63 (0.27-1.472)
AFP (25 μg/L)		

NLR: Neutrophil lymphocyte ratio; PLR: Platelet-lymphocyte ratio; LMR: Lymphocyte-monocyte ratio; AFP: Alpha feto protein; MELD: Model for end-stage liver disease; INR: International normalized ratio; HE: Hepatic encephalopathy.

Seventy-nine (51.6%) patients received pre-transplant artificial liver support. The median international normalized ratio, total bilirubin, MELD score and Child Turcotte Pugh score were 2.5, 337 μmol/L, 28 and 11, respectively. Increased alpha fetoprotein and serum creatinine (Scr) were detected in 50 and 24 patients, respectively. A total of 57 (37.25%) subjects suffered hepatic encephalopathy at diagnosis. DCDLT and LDLT were performed in 129 and 24 patients, respectively. The median total ischemia time was 254 min.

Death was confirmed for 30 patients, and 6 patients underwent a second LT during follow-up. The main cause of death, which occurred in 20 patients, was sepsis/multi-organ dysfunction. Other causes of death included liver failure secondary to chronic rejection in 2 patients, gastrointestinal bleeding in 3 patients, recurrent hepatitis B in 2 patients, biliary complications in 2 patients and heart attack in 1 patient. The median follow-up time was 47.7 mo (range: 0.01-121 mo). The 1-, 3- and 5-year OS rates were 82.9%, 80.4% and 79.4%, respectively, and the GS rates were 81.7%, 76.8% and 75.8%, respectively.

Suitable cut-off value for NLR

The peripheral WBC, neutrophil, lymphocyte and monocyte counts, albumin, NLR, PLR and LMR are commonly considered as a reflection of immune function. Hence, these blood parameters were selected as candidates for predicting outcomes of ACLF patients after LT. The areas under the ROC curves for peripheral WBC, neutrophil, lymphocyte and monocyte counts, PLR, LMR and NLR were 0.542, 0.564, 0.397, 0.556, 0.425, 0.483 and 0.736, respectively. An NLR value of 4.6 presented a sensitivity of 76.7% and a specificity of 65.9%. This cut-off was used to divide patients into high and normal NLR groups (≥ 4.6 and < 4.6, respectively).

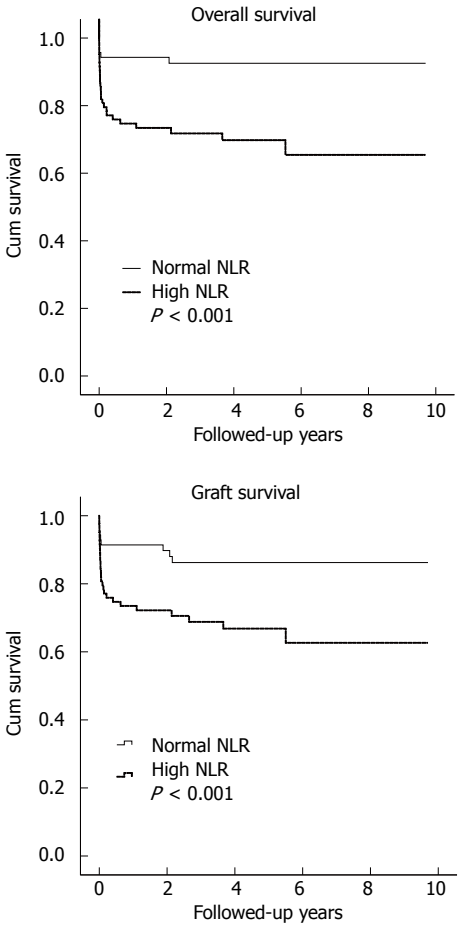


Figure 1 Kaplan-Meier results for overall survival and graft survival for patients classified according to their preoperative neutrophil-lymphocyte ratios. NLR: Neutrophil-lymphocyte ratio.

Predictive variables for OS, GS and complications

To determine whether the aforementioned blood parameters could serve as predictors for survival outcomes of ACLF patients following LT, we performed a univariate analysis. We found that an MELD score ≥ 28, NLR ≥ 4.6 and Scr > 133 were all preoperative risk factors of poor OS (Table 2). Of 153 patients, 83 (54.2%) had an NLR ≥ 4.6, and 24 (15.7%) had a high Scr. The respective 1-, 3-, and 5-year OS rates were 94.3%, 92.5% and 92.5% in the normal NLR group and 74.7%, 71.8% and 69.8% in the high NLR group ($P < 0.001$, Figure 1). In addition, the 1-, 3- and 5-year OS rates were significantly higher in the normal Scr group (86.8%, 84.9% and 84.9%, respectively) when compared with the high Scr group (62.5%, 62.5% and 55.6%, respectively); ($P < 0.001$, Figure 2). These three factors were selected for further multivariate analysis. The results showed that a MELD ≥ 28 did not reach statistical significance; however, a high NLR and increased Scr maintained their predictive value (Table 3). Patients who presented with a normal NLR and a normal Scr showed favorable survival

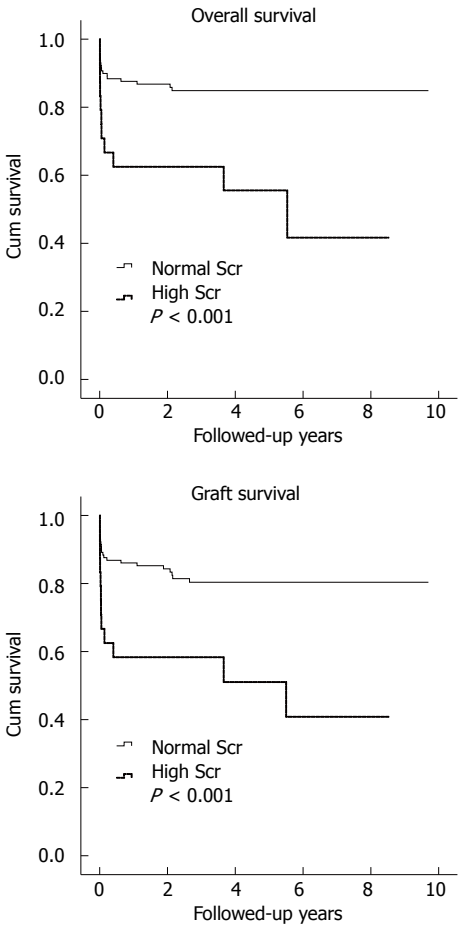


Figure 2 Kaplan-Meier results for overall survival and graft survival for patients classified according to their preoperative serum creatinine. Scr: Serum creatinine.

outcomes. The 1-, 3- and 5-year OS rates were up to 98.4%, 96.4% and 96.4%, respectively. Liver recipients with high NLRs and increased Scr presented with extremely adverse prognoses. The 1-, 3- and 5-year OS rates decreased to 60.0%, 60.0% and 50.0%, respectively (Figure 3).

The main complications after LT consisted of hyperglycemia, infectious diseases, hyperlipidemia, gastrointestinal hemorrhage, recurrent hepatitis B, biliary and neural complications, graft-*vs*-host disease and acute rejection. A total of 18 subjects showed acute rejection after LT as determined by pathologic findings according to the Banff criteria. Subsequent augmentative steroid administration was effective. The number of infectious complications in the high NLR group was greater when compared with the normal NLR group; however, there were no significant differences in other complications after LT (Figure 4).

DISCUSSION

In ACLF, both chronic and acute insults coincide. It is unclear how the survival outcome of the patient is influenced by the degree of acute and/or chronic insults in ACLF patients; however, a high mortality

Table 3 Multivariate analysis of factors affecting overall survival after liver transplant for acute-on-chronic liver failure

Variable	*P* value	HR (95%CI)
NLR ⩾ 4.6	0.003	4.305 (1.637-11.322)
Serum creatinine ⩾ 133	0.003	3.141 (1.486-6.639)
MELD ⩾ 28	0.242	1.793 (0.674-4.766)

NLR: Neutrophil-lymphocyte ratio; MELD: Model for end-stage liver disease.

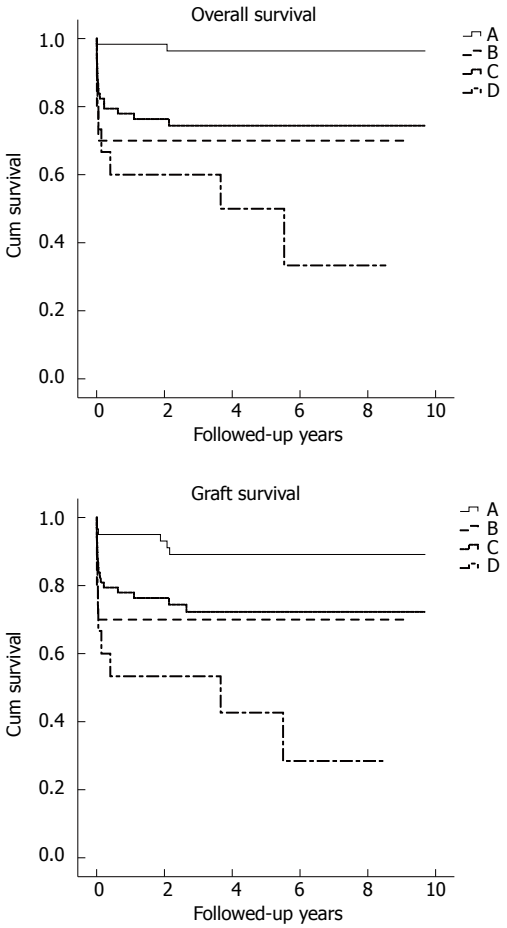


Figure 3 Kaplan-Meier results for overall survival and graft survival for patients with high serum creatinine and high neutrophil-lymphocyte ratios. The 1-, 3- and 5-year overall survival rates, respectively, were 98.4%, 96.4% and 96.4% in the A group (NLR < 4.6 and normal Scr), 66.7%, 66.7% and 66.7% in the B group (NLR < 4.6 and high Scr), 77.9%, 74.4% and 74.4% in the C group (NLR ⩾ 4.6 and normal Scr), 60.0%, 60.0% and 50.0% in the D group (NLR ⩾ 4.6 and high Scr). NLR: Neutrophil-lymphocyte ratio; Scr: Serum creatinine.

rate is clearly evident. According to the data of Jalan *et al*[1], the in-hospital mortality for ACLF ranged from 43% to 88%, and the intensive care unit (ICU) mortality ranged from 37% to 89%. Completion of LDLT or DDLT for ACLF showed encouraging post-transplant prognoses[5,11,24]. The King's College Hospital criteria are the most widely applied selection criteria for LT in acute liver failure with a high prognostic value, and it was also recommended for ACLF by APASL; however, the predictive value in ACLF requires

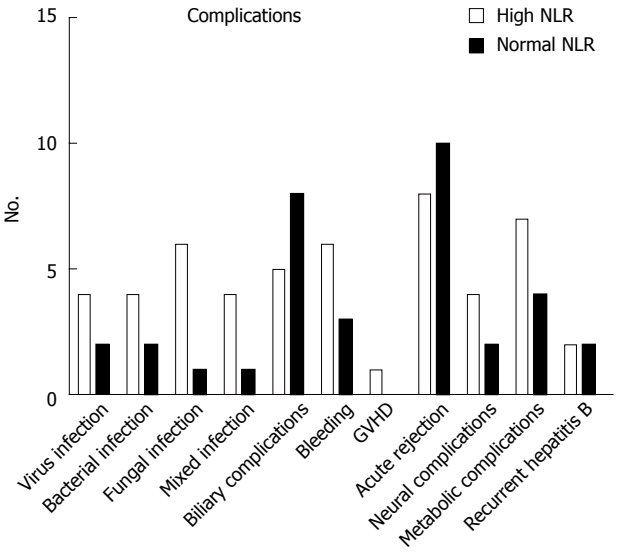


Figure 4 Incidence of postoperative complications in patients and the relationship between complications and neutrophil-lymphocyte ratio. NLR: Neutrophil-lymphocyte ratio; GVHD: Graft-*vs*-host disease.

further validation[25,26]. To facilitate graft allocation, it is important to establish clear selection criteria to define which candidates would most benefit from LT.

The imbalanced expression of both anti-inflammatory and pro-inflammatory cytokines contributes to the immunopathogenesis of ACLF[27-29]. Sen *et al*[28] described that multiple pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-2, IL-6, and IL-8, were elevated in ACLF patients. Zou *et al*[29] revealed that interferon (INF)-γ, TNF-α and IL-10 were markedly up-regulated in ACLF when compared with chronic hepatitis B patients and normal controls. As a marker of immune disorders, patients with high NLRs presented worse clinical conditions before LT in our study. Furthermore, we found that patients with normal NLRs presented better survival outcomes when compared with patients that had elevated NLRs. However, other inflammatory markers did not show predictive effects. This study is the first to identify a relationship between increased NLR and poor outcomes of patients with ACLF after LT. The 5-year survival rate reached 92.5% in LT patients with normal NLRs, which validated the use of NLR as an indicator for selecting LT candidates. Although 4.6 was the optimal cut-off value in our study, the cut-off value of NLR has varied in previously reported articles; however, as expected, a greater value correlates with a worse prognosis. Therefore, the results and the optimal value of the NLR need to be further confirmed through prospective studies with larger sample sizes.

The precise mechanism underlying how the NLR affects OS remains unclear. We found that the main cause of death was sepsis/multiple organ failure. Of the 25 subjects with high NLRs who died, 18 (72%) showed sepsis/multiple organ failure. Furthermore, elevated NLRs were associated with infectious complications. A total of 18 patients (21.69%) presented with infectious complications postoperatively in the high NLR group; however, only 6 (8.57%) cases presented with this complication in the normal NLR group (P = 0.026). These results provide a possible explanation for high NLR resulting in a worse prognosis. Furthermore, previous studies revealed that SIRS and immunological dissonance were associated with multiple organ dysfunction syndrome and secondary infectious complications[30]. In our study, the majority of study subjects with high NLRs also possessed lower lymphocyte counts and higher neutrophil counts compared with normal NLR patients. Therefore, marked neutrophilia and lymphocytopenia were considered to be physiological responses of the immune system to various stressful events, and NLR can express the severity of affliction. Zahorec[16] suggested that NLR can be routinely applied to clinical ICU practice as an additional index for infection. Lymphocytes have an important role in the host immune system, and a lymphocytopenia-impaired immune response to pathogens showed a positive correlation with bacteremia[16,31,32]. However, patients with acute or chronic hepatitis commonly presented with intestinal endotoxemia[33,34]. Several reports have disclosed that endotoxins significantly decrease the phagocytic capacity of neutrophils and induce a functionally heterogeneous neutrophil compartment that increases the susceptibility to infection[35]. In addition, Yang *et al*[36] demonstrated that neutrophils unexpectedly inhibited protective immune responses in fatal bacterial infection-induced toxic shock. These data explain why high NLR patients with pro-inflammatory milieu are prone to infection.

A high NLR is currently regarded as an available indicator of SIRS because it closely correlates with the presence of system inflammation[16]. SIRS is a common insult that precipitates liver dysfunction in a patient with previously compensated liver diseases. Moreover, the mortality rates of patients with cirrhosis or acute liver failure are higher with the presentation of SIRS[37-39]. SIRS is an earlier stage of multiple organ dysfunction and represents serious immune response dysfunction; thus, the presence of SIRS promotes postoperative infection and negative survival outcomes. The factors affecting the NLR consisted of underlying liver diseases, infection and steroidal drugs. Patients with these factors were excluded from the study. Therefore, we did not assess the relationship between NLR and SIRS in this study due to the absence of complete white blood cell counts.

In addition to the elevated NLR, high Scr was also an independent adverse factor for prognosis in ACLF patients after LT. Of the 24 patients with high Scr levels, 13 (54.2%) died. Moreover, liver recipients with high NLRs and increased Scr presented the worst survival outcomes. The 1-, 3- and 5- year survival rates were 60.0%, 60.0% and 50.0%, respectively. Although there was poor sensitivity for Scr to determine the extent of renal dysfunction[40],

the results revealed that markers of pre-transplant extrahepatic organ dysfunction can affect LT prognosis. Thus, these markers combined with NLR would provide effective predictive value.

Notably, there were several inevitable limitations of this study. First, only 24 (15.7%) liver recipients showed high Scr levels. Further analyses and larger sample sizes are needed. In addition, lymphocyte and neutrophil subsets were not routinely measured before LT. Hence, basic contributing mechanisms require further assessment.

In summary, high NLR corresponded to the severity of pre-transplant chronic liver diseases and immune disorders, and it showed powerful predictive value compared with general inflammatory markers. ACLF patients with high NLRs presented poorer OS and GS after LT. Neutrophil paralysis and lymphocytepenia promote infections that may result in poorer outcomes in ACLF patients with high NLRs following LT.

COMMENTS

Background

Liver transplantation (LT) is an optimal choice for patients with acute on chronic liver failure (ACLF), with reported survival rates of around 80%. The criteria of LT for ACLF are according to acute liver failure, and about 20% of liver recipients still have poor survival outcomes. In China, because of a great many patients with hepatitis B, liver donation is far away from filling in the need of liver transplantation. Therefore, improving the prognosis of LT is a hot issue. Recently published data revealed that immune response played an important role in progression of ACLF. Thus, finding a precise marker of immune response that is correlated with outcomes of LT potentially improves the criteria of LT for ACLF.

Research frontiers

Neutrophil amplification and lymphocytopenia are physiological responses to adverse stressful events, and a high neutrophil-lymphocyte ratio (NLR) is a new precise marker of inflammation. An elevated NLR inversely correlates with the overall and cancer-specific survival rates of various malignancies and the prognoses of non-tumorous diseases. In addition, researchers explored the feasibility of expanding the LT pool for hepatic carcinoma using the NLR.

Innovations and breakthroughs

The area under the receiver operating characteristic curve for NLR was 0.736 and an NLR value of 4.6 presented a sensitivity of 76.7% and a specificity of 65.9%. Using a Kaplan-Meier curve analysis and univariate and multivariate Cox regression models, we defined that a high NLR was a significant predictor of poor outcomes for LT. The 1-, 3-, and 5-year overall survival rates were 94.3%, 92.5% and 92.5%, respectively, in the normal NLR group and 74.7%, 71.8% and 69.8%, respectively, in patients with high NLR ($P < 0.001$).

Applications

This study showed that liver recipients with high NLRs had a tendency for infection and presented poorer survival outcomes after LT. These results implied that up-regulated NLR could be used as an indicator of antibiotic prophylaxis and improve the criteria of LT for ACLF to scientifically allocate the donor livers or expand the LT pool.

Terminology

NLR means neutrophil-lymphocyte ratio, which is a new marker of immune response.

Peer-review

This is a nice study. Results would have clinical relevance if alternative treatments other than LT were offered to high NLR patients. Furthermore, the commonly assessed marker creatinine is equally predictive.

REFERENCES

1 **Jalan R**, Gines P, Olson JC, Mookerjee RP, Moreau R, Garcia-Tsao G, Arroyo V, Kamath PS. Acute-on chronic liver failure. *J Hepatol* 2012; **57**: 1336-1348 [PMID: 22750750 DOI: 10.1016/j.jhep.2012.06.026]

2 **Zhang Z**, Zou ZS, Fu JL, Cai L, Jin L, Liu YJ, Wang FS. Severe dendritic cell perturbation is actively involved in the pathogenesis of acute-on-chronic hepatitis B liver failure. *J Hepatol* 2008; **49**: 396-406 [PMID: 18644645 DOI: 10.1016/j.jhep.2008.05.017]

3 **Sarin SK**, Kumar A, Almeida JA, Chawla YK, Fan ST, Garg H, de Silva HJ, Hamid SS, Jalan R, Komolmit P, Lau GK, Liu Q, Madan K, Mohamed R, Ning Q, Rahman S, Rastogi A, Riordan SM, Sakhuja P, Samuel D, Shah S, Sharma BC, Sharma P, Takikawa Y, Thapa BR, Wai CT, Yuen MF. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). *Hepatol Int* 2009; **3**: 269-282 [PMID: 19669378 DOI: 10.1007/s12072-008-9106-x]

4 **Stärkel P**, Horsmans Y, Geubel A, Ciccarelli O, Goubau P, Rahier J, Lerut J. Favorable outcome of orthotopic liver transplantation in a patient with subacute liver failure due to the emergence of a hepatitis B YMDD escape mutant virus. *J Hepatol* 2001; **35**: 679-681 [PMID: 11690717 DOI: 10.1016/S0168-8278(01)00178-7]

5 **Liu CL**, Fan ST, Lo CM, Wei WI, Yong BH, Lai CL, Wong J. Live-donor liver transplantation for acute-on-chronic hepatitis B liver failure. *Transplantation* 2003; **76**: 1174-1179 [PMID: 14578749 DOI: 10.1097/01.TP.0000087341.88471.E5]

6 **Cholongitas E**, Senzolo M, Patch D, Kwong K, Nikolopoulou V, Leandro G, Shaw S, Burroughs AK. Risk factors, sequential organ failure assessment and model for end-stage liver disease scores for predicting short term mortality in cirrhotic patients admitted to intensive care unit. *Aliment Pharmacol Ther* 2006; **23**: 883-893 [PMID: 16573791 DOI: 10.1111/j.1365-2036.2006.02842.x]

7 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]

8 **Pugh RN**, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913 DOI: 10.1002/bjs.1800600817]

9 **Knaus WA**, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818-829 [PMID: 3928249 DOI: 10.1097/00003246-198510000-00009]

10 **Vincent JL**, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996; **22**: 707-710 [PMID: 8844239 DOI: 10.1007/BF01709751]

11 **Duan BW**, Lu SC, Wang ML, Liu JN, Chi P, Lai W, Wu JS, Guo QL, Lin DD, Liu Y, Zeng DB, Li CY, Meng QH, Ding HG, Chen XY, Liao HY, Ma LQ, Chen Y, Zhang J, Xiang HP, Duan ZP, Li N. Liver transplantation in acute-on-chronic liver failure patients with high model for end-stage liver disease (MELD) scores: a single center experience of 100 consecutive cases. *J Surg Res* 2013; **183**: 936-943 [PMID: 23558257 DOI: 10.1016/j.jss.2013.03.008]

12 **Jalan R**, Williams R. Acute-on-chronic liver failure: pathophysiological basis of therapeutic options. *Blood Purif* 2002; **20**: 252-261 [PMID: 11867872 DOI: 10.1159/000047017]

13 **Mookerjee RP**, Sen S, Davies NA, Hodges SJ, Williams R, Jalan R. Tumour necrosis factor alpha is an important mediator of portal and systemic haemodynamic derangements in alcoholic hepatitis. *Gut* 2003; **52**: 1182-1187 [PMID: 12865279 DOI: 10.1136/gut.52.8.1182]

14 **Thabut D**, Massard J, Gangloff A, Carbonell N, Francoz C, Nguyen-Khac E, Duhamel C, Lebrec D, Poynard T, Moreau R. Model for end-stage liver disease score and systemic inflammatory response are major prognostic factors in patients with cirrhosis and acute functional renal failure. *Hepatology* 2007; **46**: 1872-1882

[PMID: 17972337 DOI: 10.1002/hep.21920]
15 American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; **20**: 864-874 [PMID: 1597042]
16 **Zahorec R**. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy* 2001; **102**: 5-14 [PMID: 11723675]
17 **Kayadibi H**, Sertoglu E, Uyanik M, Tapan S. Neutrophil-lymphocyte ratio is useful for the prognosis of patients with hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 9631-9632 [PMID: 25071363 DOI: 10.3748/wjg.v20.i28.9631]
18 **Li X**, Chen ZH, Ma XK, Chen J, Wu DH, Lin Q, Dong M, Wei L, Wang TT, Ruan DY, Lin ZX, Xing YF, Deng Y, Wu XY, Wen JY. Neutrophil-to-lymphocyte ratio acts as a prognostic factor for patients with advanced hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 11057-11063 [PMID: 25095975 DOI: 10.1007/s13277-014-2360-8]
19 **Varol E**, Bas HA, Aksoy F, Ari H, Ozaydin M. Relationship Between Neutrophil-Lymphocyte Ratio and Isolated Low High-Density Lipoprotein Cholesterol. *Angiology* 2013; **65**: 630-633 [PMID: 23921506 DOI: 10.1177/0003319713497992]
20 **Biyik M**, Ucar R, Solak Y, Gungor G, Polat I, Gaipov A, Cakir OO, Ataseven H, Demir A, Turk S, Polat H. Blood neutrophil-to-lymphocyte ratio independently predicts survival in patients with liver cirrhosis. *Eur J Gastroenterol Hepatol* 2013; **25**: 435-441 [PMID: 23249602 DOI: 10.1097/MEG.0b013e32835c2af3]
21 **Gibson PH**, Croal BL, Cuthbertson BH, Small GR, Ifezulike AI, Gibson G, Jeffrey RR, Buchan KG, El-Shafei H, Hillis GS. Preoperative neutrophil-lymphocyte ratio and outcome from coronary artery bypass grafting. *Am Heart J* 2007; **154**: 995-1002 [PMID: 17967611 DOI: 10.1016/j.ahj.2007.06.043]
22 **Motomura T**, Shirabe K, Mano Y, Muto J, Toshima T, Umemoto Y, Fukuhara T, Uchiyama H, Ikegami T, Yoshizumi T, Soejima Y, Maehara Y. Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. *J Hepatol* 2013; **58**: 58-64 [PMID: 22925812 DOI: 10.1016/j.jhep.2012.08.017]
23 **Limaye AR**, Clark V, Soldevila-Pico C, Morelli G, Suman A, Firpi R, Nelson DR, Cabrera R. Neutrophil-lymphocyte ratio predicts overall and recurrence-free survival after liver transplantation for hepatocellular carcinoma. *Hepatol Res* 2013; **43**: 757-764 [PMID: 23193965 DOI: 10.1111/hepr.12019]
24 **Chan AC**, Fan ST, Lo CM, Liu CL, Chan SC, Ng KK, Yong BH, Chiu A, Lam BK. Liver transplantation for acute-on-chronic liver failure. *Hepatol Int* 2009; **3**: 571-581 [PMID: 19680733 DOI: 10.1007/s12072-009-9148-8]
25 **Polson J**, Lee WM. AASLD position paper: the management of acute liver failure. *Hepatology* 2005; **41**: 1179-1197 [PMID: 15841455 DOI: 10.1002/hep.20703]
26 **Cholongitas E**, Theocharidou E, Vasianopoulou P, Betrosian A, Shaw S, Patch D, O'Beirne J, Agarwal B, Burroughs AK. Comparison of the sequential organ failure assessment score with the King's College Hospital criteria and the model for end-stage liver disease score for the prognosis of acetaminophen-induced acute liver failure. *Liver Transpl* 2012; **18**: 405-412 [PMID: 22213443 DOI: 10.1002/lt.23370]
27 **Wasmuth HE**, Kunz D, Yagmur E, Timmer-Stranghöner A, Vidacek D, Siewert E, Bach J, Geier A, Purucker EA, Gressner AM, Matern S, Lammert F. Patients with acute on chronic liver failure display "sepsis-like" immune paralysis. *J Hepatol* 2005; **42**: 195-201 [PMID: 15664244 DOI: 10.1016/j.jhep.2004.10.019]
28 **Sen S**, Davies NA, Mookerjee RP, Cheshire LM, Hodges SJ, Williams R, Jalan R. Pathophysiological effects of albumin dialysis in acute-on-chronic liver failure: a randomized controlled study. *Liver Transpl* 2004; **10**: 1109-1119 [PMID: 15350001 DOI: 10.1002/lt.20236]
29 **Zou Z**, Li B, Xu D, Zhang Z, Zhao JM, Zhou G, Sun Y, Huang L, Fu J, Yang Y, Jin L, Zhang W, Zhao J, Sun Y, Xin S, Wang FS. Imbalanced intrahepatic cytokine expression of interferon-gamma, tumor necrosis factor-alpha, and interleukin-10 in patients with acute-on-chronic liver failure associated with hepatitis B virus infection. *J Clin Gastroenterol* 2009; **43**: 182-190 [PMID: 18633332 DOI: 10.1097/MCG.0b013e3181624464]
30 **Bone RC**. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS) *Ann Intern Med* 1996; **125**: 680-687 [PMID: 8849154 DOI: 10.7326/0003-4819-125-8-199610150-00009]
31 **Hawkins CA**, Collignon P, Adams DN, Bowden FJ, Cook MC. Profound lymphopenia and bacteraemia. *Intern Med J* 2006; **36**: 385-388 [PMID: 16732866 DOI: 10.1111/j.1445-5994.2006.01076.x]
32 **de Jager CP**, van Wijk PT, Mathoera RB, de Jongh-Leuvenink J, van der Poll T, Wever PC. Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Crit Care* 2010; **14**: R192 [PMID: 21034463 DOI: 10.1186/cc9309]
33 **Uchihara M**, Izumi N, Sato C, Marumo F. Clinical significance of elevated plasma endothelin concentration in patients with cirrhosis. *Hepatology* 1992; **16**: 95-99 [PMID: 1535610 DOI: 10.1002/hep.1840160117]
34 **Han DW**. Intestinal endotoxemia as a pathogenetic mechanism in liver failure. *World J Gastroenterol* 2002; **8**: 961-965 [PMID: 12439906]
35 **Pillay J**, Ramakers BP, Kamp VM, Loi AL, Lam SW, Hietbrink F, Leenen LP, Tool AT, Pickkers P, Koenderman L. Functional heterogeneity and differential priming of circulating neutrophils in human experimental endotoxemia. *J Leukoc Biol* 2010; **88**: 211-220 [PMID: 20400675 DOI: 10.1189/jlb.1209793]
36 **Yang Q**, Ghose P, Ismail N. Neutrophils mediate immunopathology and negatively regulate protective immune responses during fatal bacterial infection-induced toxic shock. *Infect Immun* 2013; **81**: 1751-1763 [PMID: 23478316 DOI: 10.1128/IAI.01409-12]
37 **Leithead JA**, Ferguson JW, Bates CM, Davidson JS, Lee A, Bathgate AJ, Hayes PC, Simpson KJ. The systemic inflammatory response syndrome is predictive of renal dysfunction in patients with non-paracetamol-induced acute liver failure. *Gut* 2009; **58**: 443-449 [PMID: 19001057 DOI: 10.1136/gut.2008.154120]
38 **Rolando N**, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. *Hepatology* 2000; **32**: 734-739 [PMID: 11003617 DOI: 10.1053/jhep.2000.17687]
39 **Cazzaniga M**, Dionigi E, Gobbo G, Fioretti A, Monti V, Salerno F. The systemic inflammatory response syndrome in cirrhotic patients: relationship with their in-hospital outcome. *J Hepatol* 2009; **51**: 475-482 [PMID: 19560225 DOI: 10.1016/j.jhep.2009.04.017]
40 **Tomlanovich S**, Golbetz H, Perlroth M, Stinson E, Myers BD. Limitations of creatinine in quantifying the severity of cyclosporine-induced chronic nephropathy. *Am J Kidney Dis* 1986; **8**: 332-337 [PMID: 3538857 DOI: 10.1016/S0272-6386(86)80107-X]

P- Reviewer: Boscá L, Sijens PE **S- Editor**: Ma YJ
L- Editor: Wang TQ **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3325

World J Gastroenterol 2015 March 21; 21(11): 3325-3329
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Lack of correlation between Treg quantification assays in inflammatory bowel disease patients

Gunnar Brandhorst, Darinka Todorova Petrova, Sebastian Weigand, Christoph Eberle, Nicolas von Ahsen, Jessica Schmitz, Frank Christian Schultze, Dirk Raddatz, Michael Karaus, Michael Oellerich, Philip D Walson

Gunnar Brandhorst, Darinka Todorova Petrova, Sebastian Weigand, Christoph Eberle, Nicolas von Ahsen, Department of Clinical Chemistry, University Medical Center, 37075 Goettingen, Germany
Darinka Todorova Petrova, Jessica Schmitz, Michael Oellerich, Philip D Walson, Department of Clinical Pharmacology, University Medical Center, 37075 Goettingen, Germany
Jessica Schmitz, Chronix Biomedical, 37073 Goettingen, Germany
Frank Christian Schultze, Dirk Raddatz, Clinics of Gastroenterology and Endocrinology, University Medical Center, 37075 Goettingen, Germany
Michael Karaus, Department of Internal Medicine, Evangelisches Krankenhaus Göttingen-Weende, 37075 Goettingen, Germany
Author contributions: Brandhorst G and Petrova DT contributed equally to this work; Brandhorst G, von Ahsen N, Raddatz D, Karaus M, Oellerich M and Walson PD designed the research and supervised the experiments; Weigand S and Eberle C performed the research; Brandhorst G, Petrova DT, Schmitz J and Schultze FC analyzed the data; and Brandhorst G, Petrova DT and Walson PD wrote the paper; all authors read and approved the final manuscript.
Supported by grants from Medical Faculty of the University of Goettingen, Germany, the German Research Foundation and the Open Access Publication Funds of the Goettingen University.

Correspondence to: Darinka Todorova Petrova, PhD, Department of Clinical Chemistry, University Medical Center, Robert-Koch-Str. 40, 37075 Goettingen, Germany. darinka.petrova@med.uni-goettingen.de
Telephone: +49-551-398562
Fax: +49-551-398551
Received: September 6, 2014
Peer-review started: September 7, 2014
First decision: October 14, 2014
Revised: November 3, 2014
Accepted: December 8, 2014
Article in press: December 8, 2014
Published online: March 21, 2015

Abstract

AIM: To compare the number of regulatory T-cells (Tregs) measured by flow cytometry with those obtained using a real-time quantitative PCR (qPCR) method in patients suffering from inflammatory bowel disease (IBD).

METHODS: Tregs percentages obtained by both flow cytometry and qPCR methods in 35 adult IBD patients, 18 out of them with Crohn´s disease (CD) and 17 with ulcerative colitis (UC) were compared to each other as well as to scores on two IBD activity questionnaires using the Harvey Bradshaw Index (HBI) for CD patients and the Simple Colitis Clinical Activity Index (SCCAI) for UC patients. The Treg percentages by flow cytometry were defined as $CD4^{+}CD25^{high}CD127^{low}FOXP3^{+}$ cells in peripheral blood mononuclear cells, whereas the Treg percentages by qPCR method were determined as FOXP3 promoter demethylation in genomic DNA.

RESULTS: We found an average of 1.56% ± 0.78% Tregs by using flow cytometry, compared to 1.07% ± 0.53% Tregs by using qPCR in adult IBD patients. There were no significant correlations between either the percentages of Tregs measured by flow cytometry or qPCR and the HBI or SCCAI questionnaire scores in CD or UC patients, respectively. In addition, there was no correlation between Treg percentages measured by qPCR and those measured by flow cytometry (r = -0.06, P = 0.73; Spearman Rho). These data suggest that, either Treg-related immune function or the clinical scores in these IBD patients did not accurately reflect actual disease activity. Until the cause(s) for these differences are more clearly defined, the results

suggest caution in interpreting studies of Tregs in various inflammatory disorders.

CONCLUSION: The two methods did not produce equivalent measures of the percentage of total Tregs in the IBD patients studied which is consistent with the conclusion that Tregs subtypes are not equally detected by these two assays.

Key words: Regulatory T-cells; Inflammatory bowel disease; Crohn´s disease; Ulcerative colitis; Method comparison

Core tip: In our study neither regulatory T-cells (Tregs) percentages measured by flow cytometry defined as $CD4^+CD25^{high}CD127^{low}FOXP3^+$ cells in peripheral blood mononuclear cells or by real-time PCR measured as forkhead box P3 promoter demethylation in genomic DNA correlated with self-reported inflammatory bowel disease activity. This suggests that either Treg-related immune function or the clinical scores did not accurately reflect actual disease activity. We conclude that natural and induced Tregs are not equally detected by the assays applied.

Brandhorst G, Petrova DT, Weigand S, Eberle C, von Ahsen N, Schmitz J, Schultze FC, Raddatz D, Karaus M, Oellerich M, Walson PD. Lack of correlation between Treg quantification assays in inflammatory bowel disease patients. *World J Gastroenterol* 2015; 21(11): 3325-3329 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3325.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3325

INTRODUCTION

The immune system has been postulated to be involved in the pathogenesis of inflammatory bowel disease (IBD). Either IBD associated immune dysfunction leads to excessive responses to normal intestinal microflora or changes in the intestinal microflora or epithelial barrier function somehow lead to exaggerated or abnormal reactions by the mucosal immune system[1]. Regardless of whether immune changes are the cause of or the result of IBD, the percentage of regulatory T-cells (Tregs) has been used as a marker of immune function in IBD patients[2] and some authors have suggested that Treg imbalances correlate with IBD activity[3].

Expression of the forkhead box P3 (*FOXP3*) gene has been claimed to be a specific marker of Tregs[4] and a number of both research and commercial FOXP3 based methods have been used to assess Treg percentages in whole blood. However, contrary to what has been reported to be true in murine models[5] and in human cord blood[6] a number of Treg subtypes have been identified in human whole blood[7,8]. The two main subpopulations in humans seem to be thymus-derived natural Tregs (nTregs) and peripherally generated induced Tregs (iTregs). While many different biomarkers have been proposed to differentiate between nTregs and iTregs[9] these assays vary in their ability to correctly identify these two subpopulations[10]. To our knowledge Treg percentages measured by different methods in the same IBD patients have not been adequately assessed.

The purpose of the studies reported here was to compare the Treg percentages measured by flow cytometry to those obtained with a methylation sensitive, real-time PCR method specific for detection of the Treg-specific demethylated region (TSDR) in the same adult IBD patients and to compare the results of both methods to self-reported IBD activity assessed by approved questionnaires.

MATERIALS AND METHODS

This work was part of a larger study approved by the institutional review board and designed to evaluate whether a number of laboratory measures of immune function could be used as surrogate markers of disease activity in adults with either Crohn´s disease (CD) or ulcerative colitis (UC). Written informed consent was obtained from all patients before enrolment[11]. Treg percentages obtained by both flow cytometry and quantitative PCR (qPCR) in 35 adult IBD patients (18 with CD and 17 with UC) were compared to each other as well as to results of two IBD self-assessing activity questionnaires according to the Harvey Bradshaw Index (HBI) for CD patients and the Simple Colitis Clinical Activity Index (SCCAI) for UC patients. Patients were not preselected by their disease activity.

Flow cytometry analysis was performed as described previously[12]. The Treg percentages were defined as $CD4^+CD25^{high}CD127^{low}FOXP3^+$ cells. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using the Lymphoprep®-protocol (Axis-Shield; Oslo, Norway) and stored at -80 ℃ in 10% (v/v) dimethylsulfoxide and 90% (v/v) bovine serum albumin until analysis. In order to control the pre-analytical steps, it was verified that the freezing and thawing of PBMCs did not affect the flow cytometry results in comparison to fresh samples. PBMCs were incubated with 20 μL anti-human CD4-FITC (Catalogue number # 555346, all conjugates used by Becton Dickinson Pharmingen, Heidelberg, Germany), 5 μL anti-human CD25-PE-Cy7 (# 560920) and 20 μL anti-human CD127-Alexa Fluor 647 (# 558558). After cell fixation and membrane permeabilization according to the manufacturer´s protocol, 20 μL FOXP3-PE (clone 259D/C7) and corresponding mouse isotype control antibodies were added. The quantification of regulatory T cells was done using an 8-color flow cytometer (FACS Canto Ⅱ, Becton Dickinson; Germany). $CD4^+CD25^{high}CD127^{low}$ T-cells were gated out

Table 1 Regulatory T-cells percentages obtained by both flow cytometry and real-time PCR methods in 35 patients with inflammatory bowel disease

Subjects	*n*	Age (yr)	Flow cytometry (%)	qPCR (%)	*r*	*P* value
		Average ± SD	Average ± SD	Average ± SD		
Patients with Crohn´s disease	18	38 ± 10	1.62 ± 0.62	1.05 ± 0.56	-0.10	0.68
Female	10	39 ± 11	1.44 ± 0.66	1.00 ± 0.70	-0.18	0.61
Male	8	37 ± 9	1.83 ± 0.53	1.12 ± 0.35	-0.30	0.47
Patients with ulcerative colitis	17	43 ± 17	1.50 ± 0.93	1.10 ± 0.52	-0.05	0.85
Female	8	45 ± 14	1.39 ± 0.63	0.99 ± 0.45	-0.06	0.89
Male	9	41 ± 20	1.60 ± 1.17	1.19 ± 0.58	-0.03	0.93
Current drug therapy						
Infliximab	4	46 ± 11	1.65 ± 0.97	0.98 ± 0.34	0.20	0.80
Azathiaprine	14	41 ± 18	1.57 ± 0.81	1.07 ± 0.60	-0.21	0.48
Systemic corticosteroids	9	39 ± 12	1.73 ± 0.98	0.91 ± 0.40	-0.06	0.88

Data stratification: patients with Crohn´s disease and ulcerative colitis, as well as applied drug therapy. Correlation coefficients (*r*) and respective *P* values were calculated according to Spearman Rho.

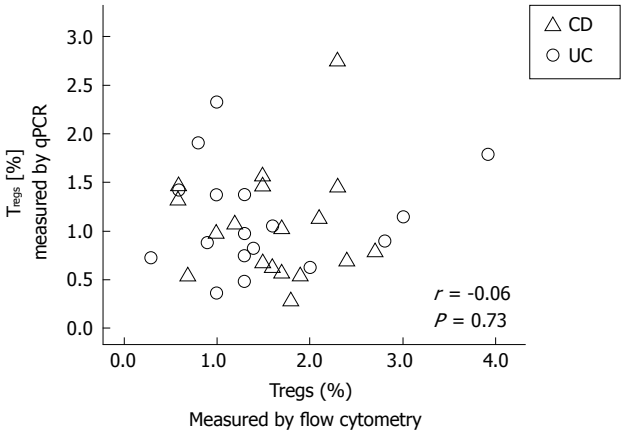


Figure 1 Lack of correlation between the percentages of regulatory T-cells measured by flow cytometry and qPCR in 35 patients with inflammatory bowel disease (*r* = -0.06, *P* = 0.73; Spearman Rho). CD: Crohn´s disease; UC: Ulcerative colitis.

of the lymphocyte region (forward/sideward scatter). From these, the intersection between $CD4^+CD25^{high}$ and $CD25^{high}CD127^{low}$ gates was formed followed by the identification of Treg cells according to their level of FOXP3 expression. Data were analyzed with BD FACS Diva software (Becton Dickinson) to identify the percentage of $CD4^+CD25^{high}CD127^{low}FOXP3^+$ cells.

As a second method of assessing the Treg percentage the FOXP3 promoter demethylation signature was determined using methylation-sensitive real-time PCR as previously described[6,13,14] and adapted in our laboratory[15]. Briefly, genomic DNA was isolated by NucleoSpin® columns (Macherey-Nagel; Düren, Germany) and treated with bisulfite for conversion of unmethylated cytosine into uracil (EZ DNA Methylation Gold™, Zymo Research, Irvine, California). After quantification in triplicates of methylated and unmethylated FOXP3-specific PCR products by use of real-time PCR and methylation-specific primers on a LightCycler® 480 (Roche Diagnostics; Mannheim, Germany), the FOXP3 demethylation status was calculated as a ratio. Due to X-chromosomal inactivation of the FOXP3 gene the results for female patients were corrected by a factor of two.

RESULTS

We found an average of 1.56% Tregs (SD: 0.78%) by using flow cytometry, compared to 1.07% Tregs (SD: 0.53%) by using qPCR in these adult IBD patients (Table 1). There were no statistically significant correlations between either the flow cytometry or qPCR measured percentages of Tregs and the HBI or SCCAI questionnaire scores in CD or UC patients, respectively. There were no significant differences between these correlations for either male *vs* female patients, the presence or absence of remission, or the drug therapies currently used (Table 1). In addition, there was no significant correlation between Treg percentages measured by qPCR and those measured with the flow cytometry (r = -0.06, P = 0.73; Spearman Rho); (Figure 1).

DISCUSSION

These data suggest that, at least in this small cohort of IBD patients, either Treg-related immune function or the clinical scores did not accurately reflect actual disease activity. It is possible that either a different scoring system or measurement of tissue *e.g.*, from intestine biopsies rather than circulating Treg percentages would have been more predictive[3,16-18]. Interestingly, in a small study that included septic patients a weak correlation between flow cytometry and demethylation PCR methods could be demonstrated[19]. However, the lack of correlation between the two measures of Treg percentages is consistent with studies that suggest that FOXP3 activity is not confined to $CD4^+CD25^{high}CD127^{low}$ cells and can be expressed in non-Treg cells[18,20]. This is also consistent with reports that the flow cytometry methods that have been previously used to identify Tregs are incapable of separating induced from natural Tregs[17]. More recently Neuropilin 1 (Nrp1) expression has been proposed as a method capable of distinguishing between nTregs and iTregs[21]. Measuring

demethylation status at the FOXP3 locus using TSDR may also aid in the differentiation of Treg subtypes due to the different degree of methylation in nTregs and iTregs[10]. Thus TSDR demethylation assays would be expected to identify primarily nTregs which would explain why the two assays found different percentages of Tregs in these IBD patients. Differences can also be caused by analytical interference from drug therapy. For example basiliximab has been shown to interfere with the detection of CD25 in flow cytometry assays[22]. However, for tumour necrosis factor alpha antibodies like infliximab are unlikely to interfere due to their different mode of action.

In the present preliminary study a cohort selection effect might be a limiting factor. In addition to self-assessment scores the disease activity could be assessed using other methods including endoscopy and/or by more objective, cheaper and conventional biochemical markers for inflammation such as fecal calprotectin, C-reactive protein in serum, platelets, leukocytosis, IL-6, *etc.* Determination of Treg populations in peripheral blood would be an expensive routine measure of disease activity. Finally, Treg proportions in the blood may not really represent Treg proportions in the lamina propria. The determination of Treg subtypes in the inflamed mucosa might have more pathogenic relevance.

Until the cause(s) for these differences are more clearly defined, the results suggest caution in interpreting studies of Tregs in various inflammatory disorders.

In conclusion, in this study neither Treg percentages in whole blood measured by flow cytometry or qPCR correlated with self-reported disease activity. This suggests that either Treg-related immune function or the clinical scores did not accurately reflect actual disease activity. Additionally, the flow cytometry and qPCR methods did not produce equivalent measures of the percentage of Tregs in these 35 adult IBD patients which is consistent with the conclusion that Tregs subtypes are not equally detected by these two assays.

ACKNOWLEDGMENTS

The technical help of R. Andag, J. Engelmayer, S. Götze, and C. Scholz (all from the Department of Clinical Chemistry, Goettingen, Germany) is greatly appreciated. Furthermore, it was part of the Inaugural Dissertation for the doctoral degree for Sebastian Weigand.

COMMENTS

Background

The immune system has been postulated to be involved in the pathogenesis of inflammatory bowel disease (IBD).

Research frontiers

Regulatory T-cell (Treg) percentages measured by different methods in the same IBD patients have not been adequately assessed.

Innovations and breakthroughs

Either Treg-related immune function or the clinical scores in these IBD patients did not accurately reflect actual disease activity.

Applications

The lack of correlation between these two assays for quantification of Tregs suggests caution in interpreting studies of Tregs in various inflammatory disorders.

Terminology

Expression of the forkhead box P3 (*FOXP3*) gene has been claimed to be a specific marker of Tregs.

Peer-review

The authors clearly show that there is poor correlation between two different metods for measuring Tregs in perfiferal blood. Thus, studies on Tregs in various inflammatory disorders should be read with great caution.

REFERENCES

1 **Strober W**, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007; **117**: 514-521 [PMID: 17332878]

2 **Himmel ME**, Yao Y, Orban PC, Steiner TS, Levings MK. Regulatory T-cell therapy for inflammatory bowel disease: more questions than answers. *Immunology* 2012; **136**: 115-122 [PMID: 22348589 DOI: 10.1111/j.1365-2567.2012.03572.x]

3 **Maul J**, Zeitz M. Ulcerative colitis: immune function, tissue fibrosis and current therapeutic considerations. *Langenbecks Arch Surg* 2012; **397**: 1-10 [PMID: 21479621 DOI: 10.1007/s00423-011-0789-4]

4 **Dummer CD**, Carpio VN, Gonçalves LF, Manfro RC, Veronese FV. FOXP3+ regulatory T cells: from suppression of rejection to induction of renal allograft tolerance. *Transpl Immunol* 2012; **26**: 1-10 [PMID: 21939765 DOI: 10.1016/j.trim.2011.08.009]

5 **Brunkow ME**, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001; **27**: 68-73 [PMID: 11138001]

6 **Liu J**, Lluis A, Illi S, Layland L, Olek S, von Mutius E, Schaub B. T regulatory cells in cord blood--FOXP3 demethylation as reliable quantitative marker. *PLoS One* 2010; **5**: e13267 [PMID: 20967272 DOI: 10.1371/journal.pone.0013267]

7 **Baecher-Allan C**, Brown JA, Freeman GJ, Hafler DA. CD4+CD25high regulatory cells in human peripheral blood. *J Immunol* 2001; **167**: 1245-1253 [PMID: 11466340]

8 **Morgan ME**, van Bilsen JH, Bakker AM, Heemskerk B, Schilham MW, Hartgers FC, Elferink BG, van der Zanden L, de Vries RR, Huizinga TW, Ottenhoff TH, Toes RE. Expression of FOXP3 mRNA is not confined to CD4+CD25+ T regulatory cells in humans. *Hum Immunol* 2005; **66**: 13-20 [PMID: 15620457]

9 **Schmitt EG**, Williams CB. Generation and function of induced regulatory T cells. *Front Immunol* 2013; **4**: 152 [PMID: 23801990 DOI: 10.3389/fimmu.2013.00152]

10 **Lin X**, Chen M, Liu Y, Guo Z, He X, Brand D, Zheng SG. Advances in distinguishing natural from induced Foxp3(+) regulatory T cells. *Int J Clin Exp Pathol* 2013; **6**: 116-123 [PMID: 23329997]

11 **Brandhorst G**, Weigand S, Eberle C, Raddatz D, Karaus M, Oellerich M, Walson PD. CD4+ immune response as a potential biomarker of patient reported inflammatory bowel disease (IBD) activity. *Clin Chim Acta* 2013; **421**: 31-33 [PMID: 23485644 DOI: 10.1016/j.cca.2013.02.016]

12 **Wagner NM**, Brandhorst G, Czepluch F, Lankeit M, Eberle C, Herzberg S, Faustin V, Riggert J, Oellerich M, Hasenfuss G, Konstantinides S, Schäfer K. Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. *Obesity* (Silver Spring) 2013; **21**: 461-468 [PMID: 23592653 DOI: 10.1002/oby.20087]

13 **Liu W**, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, Gottlieb PA, Kapranov P, Gingeras TR, Fazekas de St Groth B, Clayberger C,

Soper DM, Ziegler SF, Bluestone JA. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *J Exp Med* 2006; **203**: 1701-1711 [PMID: 16818678]

14 **Wieczorek G**, Asemissen A, Model F, Turbachova I, Floess S, Liebenberg V, Baron U, Stauch D, Kotsch K, Pratschke J, Hamann A, Loddenkemper C, Stein H, Volk HD, Hoffmüller U, Grützkau A, Mustea A, Huehn J, Scheibenbogen C, Olek S. Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue. *Cancer Res* 2009; **69**: 599-608 [PMID: 19147574 DOI: 10.1158/0008-5472.CAN-08-2361]

15 **Schultze FC**, Andag R, Alwahsh SM, Toncheva D, Maslyankov S, Yaramov N, von Ahsen N, Brandhorst G, Walson PD, Oellerich M, Petrova DT. FoxP3 demethylation is increased in human colorectal cancer and rat cholangiocarcinoma tissue. *Clin Biochem* 2014; **47**: 201-205 [PMID: 24291052 DOI: 10.1016/j.clinbiochem.2013.11.013]

16 **Hölttä V**, Sipponen T, Westerholm-Ormio M, Salo HM, Kolho KL, Färkkilä M, Savilahti E, Vaarala O, Klemetti P. In Crohn's Disease, Anti-TNF-α Treatment Changes the Balance between Mucosal IL-17, FOXP3, and CD4 Cells. *ISRN Gastroenterol* 2012; **2012**: 505432 [PMID: 22778976 DOI: 10.5402/2012/505432]

17 **Shalev I**, Selzner N, Shyu W, Grant D, Levy G. Role of regulatory T cells in the promotion of transplant tolerance. *Liver Transpl* 2012; **18**: 761-770 [PMID: 22523007 DOI: 10.1002/lt.23458]

18 **Peterson RA**. Regulatory T-cells: diverse phenotypes integral to immune homeostasis and suppression. *Toxicol Pathol* 2012; **40**: 186-204 [PMID: 22222887 DOI: 10.1177/0192623311430693]

19 **Tatura R**, Zeschnigk M, Adamzik M, Probst-Kepper M, Buer J, Kehrmann J. Quantification of regulatory T cells in septic patients by real-time PCR-based methylation assay and flow cytometry. *PLoS One* 2012; **7**: e49962 [PMID: 23209626 DOI: 10.1371/journal.pone.0049962]

20 **Litjens NH**, Boer K, Betjes MG. Identification of circulating human antigen-reactive CD4+ FOXP3+ natural regulatory T cells. *J Immunol* 2012; **188**: 1083-1090 [PMID: 22190183 DOI: 10.4049/jimmunol.1101974]

21 **Bruder D**, Probst-Kepper M, Westendorf AM, Geffers R, Beissert S, Loser K, von Boehmer H, Buer J, Hansen W. Neuropilin-1: a surface marker of regulatory T cells. *Eur J Immunol* 2004; **34**: 623-630 [PMID: 14991591]

22 **Abadja F**, Alamartine E, Berthoux F, Mariat C, Genin C, Lambert C. Quantification of circulating regulatory T cells by flow cytometry in kidney transplant patients after basiliximab induction therapy. *Transplantation* 2010; **89**: 366-368 [PMID: 20145530 DOI: 10.1097/TP.0b013e3181bbbd67]

P- Reviewer: Sorrentino D, Tommasini A **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3330

World J Gastroenterol 2015 March 21; 21(11): 3330-3336
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Recurrent anal fistulae: Limited surgery supported by stem cells

Damian Garcia-Olmo, Hector Guadalajara, Ines Rubio-Perez, Maria Dolores Herreros, Paloma de-la-Quintana, Mariano Garcia-Arranz

Damian Garcia-Olmo, Hector Guadalajara, Maria Dolores Herreros, Mariano Garcia-Arranz, Hospital Fundación Jimenez Díaz, IIS-FJD, 28040 Madrid, Spain
Damian Garcia-Olmo, Maria Dolores Herreros, Colorectal Surgery Unit, University Hospital Fundación JiménezDíaz, 28040 Madrid, Spain
Ines Rubio-Perez, Paloma de-la-Quintana, Colorectal Surgery Unit, La Paz University Hospital, 28046 Madrid, Spain
Mariano Garcia-Arranz, Cell Therapy Laboratory, University Hospital Fundación Jiménez Díaz, 28040 Madrid, Spain
Author contributions: Garcia-Olmo D and Guadalajara H contributed equally to the design of the study, performed the surgical procedures, and acquired and analyzed all data, supervised the project, and wrote the first draft of the paper; Rubio-Perez I collaborated in the analysis and interpretation of data, critically revised the main text and content, and wrote the final version of the paper; Herreros MD collaborated in design and conception of the study and performed the surgical interventions; de-la-Quintana P contributed to the first acquisition of data from patients in the outpatient clinics and their analysis; Garcia-Arranz M provided cell resources and managed all regulatory and legal aspects related to the study, participating in design and conception, and contributed to the revision of contents related to cell behavior and physiology; all authors revised and approved the final version to be published.
Ethics approval: This work recruited patients under a Compassionate-use Program, which is legislated by the Royal Spanish Decree 1015/2009, 19th of July. We had to make an individual request for every patient to the Spanish Agency for Medicines and Health Products (AEMPS).
Informed consent: Consents were signed by the attending surgeons and the patients.
Conflict-of-interest: García-Olmo D and Garcia-Arranz M, have applied for two patents related with this study entitled "Identification and isolation of multipotent cells from non-osteochondral mesenchymal tissue" (WO 2006/057649) and "Use of adipose tissue-derived stromal stem cells in treating fistula" (WO 2006/136244). García-Olmo D is a member of the Advisory Board of Tigenix SAU. This manuscript has not been published nor has been presented as a podium/poster presentation in a scientific meeting.
Data sharing: Participants gave informed consent for sharing of patient data. The risk of identification is very low, as the research is committed to keeping the anonymity of the patients.

Correspondence to: Hector Guadalajara, MD, PhD, Hospital Fundación Jimenez Díaz, IIS-FJD, Avda, Reyes Católicos 2, 28040 Madrid, Spain. hector.guadalajara@fjd.es
Telephone: +34-91-5504822
Fax: +34-91-5504849
Received: October 2, 2014
Peer-review started: October 3, 2014
First decision: October 29, 2014
Revised: November 18, 2014
Accepted: January 16, 2015
Article in press: January 16, 2015
Published online: March 21, 2015

Abstract

AIM: To study the results of stem-cell therapy under a Compassionate-use Program for patients with recurrent anal fistulae.

METHODS: Under controlled circumstances, and approved by European and Spanish laws, a Compassionate-use Program allows the use of stem-cell therapy for patients with very complex anal fistulae. Candidates had previously undergone multiple surgical interventions that had failed to resolve the fistulae, and presented symptomatic recurrence. The intervention consisted of limited surgery (with closure of the internal opening), followed by local implant of stem cells in the fistula-

tract wall. Autologous expanded adipose-derived stem cells were the main cell type selected for implant. The first evaluation was performed on the 8th postoperative week; outcome was classified as response or partial response. Evaluation one year after the intervention confirmed if complete healing of the fistula was achieved.

RESULTS: Ten patients (8 male) with highly recurrent and complex fistulae were treated (mean age: 49 years, range: 28-76 years). Seven cases were non-Crohn's fistulae, and three were Crohn's-associated fistulae. Previous surgical attempts ranged from 3 to 12. Two patients presented with preoperative incontinence (Wexner scores of 12 and 13 points). After the intervention, six patients showed clinical response on the 8th postoperative week, with a complete cessation of suppuration from the fistula. Three patients presented a partial response, with an evident decrease in suppuration. A year later, six patients (60%) remained healed, with complete reepithelization of the external opening. Postoperative Wexner Scores were 0 in six cases. The two patients with previous incontinence improved their scores from 12 to 8 points and from 13 to 5 points. No adverse reactions or complications related to stem-cell therapy were reported during the study period.

CONCLUSION: Stem cells are safe and useful for treating anal fistulae. Healing can be achieved in severe cases, sparing fecal incontinence risk, and improving previous scoring.

Key words: Adipose-derived stem cells; Cell therapy; Compassionate use; Crohn's disease; Fistula-in-ano

Core tip: Our group has performed various clinical trials with adipose stem cells. Patients with very complex fistulae, multiple previous surgeries, and treatment failure are generally not able to enter these studies despite the benefit and "last chance" of cure. We present the results of a Compassionate-use Program, which enabled the application of stem-cell therapy to these patients, under strict regulations. Ten patients were treated, and after one year of follow-up, we conclude that adipose stem cells are effective and safe, and 60% of the patients achieved complete healing.

Garcia-Olmo D, Guadalajara H, Rubio-Perez I, Herreros MD, de-la-Quintana P, Garcia-Arranz M. Recurrent anal fistulae: Limited surgery supported by stem cells. *World J Gastroenterol* 2015; 21(11): 3330-3336 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3330.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3330

INTRODUCTION

A limited surgical treatment in recurrent perianal fistulae often results in new recurrence, whereas there is a high risk of fecal incontinence if an extensive surgical treatment is performed[1-3]. The use of stem cells to treat complex fistulae is a promising area of research[4,5], for they may help to regenerate damaged perianal tissue. Especially in Crohn's disease, the presence of these cells could favor healing through anti-inflammatory and immunomodulatory effects[6-9]. Various randomized controlled trials using stem cells for the treatment of anal fistulae have already been conducted, and all of them show an excellent safety profile. Nevertheless, the real efficacy is currently difficutl to assess[5]. A recent Spanish study revealed that the mean annual global cost of conventional treatments for patients with Crohn's disease and perianal fistulae is > € 8000/year[10].

According to current regulatory issues at the time (2002), our team started a clinical trial process in order to test the ability of adipose-derived stem cells (ASC) to improve healing in complex perianal fistulae, including those associated with Crohn's disease. The chosen cell source was adipose tissue because the harvesting process for ASC following liposuction was simple and could be performed in our on-site laboratory[11]. To the date, we have finished a complete clinical trial process: a pilot study[12], and Phase Ⅱ[13] and Phase Ⅲ clinical trials[14]. Although a complex perianal fistula is the worst scenario, we observed satisfactory healing in our patients, without associated fecal incontinence. We are currently developing novel clinical trials directed to test different strategies in order to improve our results[5].

Some of the patients with multi-recurrent anal fistulae did not meet the strict eligibility criteria of clinical trials or were scheduled in control groups. The only option to treat these fistulae with stem cells was by "compassionate use". To achieve this, the European regulatory laws and the Spanish Medicine Agency guidelines were followed in order to obtain regulatory permissions. Under the Compassionate-use Program, the surgical technique and the cells' lineage is tailored for each patient, reinforcing the possibilities of cure, as opposed to the clinical trial setting. In these special cases, we performed minimal surgical maneuvers (limited surgery) directed to the conditioning of the surgical field, followed by implant of cells, in order to improve healing. This strategy enabled us to avoid anal sphincter injury and facilitated cell homing[6].

The aim of this paper is to report our experience in a clinical trial- complementary Compassionate-use Program, and discuss the possible clinical uses of stem cells in the future, focusing on the treatment of complex and recurrent perianal fistulae.

Table 1 Study data

ID	Sex	Age (yr)	Crohn's disease	Park's classification	Previous surgical attempts	Initial incontinence score (Wexner)	Surgical technique	Fibrin glue	Cells	Response 8th week	Incontinence score 8th week	Healing one year after
1	Male	58	No	Ⅲ	8	1	Flap + deep curettage	No	eASC	Yes	0	Yes
2	Male	43	No	Ⅱ	4	Unknown	IO closure + partial fistulectomy	Yes	eASC	Yes	4	No
3	Male	76	No	Ⅲ	5	0	IO closure + deep curettage	Yes	eASC	Partial	0	No
4	Male	57	No	Ⅲ	12	0	IO closure + deep curettage	Yes	eASC	Yes	0	Yes
5	Female	45	Yes	Ⅳ (multiple tracts)	6	12	IO closure + deep curettage	Yes	eASC	Partial	8	Yes
6	Male	35	Yes	Ⅱ (stenosis)	5	Unknown	IO closure + deep curettage	No	eASC	No	0	No
7	Male	40	Yes	Ⅲ	3	Unknown	IO closure + deep curettage	Yes	eASC	Yes	0	Yes
8	Male	59	No	Ⅱ	11	13	Flap + deep curettage	No	eASC Allog	Partial	5	No
9	Male	50	No	I	3	Unknown	Fistulotomy	Yes	eASC	Yes	0	Yes
10	Female	28	No	Ⅲ	5 + ileostomy	Not evaluable	Flap + partial fistulectomy	Yes	SVF	Yes	Not evaluable	Yes

IO: Internal opening; eASC: Expanded adult stem cells; Allog: Allogeneic; SVF: Stromal vascular fraction.

MATERIALS AND METHODS

We present an observational study, including 10 patients (8 male and 2 female) with recurrent perianal fistulae who had previously undergone at least three surgical interventions (maximum: 12, average: 6.2), with failure to resolve the fistula. The mean age of the patients was 49 years, and ranged from 28 to 76 years (Table 1). Seven patients presented complex non-Crohn's fistulae (four were Parks' type Ⅲ)[15] and three patients had Crohn's-associated perianal fistulae. Two of these patients complained of fecal incontinence at the moment of enrollment in this study, with a Wexner Score[16] > 10.

Autologous expanded adipose-derived stem cells (eASC) were selected in eight cases. Another case was treated using stromal vascular fraction (SVF) and in the last one, allogeneic adipose derived stem cells (Allo-eASC) were employed.

Both eASC (autologous and allogeneic) and SVF protocols were approved by the Ethics Committee of La Paz University Hospital in accordance with Spanish law, and by the Spanish Medical Agency according to European Medicine Agency (EMA) guidelines. All patients signed a detailed informed consent prior to any intervention, which included permission for data publication. Our institutional Committee on Human Experimentation (La Paz University Hospital) supervised all interventions performed. All ethical standards were in accord with those of the Helsinki Declaration (1975).

SVF from lipoaspirate

The liposuction was performed by a plastic surgeon and obtained 80-100 mL of fat. Phosphate buffered saline (PBS; Gibco of Thermo Fisher Scientific, Waltham, MA, United States) was used to wash the raw lipoaspirate and remove local anesthetics and cells. To extract the cellular fraction, the washed fat was digested with type Ⅰ collagenase (Gibco) at a final concentration of 0.075% in saline solution at 37 ℃ for 45 min.

Collagenase was inactivated with Dulbecco's modified Eagle's medium (DMEM; Gibco) containing fetal bovine serum (10% v/v). Cells in suspension were then centrifuged for 10 min (250 × *g*) and PBS was used again to wash the pellet. Centrifugation was repeated and afterwards the remaining erythrocytes were lysed by treating the suspension with ammonium chloride 160 mmol/L for 10 min at room temperature. To conclude the cellular extraction, a final wash and a filtration of the product through a 40 μm nylon mesh was performed.

Before injection, cells were suspended in sterile ringer-lactate solution (Griffols S.A., Barcelona, Spain). Morphologic determinations and phenotypic analyses were performed during product obtention. Data are partly published in García-Olmo *et al*[12]. The cell viability was always > 95% as determined by trypan-blue (Sigma-Aldrich, St Louis, MO, United States).

Autologous stem cell expansion and preparation for implantation

The released cellular fraction (SVF) was seeded at 2-3 × 10^4 cells/cm^2. Culture was carried out in DMEM with 10% fetal bovine serum and 1% ampicillin/streptomycin. No additional supplements were added. The atmospheric conditions were 37 ℃ with a 5% CO_2 atmosphere.

Cells were re-plated once 80% confluency was confirmed; their prior detachment was performed by trypsinization (trypsin-EDTA; Gibco). This cycle was repeated up to three times until the required number of cells for implantation was obtained. Due to logistics and personal issues, in two cases the cells were then frozen for preservation.

Morphologic determinations and phenotypic analyses were performed by flow cytometry during

expansion. Mycoplasma was detected using a Myco Alert Mycoplasma Detection Kit (Cambrex Corp., East Rutherford, NJ, United States). Data are partly published in García-Olmo *et al*[12].

At least one week before the surgical intervention was scheduled, expanded ASCs were prepared (washed with PBS, trypsinized, and centrifuged). Their viability was checked (> 95%), and finally the cells were resuspended in ringer-lactate solution (Griffols S.A.) at the desired volume and concentration (depending on the fistula) for their immediate use.

Allogeneic stem cell expansion and preparation for implantation

These cells were manufactured from donors by Tigenix SAU (Madrid, Spain) according to EMA permissions and regulations from healthy donors. The expansion protocol was similar to that of autologous procedures.

Treatment procedure and evaluation of healing

All surgical procedures were performed at La Paz University Hospital (Madrid), by the same team of surgeons, belonging to the Colorectal Surgery Unit.

In all cases, a deep curettage of the tracts was first performed, and then the ASC suspension (50%) was injected through a long, fine needle into the tract walls. The injections were superficial; not deeper than 2 mm. In seven cases, the fistulous tract was sealed with fibrin glue (Baxter Inc., Deerfield, IL, United States) containing a portion (1 mL) of the cells. The fibrin glue was used as a sealant to finalize the procedure in order to ensure cells remained in the fistulous area. The main reason for injecting a percentage of the ASC into the fibrin glue was to have a reservoir in the area so they could act for longer. However, recent investigations indicate that cells alone are sufficient for a therapeutic effect[17,18].

In very complex perianal fistulae, a partial fistulectomy was performed without removing intra-sphincteric tracts. The closure of the internal opening was achieved by stitches in six cases and by a mucosal advancement-flap in three cases. In the remaining patient, a fistulotomy was performed.

Treatment outcomes

A first evaluation was performed on the 8th post-toperative week, and a final evaluation was scheduled one year after the procedure (although patients attended the outpatient clinic in between, at variable intervals). Response was defined as a complete cessation of suppuration on week 8, despite not achieving a complete re-epithelization. Partial response was defined as an evident decrease in suppuration. Healing was defined as no suppuration from the external orifice, achieving a complete re-epithelization after one year of follow-up. These intervals of time for follow-up were selected following published data about the best periods for long-term fistula follow-up evaluation[19].

RESULTS

Of the ten highly recurrent perianal fistulae treated, 6/10 (60%) showed a clinical response 8 wk after the procedure, and 3/10 (30%) showed a partial response. One year later, 6/10 (60%) remained healed, with the external opening being completely epithelialized (Table 1). Postoperative results of Wexner Scores for Incontinence[16] were 0 in 6 cases. In the two patients with previous fecal incontinence, the scoring improved from 12 to 8 and from 13 to 5. No adverse reactions or complications related to stem-cell therapy were reported during the study period.

No statistical relationships have been established between the use of fibrin glue, surgical approach or cell lineage, due to the small number and variability of patients.

DISCUSSION

Following strict regulations, we treated ten patients with recurrent perianal fistulae, achieving a 90% response and a complete healing after one year in six cases, with no associated incontinence. Moreover, in two cases, previous incontinence was reduced. It is important to remark that the performance of this therapeutic strategy does not produce injury to the anal sphincter, because intrasphincteric-tract resection is not required. Although this is not a randomized controlled trial, the results are similar to those already published[12-14].

ASCs enlarge the therapeutic arsenal for anal fistulae, and can be considered an interesting tool for the regeneration/repair of wounds or chronically damaged tissues. The specific mechanism of action of ASCs is still under study, but it has been widely demonstrated that these cells improve healing[6]. Two different biologic effects are responsible for this healing effect: proliferation and differentiation on the one hand, and immune regulation and local suppression of inflammation on the other[6].

According to the EMA, ASC treatments in the EU should be administered only under clinical trials or other controlled conditions, such as Compassionate-use Programs. It is important to remark that all clinical uses of stem cells outside of these regulatory conditions are considered illegal. This is clearly stated in the law RD 1015/2009. In this way, in February 2011, the EMA published a report on stem cell-based medicinal products. It expressed concerns about the unregulated use of medicinal products containing ASCs.

In this context, one of the limitations of our study is the small number of patients included. We believed that a limited surgical treatment supported by ASC could be beneficial for these patients; but only those that did not meet the inclusion criteria or were

Table 2 Published studies on stem cell therapy for anal fistulae

Ref.	Year	Condition	Study design	Cell source	Cell quantity (dose)	Intervention model
García-Olmo *et al*[11]	2003	Recto-vaginal fistula in Crohn's disease	Case report	Autologous eASC	1×10^7	Single arm
García-Olmo *et al*[12]	2005	Enterocutaneous, recto-vaginal, perianal fistula in Crohn's disease	Phase Ⅰ	Autologous eASC	1-3 × 10^7 re-suspended in fibrin glue	Single arm
García-Olmo *et al*[13]	2009	Perianal fistula with or without Crohn's disease	Phase Ⅱ	Autologous eASC	Not specified	Two arms: fibrin glue, fibrin glue + eASC
García-Olmo *et al*[22]	2010	Recto-vaginal fistula in Crohn's disease	Case report	Allogenic eASC	Not specified	Single arm
Ciccocioppo *et al*[25]	2011	Enterocutaneous and complex perianal fistula in Crohn's disease	Case report	Expanded autologous bone marrow	5×10^7	Single arm
Cho *et al*[24]	2012	Perianal fistula in Crohn's disease	Phase Ⅰ	Autologous eASC	Not specified	Single arm: dose escalation study
Herreros *et al*[14]	2012	Complex perianal fistula without Crohn's disease	Phase Ⅲ	Autologous eASC	2×10^7 then 4×10^7 if no effect	Three arms: fibrin glue, eASC, fibrin glue + eASC
Herreros *et al*[14]	2012	Complex perianal fistula without Crohn's disease	Observational	Autologous eASC	2×10^7 then 4×10^7 if no effect	Three arms: fibrin glue, eASC, fibrin glue + eASC
Guadalajara *et al*[23]	2012	Perianal fistula with or without Crohn's disease	Observational	Autologous eASC	Not specified	Two arms: fibrin glue, fibrin glue + eASC
de la Portilla *et al*[17]	2012	Perianal fistula in Crohn's disease	Phase Ⅰ / Ⅱ	Allogeneic eASC	2×10^7 then 4×10^7 if no effect	Single arm
Lee *et al*[20]	2013	Perianal fistula in Crohn's disease	Phase Ⅱ	Autologous eASC	Depending on the fistula. Re-dosing (1.5 times) if no effect	Single arm

SAE: Serious adverse events (those requiring hospital admission > 24 h); eASC: Expanded adult stem cells.

scheduled in control groups of our clinical trials could be selected for the present study.

Various randomized controlled trials using ASCs for the treatment of anal fistulae have been conducted, and all of them show an excellent safety profile. Nevertheless, the actual efficacy is difficult to assess. To date, there are 11 published papers including data on stem cell-based treatment of anal fistulae (Table 2). The first one was published in 2003[11], and the last was published very recently, in 2013[20]. Eight of these have been published by Spanish groups[11-14,17,21-23], two other papers come from South Korea[20,24], and one from Italy[25]. The majority refer to ASC treatment of Crohn's disease-related fistulae. The Italian study was the only one to select bone marrow as the ASC source for the treated fistula[25]. The rest of the studies employed autologous or allogeneic cells from adipose tissue. In all studies, cells were expanded, and a wide range of doses applied. Except for the Italian study[25], all procedures included closure of the internal opening, and in all cases the cell injections were intra-lesional. Over three hundred patients have been enrolled in these studies and the most important result is the assurance of the excellent safety profile of stem cells: no serious cell-related adverse events were described. Regarding efficacy, results show very different profiles, but about 40%-60% of patients achieved healing (Table 3).

The cost of the treatment is another important issue. Nowadays, the production cost of expanded ASCs (Good Manufacturing Practice compliant) can range from € 8000-12000, though the production could be reduced to € 3000-4000 without expansion[13]. We estimate that a high scale industrial production could significantly reduce the expenses. A reasonable strategy that we propose, considering the high cost of cell expansion and our previous results, would be to apply a first treatment with SVF, freeze a portion of the cells obtained, and propose a second treatment with expanded cells in cases achieving no response after eight weeks.

Treatment of recurrent fistulae is a difficult surgical challenge. In these patients, the pursuit of healing usually involves multiple operations, with a subsequent perianal scarring and distortion. In the most complex cases, the condition is worsened by the accompanying fecal incontinence. Therefore, these individuals are progressively more difficult to treat, resulting in the exasperation of both the patient and the surgeon[26]. In these cases, wound healing is a critical issue, and indeed, new approaches are needed. For the reasons outlined earlier, we believe that once available, ASCs will fulfill a clear and previously unmet medical need, helping to improve the healing and hence the quality of life of patients with recurrent perianal fistulae.

In conclusion, limited surgery supported by ASCs may constitute as a new therapeutic strategy in the treatment of recurrent fistulae.

ACKNOWLEDGMENTS

The authors thank Carolina Gonzalez and Tihomir

Table 3 Outcomes of published clinical experience of stem cell treatment for anal fistula

Ref.	Procedure	No. of patients treated	Healed (*n*)	Follow-up (mo)	Recurrence (*n*)	SAE (*n*)
García-Olmo *et al*[11]	Closure of IO. Injection in site, without fibrin glue	1	1	3	0	0
García-Olmo *et al*[12]	Cells resuspended in fibrin glue. Injection in site	9	6	12	Not specified	0
García-Olmo *et al*[13]	Closure of IO. Injection in site	Fibrin glue: 25 Fibrin glue + eASC: 24	Fibrin glue: 3 Fibrin glue + eASC: 17	12	Fibrin glue: 0 Fibrin glue + eASC: 2	4 (1 related to fibrin glue, others unrelated)
García-Olmo *et al*[22]	Closure of IO. Injection in site, without fibrin glue	1	1	36	1	0
Ciccocioppo *et al*[25]	Four injections in site	10	7	12	0	0
Cho *et al*[24]	Closure of IO and fibrin glue. Injection in site	9	3	15	0	0
Herreros *et al*[14]	Closure of IO. Injection in site	eASC: 64 Fibrin glue + eASC: 60 Fibrin glue: 59	eASC: 27 Fibrin glue + eASC: 24 Fibrin glue: 23	6	eASC: 0 Fibrin glue + eASC: 4 Fibrin glue: 0	4 unrelated to study treatment
Herreros *et al*[14]	Closure of IO. Injection in site	Not specified	eASC: 57% Fibrin glue+ eASC: 52.4% Fibrin glue: 37.3%	12	Not specified	1 unrelated to study treatment
Guadalajara *et al*[23]	Closure of IO. Injection in site	Fibrin glue: 13 Fibrin glue + eASC: 21	Fibrin glue: 3 Fibrin glue + eASC: 10	38	Fibrin glue: 1 Fibrin glue + eASC: 5	0
de la Portilla *et al*[17]	Closure of IO. Injection in site, without fibrin glue	24	9	4	Not specified	2 unrelated to study treatment
Lee *et al*[20]	Injection in site and fibrin glue	43	27	12	4	0

IO: Internal opening; SAE: Serious adverse events (those requiring hospital admission > 24 h); eASC: Expanded adult stem cells.

Georgiev for their collaboration in all our Adipose Stem Cells Trials and Studies, assisting in different technical aspects and taking care of the patients.

COMMENTS

Background

The concept of stem cell therapy came from the possibility of obtaining an immature cell that could differentiate into a specific lineage if placed in the correct environment. One of the first examples was the transplantation of stem cells in hematologic patients, which could achieve the regeneration of normal marrow, and is now widely used. Following this idea, researchers raised the question of whether or not there could be different stem cells for other organs, or even embryonic cells that could develop into any of them. The study and therapeutic use of these types of cells could be the answer for many incurable injuries and diseases.

Research frontiers

Complex and recurrent anal fistulae (whether associated or not to Crohn's disease), constitute an important surgical problem, which can be difficult to solve. Many strategies have been proposed to achieve healing, including different surgical techniques, including fibrin glues and plugs. The application of stem cells in the fistula tract promotes the "closure" of the fistula by stimulating the regeneration of the tissue, both by direct growth and immunomodulatory effects. The exact mechanisms by which stem cells induce healing are still under investigation.

Innovations and breakthroughs

An increasing number of randomized controlled trials have tested the application of adipose-derived stem cells (ASCs) in perianal fistulae, with variable rates of success. In the present study, we applied ASCs to the worst patients, those with recurrent fistulae despite multiple previous treatments and interventions. In these desperate cases, even a partial response to the treatment was a success, as patients' distress was a constant after so many failures. ACSs were remarkably effective and we achieved healing in 60% after one year of follow-up.

Applications

This study, and various others in a randomized controlled trial setting, suggests that the surgical application of stem cells in anal fistula tract is a potentially therapeutic strategy that could resolve even the most recurrent and complex fistulae. In the same direction, ACSs are being used for the regeneration of skin, cartilage, bone, cornea, endothelium, *etc*. Applications to other organs, such as the heart and lung, and the nervous system have raised high expectations in the field. Research continues, and new applications are sure to develop in the near future.

Terminology

An anal fistula is an abnormal conduct communicating the anal canal with the perianal skin. The fistula tract typically breaks through the sphincters, and can have multiple ramifications. If one of the openings is blocked, an abscess occurs, which can worsen the condition. Drainage of these abscesses and surgical attempts to close the fistula can damage the muscle of the sphincters and cause fecal incontinence. The former, associated to suppuration and pain are the most common symptoms, creating a permanent discomfort for patients. Adult (somatic) stem cells are undifferentiated cells that can be found in differentiated tissue (such as bone, fat, and muscle) and have the potential to give rise to the specialized cell types present in the tissue from which they originate. A stem cell fulfills three characteristics: self-renewal capacity, differentiation potential, and *in vivo* engraftment capacity. Stem cell therapy: use of stem cells to replace those from damaged or diseased tissue. The source of the cells can either be the patient (autologous), another individual (allogeneic), or an animal (xenogeneic).

Peer-review

A good and interesting study even though it includes only ten patients. However, the results are very useful to speculate about the best current treatment of recurrent complex fistulae. It will be interesting if a randomized cross-over multicenter study can confirm these results with stem cell therapy in the complex anal fistulae.

REFERENCES

1 **García-Aguilar J**, Davey CS, Le CT, Lowry AC, Rothenberger

DA. Patient satisfaction after surgical treatment for fistula-in-ano. *Dis Colon Rectum* 2000; **43**: 1206-1212 [PMID: 11005484]

2 **Whiteford MH**, Kilkenny J, Hyman N, Buie WD, Cohen J, Orsay C, Dunn G, Perry WB, Ellis CN, Rakinic J, Gregorcyk S, Shellito P, Nelson R, Tjandra JJ, Newstead G. Practice parameters for the treatment of perianal abscess and fistula-in-ano (revised). *Dis Colon Rectum* 2005; **48**: 1337-1342 [PMID: 15933794 DOI: 10.1007/s10350-005-0055-3]

3 **Ortíz H**, Marzo J. Endorectal flap advancement repair and fistulectomy for high trans-sphincteric and suprasphincteric fistulas. *Br J Surg* 2000; **87**: 1680-1683 [PMID: 11122184 DOI: 10.1046/j.1365-2168.2000.01582.x]

4 **Garcia-Olmo D**, Garcia-Arranz M, Herreros D. Expanded adipose-derived stem cells for the treatment of complex perianal fistula including Crohn's disease. *Expert Opin Biol Ther* 2008; **8**: 1417-1423 [PMID: 18694359 DOI: 10.1517/14712598.8.9.1417]

5 **Trebol Lopez J**, Georgiev Hristov T, García-Arranz M, García-Olmo D. Stem cell therapy for digestive tract diseases: current state and future perspectives. *Stem Cells Dev* 2011; **20**: 1113-1129 [PMID: 21187000 DOI: 10.1089/scd.2010.0277]

6 **García-Gómez I**, Elvira G, Zapata AG, Lamana ML, Ramírez M, Castro JG, Arranz MG, Vicente A, Bueren J, García-Olmo D. Mesenchymal stem cells: biological properties and clinical applications. *Expert Opin Biol Ther* 2010; **10**: 1453-1468 [PMID: 20831449 DOI: 10.1517/14712598.2010.519333]

7 **Falanga V**, Iwamoto S, Chartier M, Yufit T, Butmarc J, Kouttab N, Shrayer D, Carson P. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. *Tissue Eng* 2007; **13**: 1299-1312 [PMID: 17518741 DOI: 10.1089/ten.2006.0278]

8 **Wu Y**, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 2007; **25**: 2648-2659 [PMID: 17615264 DOI: 10.1634/stemcells.2007-0226]

9 **Chen L**, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One* 2008; **3**: e1886 [PMID: 18382669 DOI: 10.1371/journal.pone.0001886]

10 **Chaparro M**, Zanotti C, Burgueño P, Vera I, Bermejo F, Marín-Jiménez I, Yela C, López P, Martín MD, Taxonera C, Botella B, Pajares R, Ponferrada A, Calvo M, Algaba A, Pérez L, Casis B, Maté J, Orofino J, Lara N, García-Losa M, Badia X, Gisbert JP. Health care costs of complex perianal fistula in Crohn's disease. *Dig Dis Sci* 2013; **58**: 3400-3406 [PMID: 24026400 DOI: 10.1007/s10620-013-2830-7]

11 **García-Olmo D**, García-Arranz M, García LG, Cuellar ES, Blanco IF, Prianes LA, Montes JA, Pinto FL, Marcos DH, García-Sancho L. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. *Int J Colorectal Dis* 2003; **18**: 451-454 [PMID: 12756590 DOI: 10.1007/s00384-003-0490-3]

12 **García-Olmo D**, García-Arranz M, Herreros D, Pascual I, Peiro C, Rodríguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum* 2005; **48**: 1416-1423 [PMID: 15933795 DOI: 10.1007/s10350-005-0052-6]

13 **Garcia-Olmo D**, Herreros D, Pascual I, Pascual JA, Del-Valle E, Zorrilla J, De-La-Quintana P, Garcia-Arranz M, Pascual M. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum* 2009; **52**: 79-86 [PMID: 19273960 DOI: 10.1007/DCR.0b013e3181973487]

14 **Herreros MD**, Garcia-Arranz M, Guadalajara H, De-La-Quintana P, Garcia-Olmo D. Autologous expanded adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistulas: a phase III randomized clinical trial (FATT 1: fistula Advanced Therapy Trial 1) and long-term evaluation. *Dis Colon Rectum* 2012; **55**: 762-772 [PMID: 22706128 DOI: 10.1097/DCR.0b013e318255364a]

15 **Parks AG**, Gordon PH, Hardcastle JD. A classification of fistula-in-ano. *Br J Surg* 1976; **63**: 1-12 [PMID: 1267867]

16 **Vaizey CJ**, Carapeti E, Cahill JA, Kamm MA. Prospective comparison of faecal incontinence grading systems. *Gut* 1999; **44**: 77-80 [PMID: 9862829]

17 **de la Portilla F**, Alba F, García-Olmo D, Herrerías JM, González FX, Galindo A. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. *Int J Colorectal Dis* 2013; **28**: 313-323 [PMID: 23053677 DOI: 10.1007/s00384-012-1581-9]

18 **Singer NG**, Caplan AI. Mesenchymal stem cells: mechanisms of inflammation. *Annu Rev Pathol* 2011; **6**: 457-478 [PMID: 21073342 DOI: 10.1146/annurev-pathol-011110-130230]

19 **Ortiz H**, Marzo M, de Miguel M, Ciga MA, Oteiza F, Armendariz P. Length of follow-up after fistulotomy and fistulectomy associated with endorectal advancement flap repair for fistula in ano. *Br J Surg* 2008; **95**: 484-487 [PMID: 18161890 DOI: 10.1002/bjs.6023]

20 **Lee WY**, Park KJ, Cho YB, Yoon SN, Song KH, Kim do S, Jung SH, Kim M, Yoo HW, Kim I, Ha H, Yu CS. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *Stem Cells* 2013; **31**: 2575-2581 [PMID: 23404825 DOI: 10.1002/stem.1357]

21 **Garcia-Olmo D**, Herreros D, Pascual M, Pascual I, De-La-Quintana P, Trebol J, Garcia-Arranz M. Treatment of enterocutaneous fistula in Crohn's Disease with adipose-derived stem cells: a comparison of protocols with and without cell expansion. *Int J Colorectal Dis* 2009; **24**: 27-30 [PMID: 18696086 DOI: 10.1007/s00384-008-0559-0]

22 **García-Olmo D**, Herreros D, De-La-Quintana P, Guadalajara H, Trébol J, Georgiev-Hristov T, García-Arranz M. Adipose-derived stem cells in Crohn's rectovaginal fistula. *Case Rep Med* 2010; **2010**: 961758 [PMID: 20224798 DOI: 10.1155/2010/961758]

23 **Guadalajara H**, Herreros D, De-La-Quintana P, Trebol J, Garcia-Arranz M, Garcia-Olmo D. Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. *Int J Colorectal Dis* 2012; **27**: 595-600 [PMID: 22065114 DOI: 10.1007/s00384-011-1350-1]

24 **Cho YB**, Lee WY, Park KJ, Kim M, Yoo HW, Yu CS. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. *Cell Transplant* 2013; **22**: 279-285 [PMID: 23006344 DOI: 10.3727/096368912X656045]

25 **Ciccocioppo R**, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, Ubezio C, Minelli A, Alvisi C, Vanoli A, Calliada F, Dionigi P, Perotti C, Locatelli F, Corazza GR. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut* 2011; **60**: 788-798 [PMID: 21257987 DOI: 10.1136/gut.2010.214841]

26 **Dudukgian H**, Abcarian H. Why do we have so much trouble treating anal fistula? *World J Gastroenterol* 2011; **17**: 3292-3296 [PMID: 21876616 DOI: 10.3748/wjg.v17.i28.3292]

P- Reviewer: Campo SMA, Hokama A **S- Editor**: Ma YJ
L- Editor: AmEditor **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3337

World J Gastroenterol 2015 March 21; 21(11): 3337-3343
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Economic and medical benefits of ultrasound screenings for gallstone disease

Hung-Ju Shen, Chung-Te Hsu, Tao-Hsin Tung

Hung-Ju Shen, Chung-Te Hsu, Tao-Hsin Tung, Cheng Hsin General Hospital, Taipei 112, Taiwan
Tao-Hsin Tung, Faculty of Public Health, School of Medicine, Fu-Jen Catholic University, Taipei 24205, Taiwan
Tao-Hsin Tung, Department of Nursing, Kang-Ning Junior College of Medical Care and Management, Taipei 114, Taiwan
Author contributions: Shen HJ, Hsu CT and Tung TH carried out the study and drafted the manuscript; Shen HJ participated in the design of the study and performed the statistical analysis; Tung TH conceived of the study, and participated in its design and coordination; all authors read and approved the final manuscript.
Supported by Grant from the National Science Council, No. NSC-98-2314-B-350-002-MY3 (in part).

Correspondence to: Tao-Hsin Tung, PhD, Cheng Hsin General Hospital, Shih-Pai, Taipei 112, Taiwan. ch2876@chgh.org.tw
Telephone: +886-2-28264400
Fax: +886-2-28264550
Received: September 23, 2014
Peer-review started: September 25, 2014
First decision: October 14, 2014
Revised: November 3, 2014
Accepted: December 8, 2014
Article in press: December 8, 2014
Published online: March 21, 2015

Abstract

AIM: To investigate whether screening for gallstone disease was economically feasible and clinically effective.

METHODS: This clinical study was initially conducted in 2002 in Taipei, Taiwan. The study cohort total included 2386 healthy adults who were voluntarily admitted to a regional teaching hospital for a physical check-up. Annual follow-up screening with ultrasound sonography for gallstone disease continued until December 31, 2007. A decision analysis using the Markov Decision Model was constructed to compare different screening regimes for gallstone disease. The economic evaluation included estimates of both the cost-effectiveness and cost-utility of screening for gallstone disease.

RESULTS: Direct costs included the cost of screening, regular clinical fees, laparoscopic cholecystectomy, and hospitalization. Indirect costs represent the loss of productivity attributable to the patient's disease state, and were estimated using the gross domestic product for 2011 in Taiwan. Longer time intervals in screening for gallstone disease were associated with the reduced efficacy and utility of screening and with increased cost. The cost per life-year gained (average cost-effectiveness ratio) for annual screening, biennial screening, 3-year screening, 4-year screening, 5-year screening, and no-screening was new Taiwan dollars (NTD) 39076, NTD 58059, NTD 72168, NTD 104488, NTD 126941, and NTD 197473, respectively ($P < 0.05$). The cost per quality-adjusted life-year gained by annual screening was NTD 40725; biennial screening, NTD 64868; 3-year screening, NTD 84532; 4-year screening, NTD 110962; 5-year screening, NTD 142053; and for the control group, NTD 202979 ($P < 0.05$). The threshold values indicated that the ultrasound sonography screening programs were highly sensitive to screening costs in a plausible range.

CONCLUSION: Routine screening regime for gallstone disease is both medically and economically valuable. Annual screening for gallstone disease should be recommended.

Key words: Decision analysis; Gallstone disease; Economic evaluation; Evidence-based medicine; Screening

Core tip: The results of this economic evaluation of screening for gallstone disease indicated that routine ultrasound sonography screening is worthwhile. We recommend that the Chinese population is screened annually for gallstone disease, regardless of whether or not they have been diagnosed with gallstone disease in the past.

Shen HJ, Hsu CT, Tung TH. Economic and medical benefits of ultrasound screenings for gallstone disease. *World J Gastroenterol* 2015; 21(11): 3337-3343 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3337.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3337

INTRODUCTION

Gallstone disease (GSD) is a common gastrointestinal disease, and > 14% of adults are affected by this disorder[1,2]. In the United States, the direct and indirect economic cost of treating GSD patients has been estimated at USD 16 billion annually, and GSD annually accounts for > 800000 hospitalizations[3,4]. Cholecystectomy is considered to be a safe treatment of choice for symptomatic GSD patients[2,5]. Nevertheless, depending on the clinical manifestations of the disease and changes in symptoms over time, expectant management might also present a valid therapeutic approach for certain GSD patients[2]. From the clinical viewpoint, early detection of the disorder through regular ultrasound sonography screening, followed by appropriate treatment regime, could avert the need for cholecystectomy.

Laparoscopic cholecystectomy is widely accepted in developed countries as the first line of treatment for uncomplicated GSD. Up to 80% of cholecystectomies are laparoscopies in such countries[6,7], which are relatively safe (mortality is < 0.2% and morbidity < 5.0%) and are highly acceptable to both patients and physicians[2,8]. In addition, previous study also indicated that laparoscopic cholecystectomy is associated with a significantly shorter hospital stay and quicker convalescence compared with open cholecystectomy[2].

GSD is an important health problem, therefore, it is matched to the Wilson criteria for routine screening. This means that the disease natural course should be understood; a recognizable latent or early symptomatic stage; a clinical test is easy to perform and interpret, reliable, accurate, acceptable, sensitive and specific; an accepted treatment recognized for the disease; it is more effective if treatment is started early; a policy on who should be treated; diagnosis and treatment are cost-effective; and case-finding should be a continuous process. The costs of screening programs and early treatment for GSD might be offset by these benefits if the early treatment was known to reduce the incidence of the disease or slow its progression and reduce the need for cholecystectomy. However, relevant cost analyses did not consider the natural history of GSD, and therefore, might have provided inaccurate estimations. The unique medical environment of Taiwan requires careful analysis of the costs and benefits before firm conclusions can be drawn and standards set. Our previous study indicated that compared with the control group, routine screening strategies for GSD reduced the necessity of cholecystectomy by approximately the following amounts: annual screening 82.9% 95%CI: 75.7%-90.4%); biennial screening 71.6% (95%CI: 57.0%-88.8%); 3-year screening 64.8% (95%CI: 46.1%-81.5%); 4-year screening 49.6% (95%CI: 23.9%-75.3%); and 5-year screening 32.1% (95%CI: -2.8%-66.7%)[9]. In this study, we further investigated whether a routine ultrasound sonography screening program is a cost-effective strategy for managing GSD in the Chinese population in Taiwan.

MATERIALS AND METHODS

Data source and collection

We recruited a study cohort to evaluate the economic implications of GSD screening in Taipei, Taiwan. The initial study cohort comprised 2386 healthy adults (1235 men and 1151 women) who were voluntarily admitted to a regional teaching hospital in Northern Taiwan to receive a physical check-up. The study was initially conducted between January and December 2002. Annual follow-up screenings for GSD continued until December 31, 2007. We analyzed information on patients who received at least two GSD screenings.

One thousand and seven (42.2%) patients in the original study group failed to complete the entire assessment series. Participants who were lost to follow-up during the 5-year period showed the following characteristics compared to those who remained: more advanced age (50.2 ± 10.8 years *vs* 45.0 ± 9.9 years, $P < 0.0001$), higher systolic blood pressure [(SBP); 127.9 ± 21.0 mmHg *vs* 120.7 ± 19.1 mmHg, $P < 0.0001$)], and higher fasting plasma glucose (102.6 ± 25.1 mg/dL *vs* 95.2 ± 24.0 mg/dL, $P < 0.0001$). All study procedures were performed in accordance with the guidelines of our Institutional Ethics Committee and adhered to the tenets of the Declaration of Helsinki. The data from all participants remained anonymous. Access to personal demographic and medical records was approved by the Human Subjects Review Board at Cheng-Hsin General Hospital, Taipei, Taiwan.

Diagnosis of GSD

In the present study, GSD was diagnosed by a panel

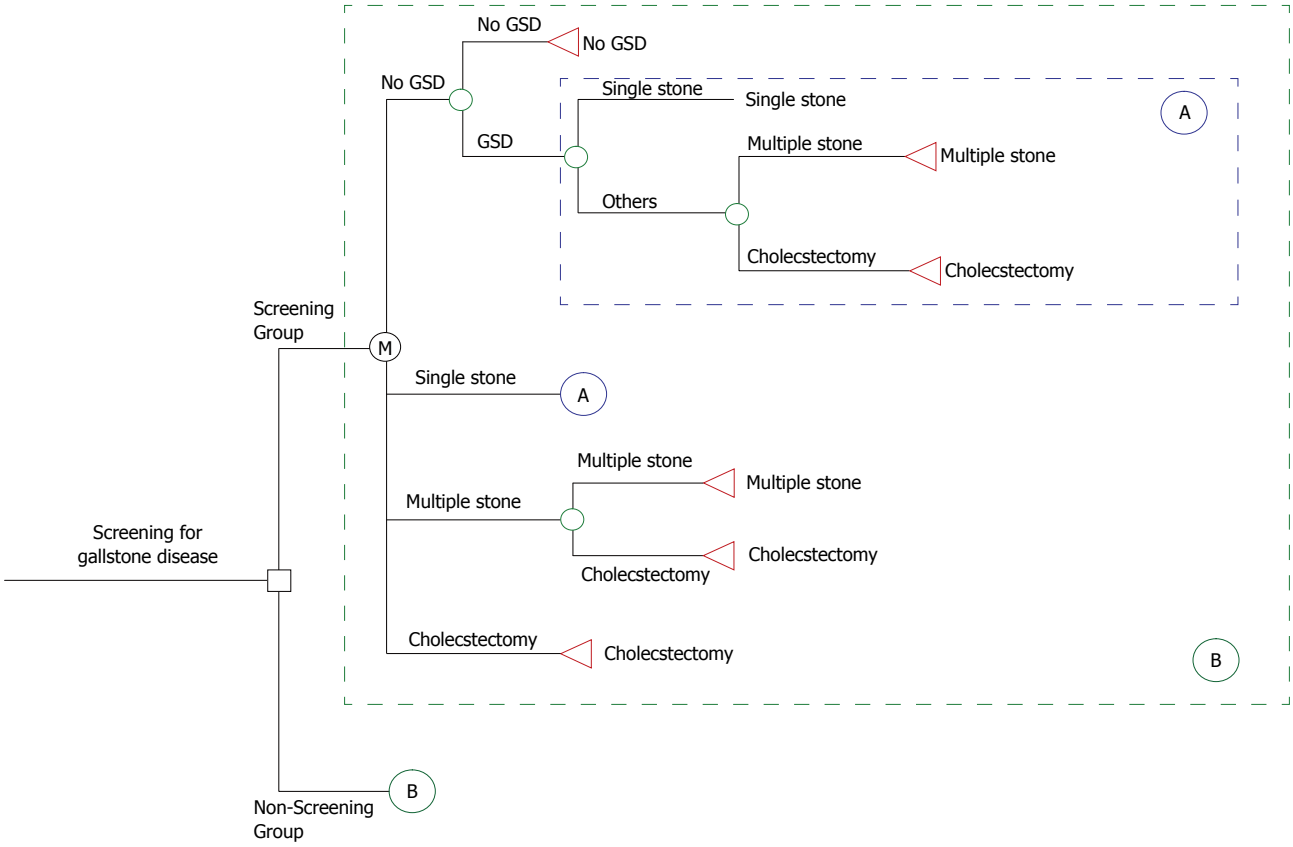


Figure 1 Markov Decision Model for two options, that is, screening and non-screening for gallstone disease. GSD: Gallstone disease.

of specialists using real-time ultrasound sonography (nemio SSA-550A; Toshiba, Japan). The abdominal region of the patients was examined after they had fasted for ⩾ 8 h, and sonography was used to identify movable hyperechoic foci with acoustic shadows. The diagnosis of GSD was classified as follows: single gallbladder stone; multiple gallbladder stones; and gallstones requiring cholecystectomy. Gallbladder polyps were not included. The study cases were identified among all types of GSD patients[10].

To determine consistent criteria for the diagnosis of GSD, the κ statistic was further used to evaluate the inter-observer reliability among various medical specialists. A pilot study was performed using the data of 100 randomly selected healthy participants; none of our patients were included in this stage. The κ value of the inter-observer reliability for the diagnosis of GSD among the medical specialists was 0.79 (95%CI: 0.61-0.95)[10].

Economic evaluation of screening for GSD

Markov decision model of screening for GSD: Decision tree analysis is a statistical technique used for selecting an optimal decision rule. A study problem is formulated in a tree-structured format, including the decision node (□), chance node (○), and value node (△). An expected value for each node is calculated, and the best decision is selected based on the expected values[11]. The economic evaluation tool in this study used to assess the GSD screening was on the basis of TreeAge software (DATA 3.5, Tree-Age, Inc., Williamstown, MA, United States) for medical decision analyses. This software uses the influence diagram approach and tree structure.

As Figure 1 shows, a decision analysis using the Markov Decision Model was constructed to compare various screening regimens for GSD with no screening group. The assumption of the no-screening group was that except for GSD screening, patients still received routine medical care until they received a cholecystectomy. According to stochastic process theory, the Markov Chain Model is determined by both the initial state and the transition matrix. This model starts with the decision to screen or not to screen a patient, and the overall expected value is based on the expected values of the end nodes rather than all nodes. As Circle A shows in Figure 1, four states of the natural history of GSD are possible for each decision, that is, no GSD, single stone, multiple stones, and requiring cholecystectomy. The initial state distribution is based on the results of this study. Transition probabilities from one state to another, representing the natural course of GSD, were derived by our previous empirical estimation. In addition, the Circle B indicated that the same disease process with Markov property between the screening and non-screening groups. For each scenario, the expected probability based on participants' aggregate experiences, using data accumulated for each stage during the 10-year follow-up were calculated.

Table 1 Cost assumptions, utility value, transition probabilities in decision analysis of screening for gallstone disease

Parameter	Value
Annual direct cost (NTD)	
Screening cost[1]	1382
Regular clinics fee[2]	509
Laparoscopic cholecystectomy	11710
Hospitalizations and others	26825
Total	40426
Annual indirect cost (NTD)	
Gross domestic product	635670
Utility (quality of life) value	
No GSD	0.92 ± 0.10
Single stone	0.90 ± 0.12
Multiple stones	0.89 ± 0.19
Cholecystectomy	0.88 ± 0.08
Annual transition probability (%)[9]	
No GSD → Single stone	5.05
Single stone → Multiple stones	10.00
Multiple stones → Cholecystectomy	13.76

[1]Screening cost includes clinician's fee, ultrasound examination, SMA-12 test, and manpower cost; [2]Regular clinics fee includes clinician's fee and pharmacist's fee. GSD: Gallstone disease; NTD: New Taiwan dollars.

Cost estimation: Both the direct and indirect costs both were analyzed. Direct costs included the cost of GSD screening, cost of regular clinical fees, and further treatment costs. For indirect costs, we included only the loss of productivity for the patient because of time off work for treatment. Due to the fact that participants were not accompanied every time by an attendant, the cost of an attendant was not considered. The average time off work for treatment was estimated by the specialists. All costs are expressed in new Taiwan dollars (NTD).

Cost-effectiveness analysis (CEA) and cost-utility analysis (CUA): We conducted a CEA to compare the cost per life-year gained by the screened patients relative to the non-screened group. To adjust for quality of life, a series of utility scores was assigned based on the CUA, as follows: no GSD, single stone, multiple stones, and requiring cholecystectomy. The time trade-off method was used to evaluate the utility value according to a standard procedure with modification[12]. A scenario was described to the participants as follows: "Imagine a situation in which you could live for 10 years with your current health status. Now imagine you were given the opportunity to restore your health status to perfect health. This opportunity could increase your quality of life, but would also decrease your chance of survival. What is the maximum number of years you would be willing to forgo so that you could receive this opportunity and have the best health for the remainder of your life?"

Then the utility value was calculated as follows: the number of years a patient was willing to trade in return for improving one's health, divided by the estimated number of years of remaining life, followed by subtracting this number from 1.0, that is, the utility value = 1.0 - (time traded/time of remaining life)[12,13]. The CUA approach was then used to compare the cost per quality-adjusted life-year (QALY) gained between the screened and non-screened groups.

Sensitivity analysis and discount rate: One-way sensitivity analyses were conducted on individual estimates to assess the effect of screening for GSD on costs, effectiveness, and utility. To account for time preference (*i.e.*, receiving a benefit earlier and incurring the cost later) we discounted all costs and benefits to the present value at 5% annually.

RESULTS

Table 1 shows the annual direct and indirect cost of GSD screening in the decision analysis. Direct costs included the cost of screening (NTD 1382), regular clinical fees (NTD 509), cost of laparoscopic cholecystectomy (NTD 11710), and cost of hospitalization and others (NTD 26825). The total annual direct costs are estimated as NTD 40426. Indirect costs represent the loss of productivity attributable to the patient's disease state, and were estimated at NTD 635670 using the gross domestic product (GDP) for 2011 in Taiwan. The utility value for no GSD, single stone, multiple stones, and cholecystectomy was 0.92 ± 0.10, 0.90 ± 0.12, 0.89 ± 0.19, and 0.88 ± 0.08, respectively. In addition, the annual transition probabilities from each stage to the next stage were as follows: no GSD to single stone, 5.05%; single stone to multiple stones, 10.00%; and multiple stones to cholecystectomy, 13.76%[9].

Table 2 shows the results of CEA for various GSD screening programs during the 10-year follow-up. Annual screening incurred the lowest cost and yielded the greatest effectiveness. The cost per life-year gained (average cost-effectiveness ratio) for annual screening, biennial screening, 3-year screening, 4-year screening, 5-year screening, and control (no-screening) was NTD 39076, NTD 58059, NTD 72168, NTD 104488, NTD 126941, and NTD 197473, respectively. Compared with the non-screened group, the screened groups showed greater effectiveness and lower costs. In other words, any screening program was more cost-effective than no program.

Table 2 also shows the results after adjusting for utility. Annual screening provided the highest QALY combined with the lowest cost. The cost per QALY gained of annual screening, biennial screening, 3-year, 4-year, and 5-year screenings, and for the no screening was NTD 40725, NTD 64868, NTD 84532, NTD 110962, NTD 142053, and NTD 202979, respectively. Compared with not screening, routine screening provided greater effectiveness at a lower economic cost. Any screening program was more cost-effective than no program at all.

Table 3 shows the sensitivity analysis of CEA and

Table 2 Cost-effectiveness analysis and cost-utility analysis for different screening programs for gallstone disease during 10 years follow-up

Screening strategy	Cost (NTD)	Effectiveness (life-years gained)	Cost/effectiveness (NTD)	ICER (compared to control group)	Utility (QALY)	Cost/ utility(NTD)	ICUR (compared to control group)
Annual	199856	5.1146	39076	Dominate[1]	4.9075	40725	Dominate[1]
Biennial	215231	3.7071	58059	Dominate[1]	3.3180	64868	Dominate[1]
3-yearly	253908	3.5183	72168	Dominate[1]	3.0037	84532	Dominate[1]
4-yearly	331477	3.1724	104488	Dominate[1]	2.9873	110962	Dominate[1]
5-yearly	368002	2.8990	126941	Dominate[1]	2.5906	142053	Dominate[1]
Control group	508117	2.5731	197473	-	2.5033	202979	-

[1]Any screening program was more cost-effective than no program. ICER: Incremental cost-effectiveness ratio; ICUR: Incremental cost-utility ratio; NTD: New Taiwan dollars; QALY: Quality-adjusted life-year.

Table 3 Sensitivity analysis of cost-effectiveness analysis and cost-utility analysis of different screening programs for Gallstone disease

Variable	Base case	Range	Threshold of CEA	Threshold of CUA
Annual screening				
Screening cost (NTD)	1382	1000-50000	41630	40082
Indirect cost (NTD)	231834	0-635670	Dominate[1]	Dominate[1]
Percentage of cholecystectomy	0.8	0.1-0.9	Dominate[1]	Dominate[1]
Biennial screening				
Screening cost (NTD)	1382	1000-50000	38771	37250
Indirect cost (NTD)	231834	0-635670	Dominate[1]	Dominate[1]
Percentage of cholecystectomy	0.8	0.1-0.9	Dominate[1]	Dominate[1]
3-yr screening				
Screening cost (NTD)	1382	1000-50000	36048	36003
Indirect cost (NTD)	231834	0-635670	Dominate[1]	Dominate[1]
Percentage of cholecystectomy	0.8	0.1-0.9	Dominate[1]	Dominate[1]
4-yr screening				
Screening cost (NTD)	1382	1000-50000	32186	31952
Indirect cost (NTD)	231834	0-635670	Dominate[1]	Dominate[1]
Percentage of cholecystectomy	0.8	0.1-0.9	Dominate[1]	Dominate[1]
5-yr screening				
Screening cost (NTD)	1382	1000-50000	29063	28579
Indirect cost (NTD)	231834	0-635670	Dominate[1]	Dominate[1]
Percentage of cholecystectomy	0.8	0.1-0.9	Dominate[1]	Dominate[1]

[1]Any screening program was more cost-effective than no program. CEA: Cost-effectiveness analysis; CUA: Cost-utility analysis; NTD: New Taiwan dollars.

CUA for various GSD screening regimes. The threshold values showed that screening programs were highly sensitive to costs within a plausible range. Compared to no screening, the threshold of CEA in annual screening, biennial screening, 3-yearly screening, 4-yearly screening, and 5-yearly screening was NTD 41630, NTD 38771, NTD 36048, NTD 32186, and NTD 29063, respectively. The screening cost threshold of CUA also decreased with increasing screening interval. For indirect cost and percentage of cholecystectomy, any screening program was more cost-effective than no program.

DISCUSSION

The well-known factors related to GSD include type 2 diabetes, obesity, and metabolic syndrome[14-19]. The presence of metabolic syndrome as an insulin resistance phenotype is related to increased morbidity in GSD[19,20]. A previous study showed that both asymptomatic and symptomatic GSD patients displayed a benign natural history. The majority of patients with severe or mild symptoms no longer experienced biliary pain during follow-up, and the rate of symptom development in asymptomatic patients was low[2]. However, participants with GSD showed increased rates of cardiovascular disease, cancer, and all-cause mortality compared with no GSD. This relationship was found among patients in the United States who were either diagnosed with GSD after an ultrasound scan, or who had received a cholecystectomy. The association was largely unexplained by multiple demographic and cardiovascular disease risk factors in this population[21]. One case-control study in Sweden revealed that more than twice as many young women who had died of cancer had received a cholecystectomy than women who had died from other causes[22]. Another study that used a progressive disease model to describe the natural history of GSD reported that the estimated mean duration for the stages of no GSD, single stone, and multiple stones was 18.18 years, 8.77 years, and 6.76 years, respectively[9]. Based on these findings, if we assume that no patients progressed

directly from having a single gallstone to receiving a cholecystectomy, the average time to progress from no GSD to requiring a cholecystectomy is about 33.7 years for the general population. This slow progression indicates that clinicians should be able to detect single or multiple stones at an early stage, and thus, reduce GSD-related mortality.

Currently, it is under discussion if cholecystectomy is suggested for patients with asymptomatic GSD. However, in symptom-free patients, it is generally conceived that surgical procedures are not recommended[23].

Evidence-based studies have suggested that screening for and treating GSD is extremely cost-effective. In Chile, a screening program for GSD in a high-risk population achieved significant benefits at low incremental costs and acceptable cost-effectiveness. The incremental cost-effectiveness ratios were as follows: universal screening and elective intervention, NTD 180; high-risk intervention, NTD 147; and selective screening strategy, NTD 481[24]. Considering both cost and efficacy, prevention programs that screened for GSD resulted in substantial federal budget savings combined with highly cost-effective health care. In the present study, the CEA and CUA showed that annual GSD screening was the most effective and efficient screening schedule. Thus, the safest and most aggressive approach to preventing GSD should include annual screening. In addition, professionals responsible for establishing standards for the quality of health care must consider the marginal benefit of frequent ultrasound sonography examinations.

Economic evaluations are commonly criticized by decision makers for ignoring budgetary constraints, which are of prime concern to decision makers. Stakeholders might encounter financial difficulties if they adopt too many cost-effective interventions in which the affordability of a program depends on the overall volume of patients[2,25]. From a clinical perspective, the annual cost of screening for GSD (including clinician fees and ultrasound examinations) is relatively low per patient at USD 29.35 or NTD 882. Our results showed that an annual screening regimen could offer greater cost-effectiveness than any longer screening intervals. However, long-term follow-up might be affected by difficulties in maintaining contact with patients; such patients might be unlikely to remember to schedule an examination after several years have passed.

The use of primary data and the calculation of both direct and indirect costs allowed us to estimate the true benefit of GSD screening more accurately than in previous studies. Nonetheless, this study was subject to certain limitations. First, we did not explicitly consider the sensitivity and specificity of the GSD screening. A greater understanding of GSD from the asymptomatic to the symptomatic stage and the ideal conditions for screening would help to determine the optimal frequency of sonography check-ups and the sensitivity and specificity of this screening tool. Second, although the κ value for inter-observer reliability appeared adequate[26], non-differential misclassification-bias identification might have influenced the results. Third, potential self-selection bias might have occurred because our study design was hospital-based and the follow-up rate was relatively low (57.8%). This is more likely caused by non-respondents with older ages and severe SBP and fasting plasma glucose than participants, that is, of it not being exactly representative of the whole general population. Fourth, because laparoscopic cholecystectomy is not performed routinely for GSD (only polyp > 1 cm), further studies will be needed to explore cholecystectomy (this study recommended it only in cases with multiple stones): such as patients with cholecystitis who required cholecystectomy with one stone or no stone. Finally, our sample size was too small to estimate certain variables that would likely affect the optimal screening intervals for GSD. These variables include comorbidity (*e.g.*, obesity or type 2 diabetes), influence of GSD development over time and among various age groups on the stage of disease, and the occurrence of complications. Further long-term studies should be conducted to clarify whether patients whose weight is well controlled, or those at an early stage of GSD, would benefit from the least frequent screening interval.

In conclusion, the results of our economic evaluation of GSD screening suggested that screening is worthwhile. We recommend that Chinese people are screened annually for GSD, regardless of whether or not they have been diagnosed with GSD in the past.

COMMENTS

Background

Gallstone disease (GSD) is an important health problem, therefore, it is matched to the Wilson criteria for routine screening. The authors investigated whether a routine ultrasound sonography screening program is a cost-effective strategy for managing GSD in the Chinese population in Taiwan.

Research frontiers

The costs of screening programs and early treatment for GSD might be offset by these benefits if early treatment reduces the incidence of the disease or slows its progression and reduces the need for cholecystectomy.

Innovations and breakthroughs

Longer time intervals in screening for GSD were associated with reduced efficacy and utility of screening and with increased cost. The cost per life-year gained (average cost-effectiveness ratio) for annual screening, biennial screening, 3-year screening, 4-year screening, 5-year screening, and no-screening was NTD 39076, NTD 58059, NTD 72168, NTD 104488, NTD 126941, and NTD 197473, respectively ($P < 0.05$). The cost per quality-adjusted life-year (QALY) gained by annual screening, NTD 40725; biennial screening, NTD 64868; 3-year screening, NTD 84532; 4-year screening, NTD 110962; 5-year screening, NTD 142053; and for the control group, NTD 202979 ($P < 0.05$). The threshold values indicated that the ultrasound sonography screening programs were highly sensitive to screening costs in a plausible range.

Applications

The results of the present economic evaluation of GSD screening suggested that screening is worthwhile. The authors recommend that Chinese people are screened annually for GSD, regardless of whether or not they have been diagnosed with it in the past.

Terminology

Considering both cost and efficacy, prevention programs that screened for GSD resulted in substantial federal budget savings combined with highly cost-effective health care. In this study, the cost-effectiveness analysis and cost-utility analysis showed that annual GSD screening was the most effective and efficient screening schedule. Thus, the safest and most aggressive approach to preventing GSD should include annual screening.

Peer-review

This manuscript purports to show that there are economic and medical benefits from ultrasound screening for gallstones. Using a Markov Decision model they calculated QALY gained by different screening regimens. The authors can clarify if the patients were accompanied every time by an attendant. If yes, then cost and wages lost of the attendant has to also be taken into consideration. Or else the authors can state that attendant was not required.

REFERENCES

1 **Portincasa P**, Moschetta A, Palasciano G. Cholesterol gallstone disease. *Lancet* 2006; **368**: 230-239 [PMID: 16844493 DOI: 10.1016/S0140-6736(06)69044-2]

2 **Festi D**, Reggiani ML, Attili AF, Loria P, Pazzi P, Scaioli E, Capodicasa S, Romano F, Roda E, Colecchia A. Natural history of gallstone disease: Expectant management or active treatment? Results from a population-based cohort study. *J Gastroenterol Hepatol* 2010; **25**: 719-724 [PMID: 20492328 DOI: 10.1111/j.1440-1746.2009.06146.x]

3 **Everhart JE**, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology* 1999; **117**: 632-639 [PMID: 10464139 DOI: 10.1016/S0016-5085(99)70456-7]

4 **Zacks SL**, Sandler RS, Rutledge R, Brown RS. A population-based cohort study comparing laparoscopic cholecystectomy and open cholecystectomy. *Am J Gastroenterol* 2002; **97**: 334-340 [PMID: 11866270 DOI: 10.1111/j.1572-0241.2002.05466.x]

5 **Roda E**, Festi D, Lezoche E, Leuschner U, Paugartner G, Sauerbruch T. Strategies in the treatment of biliary stones. *Gastroenterol Int* 2000; **13**: 7-15

6 **Teerawattananon Y**, Mugford M. Is it worth offering a routine laparoscopic cholecystectomy in developing countries? A Thailand case study. *Cost Eff Resour Alloc* 2005; **3**: 10 [PMID: 16259625 DOI: 10.1186/1478-7547-3-10]

7 **Hobbs MS**, Mai Q, Fletcher DR, Ridout SC, Knuiman MW. Impact of laparoscopic cholecystectomy on hospital utilization. *ANZ J Surg* 2004; **74**: 222-228 [PMID: 15043732 DOI: 10.1111/j.1445-2197.2004.02955.x]

8 National Institutes of Health Consensus Development Conference Statement on Gallstones and Laparoscopic Cholecystectomy. *Am J Surg* 1993; **165**: 390-398 [PMID: 8480870]

9 **Hsu CT**, Lien SY, Jiang YD, Liu JH, Shih HC, Tung TH. Screening gallstone disease by ultrasound decreases the necessity of cholecystectomy. *Asia Life Sci* 2013; **22**: 51-60

10 **Liu CM**, Tung TH, Chou P, Chen VT, Hsu CT, Chien WS, Lin YT, Lu HF, Shih HC, Liu JH. Clinical correlation of gallstone disease in a Chinese population in Taiwan: experience at Cheng Hsin General Hospital. *World J Gastroenterol* 2006; **12**: 1281-1286 [PMID: 16534886 DOI: 10.3748/wjg.v12.i8.1281]

11 **Tung TH**, Shih HC, Chen SJ, Chou P, Liu CM, Liu JH. Economic evaluation of screening for diabetic retinopathy among Chinese type 2 diabetics: a community-based study in Kinmen, Taiwan. *J Epidemiol* 2008; **18**: 225-233 [PMID: 18776707]

12 **Brown MM**, Brown GC, Sharma S, Shah G. Utility values and diabetic retinopathy. *Am J Ophthalmol* 1999; **128**: 324-330 [PMID: 10511027]

13 **Brown MM**, Brown GC, Sharma S, Busbee B, Brown H. Quality of life associated with unilateral and bilateral good vision. *Ophthalmology* 2001; **108**: 643-647; discussion 647-648 [PMID: 11297474]

14 **Chien WH**, Liu JH, Hou WY, Shen HJ, Chang TY, Tung TH. Clinical implications in the incidence and associated risk factors on gallstone disease among elderly type 2 diabetes in Kinmen, Taiwan. *Int J Gerontol* 2014; **8**: 95-99

15 **Chen JY**, Hsu CT, Liu JH, Tung TH. Clinical predictors of incident gallstone disease in a Chinese population in Taipei, Taiwan. *BMC Gastroenterol* 2014; **14**: 83 [PMID: 24775330 DOI: 10.1186/1471-230X-14-83]

16 **Grundy SM**. Metabolic syndrome scientific statement by the American Heart Association and the National Heart, Lung, and Blood Institute. *Arterioscler Thromb Vasc Biol* 2005; **25**: 2243-2244 [PMID: 16258150]

17 **Grundy SM**, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; **112**: 2735-2752 [PMID: 16157765]

18 **Eckel RH**, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2010; **375**: 181-183 [PMID: 20109902 DOI: 10.1016/S0140-6736(09)61794-3]

19 **Chen LY**, Qiao QH, Zhang SC, Chen YH, Chao GQ, Fang LZ. Metabolic syndrome and gallstone disease. *World J Gastroenterol* 2012; **18**: 4215-4220 [PMID: 22919256 DOI: 10.3748/wjg.v18.i31.4215]

20 **Cojocaru C**, Pandele GI. [Metabolic profile of patients with cholesterol gallstone disease]. *Rev Med Chir Soc Med Nat Iasi* 2010; **114**: 677-682 [PMID: 21243792]

21 **Ruhl CE**, Everhart JE. Gallstone disease is associated with increased mortality in the United States. *Gastroenterology* 2011; **140**: 508-516 [PMID: 21075109 DOI: 10.1053/j.gastro.2010.10.060]

22 **Lowenfels AB**, Domellöf L, Lindström CG, Bergman F, Monk MA, Sternby NH. Cholelithiasis, cholecystectomy, and cancer: a case-control study in Sweden. *Gastroenterology* 1982; **83**: 672-676 [PMID: 7095370]

23 **Portincasa P**, Ciaula AD, Bonfrate L, Wang DQ. Therapy of gallstone disease: What it was, what it is, what it will be. *World J Gastrointest Pharmacol Ther* 2012; **3**: 7-20 [PMID: 22577615 DOI: 10.4292/wjgpt.v3.i2.7]

24 **Puschel K**, Sullivan S, Montero J, Thompson B, Díaz A. [Cost-effectiveness analysis of a preventive program for gallbladder disease in Chile]. *Rev Med Chil* 2002; **130**: 447-459 [PMID: 12090112]

25 **Ubel PA**, Hirth RA, Chernew ME, Fendrick AM. What is the price of life and why doesn't it increase at the rate of inflation? *Arch Intern Med* 2003; **163**: 1637-1641 [PMID: 12885677 DOI: 10.1001/archinte.163.14.1637]

26 **Byrt T**. How good is that agreement? *Epidemiology* 1996; **7**: 561 [PMID: 8862998]

P- Reviewer: Kang JY, Sureka B, Vasilescu A **S- Editor**: Ma YJ
L- Editor: Kerr C **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3344

World J Gastroenterol 2015 March 21; 21(11): 3344-3350
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Weekly pattern of emergency room admissions for peptic ulcers: A population-based study

Li-Ting Kao, Ming-Chieh Tsai, Herng-Ching Lin, Femi Pai, Cha-Ze Lee

Li-Ting Kao, Graduate Institute of Life Science, National Defense Medical Center, Taipei 110, Taiwan
Ming-Chieh Tsai, Division of Gastroenterology, Department of Internal Medicine, Cathay General Hospital, Taipei 110, Taiwan
Herng-Ching Lin, Sleep Research Center, Taipei Medical University Hospital, Taipei 110, Taiwan
Femi Pai, School of Medicine, Taipei Medical University, Taipei 110, Taiwan
Cha-Ze Lee, Department of Internal Medicine, National Taiwan University Hospital and College of Medicine, Taipei 110,Taiwan
Author contributions: Kao LT and Tsai MC conceived of and designed the study; Lin HC analyzed the data; Lee CZ, Kao LT, Pai F, and Kao LT wrote the manuscript.
Ethics approval: This study was based on de-identified secondary data from the LHID2000 released to the public for research purposes, and thus was exempted from full review by the National Defense Medical Center's Internal Review Board.
Informed consent: This study retrieved data from an administrative dataset. Therefore, informed consent was not needed.
Conflict-of-interest: The authors declare no conflicts of interest.
Data sharing: The LHID2000, which is open to researchers, is available from the National Health Research Institute of Taiwan (http://nhird.nhri.org.tw/date_01.html); dataset available from nhird@nhri.org.tw.

Correspondence to: Dr. Cha-Ze Lee, Department of Internal Medicine, National Taiwan University Hospital and College of Medicine, No. 1, Changde St, Taipei 110, Taiwan. czl@ntu.edu.tw
Telephone: +886-2-2736
Fax: +886-2-23789788
Received: November 7, 2014
Peer-review started: November 8, 2014
First decision: November 26, 2014
Revised: December 9, 2014
Accepted: January 8, 2015
Article in press: January 8, 2015
Published online: March 21, 2015

Abstract

AIM: To investigate variations in the incidence of peptic ulcers (PUs) in Taiwan by day of the week within age subgroups.

METHODS: Ambulatory care data were retrieved from the Longitudinal Health Insurance Database 2000. There were 7204 subjects ≥ 18 years-old with an emergency room admission claim for the treatment of PUs, resulting in a total of 9234 emergency room visits for PUs between 2009 and 2011. Data was divided into the seven days of the week and an additional variable for holidays. One-way analysis of variance was used to examine associations among the daily mean number of PU emergency room admissions and holidays/weekends/weekdays.

RESULTS: One-way analysis of variance showed that there was a significant difference in emergency room admissions for PUs by the day of the week ($P < 0.001$), with admission more likely to occur on Sundays or holidays than weekdays within the total and working populations. The weekday patterns of admission were similar for the patients aged 18-64 years and ≥ 65 years of age. Holidays, followed by Sundays, had higher PU admissions than the mean daily PU emergency room admissions. Furthermore, inclusion of only those treated for PUs with hemorrhage or perforation, Sundays and holidays had higher mean emergency room admissions than other days. Inclusion of patients who diagnosed with *Helicobacter pylori* infection, only holidays had higher mean emergency room admissions than other days. Inclusion of patients who had been prescribed non-steroidal anti-inflammatory drugs (NSAIDs) for over

30 d, Sundays and holidays had higher mean PU ER admissions than other non-holiday weekdays.

CONCLUSION: There is a higher incidence of emergency room admission for PUs on weekends than on weekdays for the total and working populations.

Key words: Chronology; Epidemiology; Peptic ulcer; Weekly pattern

Core tip: This study used a population-based dataset in Taiwan to investigate the variations in peptic ulcer (PU) incidence by day of the week within subgroups defined by age. We identified 7204 subjects ⩾ 18 years-old with an emergency room (ER) admission claim for the treatment of PUs, resulting in a total of 9234 ER visits for PU between 2009 and 2011. There was a higher incidence of PU admission on weekends than on weekdays for the total and working populations. Furthermore, Sundays and holidays had higher mean ER admissions than other days for cases of PUs with hemorrhage or perforation.

Kao LT, Tsai MC, Lin HC, Pai F, Lee CZ. Weekly pattern of emergency room admissions for peptic ulcers: A population-based study. *World J Gastroenterol* 2015; 21(11): 3344-3350 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3344.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3344

INTRODUCTION

A peptic ulcer (PU) is a common gastrointestinal tract disorder that is mainly located in the stomach and proximal duodenum[1-3]. Previous studies show that the overall prevalence of PUs varies by country, *e.g.*, 4.1% in northern Sweden[4], 8.4% in the United States[5], and 17.2% in China[6]. In addition, some studies have reported monthly or seasonal variations in PU-related disorders among various regions or countries. An Italian study shows that the number of PU cases is lower in summer than in winter, spring, or autumn[7]. Studies from China and Japan also report that cases of hemorrhage caused by PU increase during the winter and decrease during summer[8-10]. Furthermore, researchers in Turkey have observed a peak in the number of PU incidences in winter, consistent with findings of previous studies[11].

Although prior studies have reported the same pattern of seasonal variation in PU incidence, very few studies have focused on PU onset variation with respect to the daily rhythm of people's lives. The majority of such studies were also performed in the 1980s. For example, studies in Canada and Israel show a higher incidence of perforated PUs on Tuesdays and Wednesdays[12,13], whereas a study in Scotland revealed a high incidence on Fridays and Saturdays[14], and a Norwegian study found a higher incidence on Thursdays and Fridays[15]. Thus, the results of such chronobiologic studies on the daily variation of PU incidence remain inconsistent.

The main purpose of this population-based study was to investigate the variations in PU incidence in Taiwan by day of the week within subgroups defined by age. If the weekly pattern associated with PU onset could be established, it might provide decisive information for clinicians and ultimately, guide in the development of preventive strategies.

MATERIALS AND METHODS

Data source

Ambulatory care data were retrieved from the Longitudinal Health Insurance Database (LHID2000) published by the Taiwan National Health Research Institute. The LHID2000 includes de-identified claims data on one million randomly selected enrollees of Taiwan's National Health Insurance program that was designed to be representative of the total enrolled population as of December 2000 (n = 23.72 million), and thus was exempted from full review by the National Defense Medical Center's Internal Review Board. The Taiwan National Health Research Institute and independent researchers have demonstrated the representative validity of the sample and the high data accuracy of the claims-based LHID2000 on the diagnostic and therapeutic documentation relative to patient charts[16,17].

Study sample

A total of 9234 emergency room (ER) admission claims for the treatment of PU (ICD-9-CM codes 530-531) from 7204 subjects ⩾ 18 years-old between January 2009 and December 2011 were identified. Readmission to the ER within seven days of the first visit was not counted, but treated as part of the same episode.

Statistical analysis

The statistical methods of this study were reviewed by Yi-Hua Chen from the School of Public Health of Taipei Medical University. Data analyses were performed with SPSS 10.0 (SPSS Inc., Chicago, IL, United States) statistical software. Data were divided into the seven days of the week, with an additional variable for holidays (defined as national holidays in Taiwan) falling on weekdays or weekends. The daily mean number of incidences of PU ER admissions within the week for the total and each age group (18-64 years old and ⩾ 65 years old) is presented.

Subgroup analyses were conducted to investigate daily mean incidences of PU ER admissions in patients

Table 1 Demographic characteristics of patients with peptic ulcers admitted to an emergency room in Taiwan between 2009 and 2011 (n = 7204)

Variable	*n* (%)
Age (yr)	
18-44	2426 (33.7)
45-64	2211 (30.7)
⩾ 65	2567 (35.6)
Sex	
Male	3842 (53.3)
Female	3362 (46.7)
Geographic region	
Northern	3099 (43.0)
Central	1878 (26.1)
Southern	1898 (26.4)
Eastern	329 (4.6)
Urbanization level	
1 (most urbanized)	1796 (24.9)
2	1869 (25.9)
3	1137 (15.8)
4	1182 (16.4)
5 (least urbanized)	1220 (16.9)
Monthly income	
NTD 1-15840	3308 (45.9)
NTD 15841-25000	2593 (36.0)
⩾ NTD 25001	1303 (18.1)

who had been diagnosed with *Helicobacter pylori* (*H. pylori*) infection (ICD-9-CM code 04186) and in patients who had been prescribed non-steroidal anti-inflammatory drugs (NSAIDs) for > 30 d, which are important factors for young and elderly patients with PUs[18,19].

One-way analysis of variance was used to examine associations between the daily mean number of PU ER admissions and holidays/weekend days/work days. The percentage of variation from the mean daily figures was calculated as: (specific day ER visit number - mean daily visit number)/mean daily visit number. Data are presented as mean ± SD, and statistical significance was set at $P \leqslant 0.05$.

This study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines[20].

RESULTS

Based on the distribution of sample subjects by demographic characteristics, the mean sample age was 57.5 ± 19.8 years (Table 1). Almost half (43.0%) of the sample subjects resided in northern Taiwan, where Taipei, the most populated city and region in Taiwan, is located. Moreover, the lowest income population (36.0%) was more likely to be affected with PU than the highest income population (18.1%). These results might be due to poor standards of hygiene, psychologic stress, and health risk behaviors in the low socioeconomic status population[21].

In the period of 2009-2011, there was a total of 9234 ER admissions for PU, with a mean of 8.4 ± 3.1 daily PU ER admissions. The daily mean numbers of

Table 2 Emergency room admissions by weekday of patients with peptic ulcers

Variable	Mean	SD	Days	Minimum	Maximum
Monday	8.42	2.86	149	2	18
Tuesday	7.63	2.74	153	2	15
Wednesday	7.91	3.04	152	1	15
Thursday	7.99	2.80	154	2	15
Friday	7.75	2.73	155	2	17
Saturday	8.65	3.09	150	2	18
Sunday	9.98	3.12	152	4	17
Holiday	12.07	5.01	30	5	22

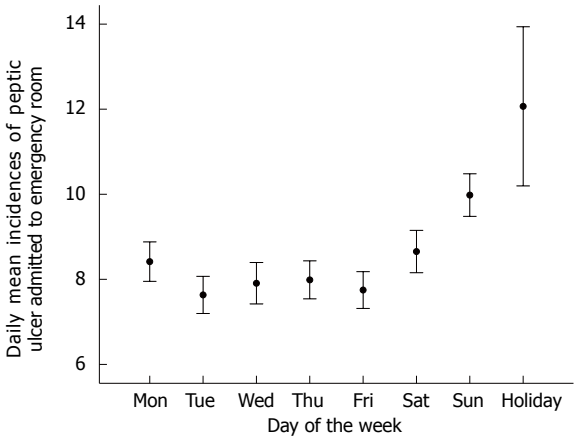


Figure 1 Daily mean incidences and 95%CI of peptic ulcer admitted to the emergency rooms.

PU ER admissions by weekday are presented in Table 2 and Figure 1. Sundays (9.98 ± 3.12) and holidays (12.07 ± 5.01) had higher mean PU ER admissions than other non-holiday weekdays.

Correspondingly, one-way analysis of variance showed a significant weekly variation of PU onset in the 18-64 years of age group ($P < 0.001$), but not for the ⩾ 65 years of age group. The daily mean incidence of PU ER admissions by age group is presented in Figure 2.

The percent variations of daily admissions of each age group from the mean daily admission during the three-year study period are shown in Figure 3. The patterns of weekday-wise variations were similar for the 18-64 and ⩾ 65 years of age groups. The patterns showed an increase on Saturdays and a peak number on holidays; Sundays and holidays had 13.4% and 37.1% higher PU ER admissions, respectively, than the mean daily PU ER admissions.

In addition, limiting the PU ER admissions to those treated for PU with hemorrhage or perforation, Sundays and holidays still had higher mean ER PU admissions than other non-holiday weekdays (Figure 4).

Table 3 presents the daily mean incidences of PU ER admissions for patients diagnosed with *H. pylori* infection. Only holidays had higher mean PU ER admissions than other non-holiday weekdays and

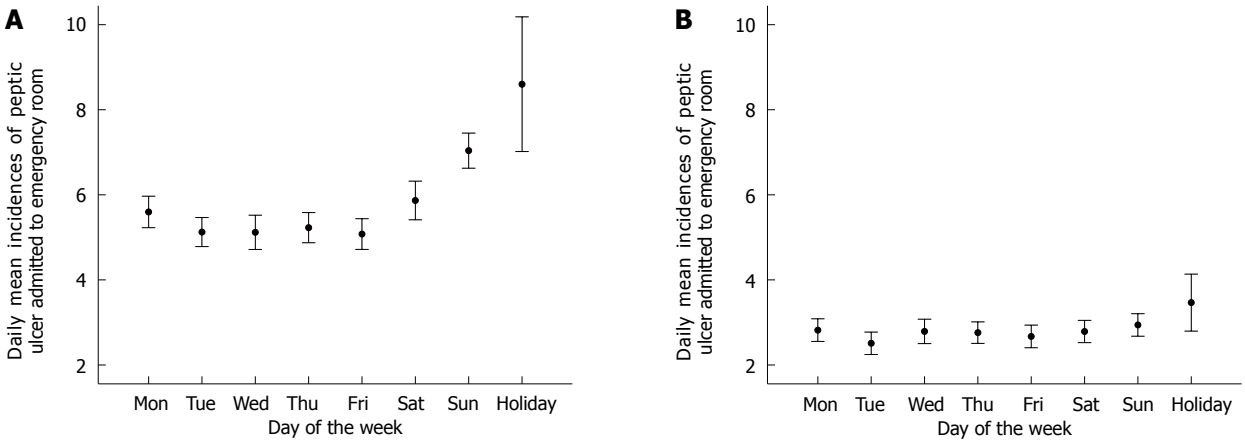


Figure 2 Daily mean incidences and 95%CI of peptic ulcer admitted to the emergency rooms, by different age groups: 18-64 (A) and ⩾ 65 years old (B).

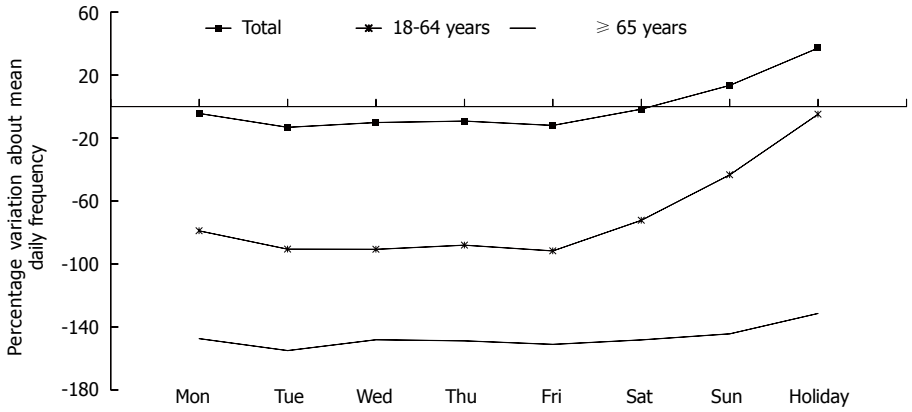


Figure 3 Mean daily average percentage variations in peptic ulcer emergency room visits according to age groups (2009-2011).

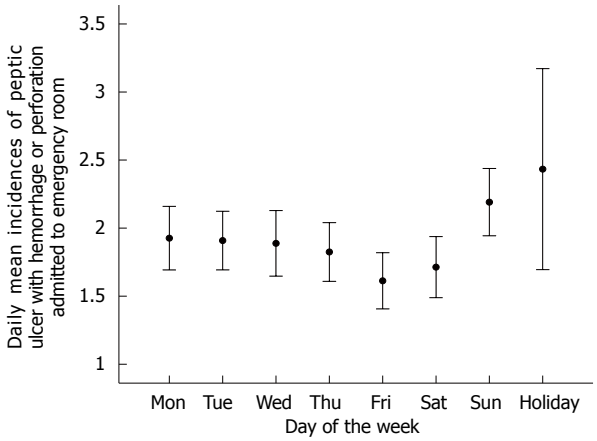


Figure 4 Daily mean incidences and 95%CI of peptic ulcer with hemorrhage or perforation admitted to emergency rooms.

weekends.

Daily mean incidences of PU ER admissions for patients who had been prescribed NSAIDs for over 30 d are shown in Table 4. Sundays and holidays had higher mean PU ER admissions than other non-holiday weekdays.

DISCUSSION

This population-based study demonstrates a statistically significant variation with days of the week for PU ER admissions. The highest ER admissions of PU with or without complications occur on Sundays and holidays. Similar patterns are also observed for ER admissions of PU with hemorrhage and perforation. Moreover, with further stratification by age, a significant weekly variation of PU onset is observed in the group aged 18-64 years, but not for the group of patients ⩾ 65 years.

Numerous studies have investigated diurnal, weekly, or seasonal variations in the onset of different diseases, such as cardiovascular diseases[7,22-24], cerebrovascular diseases[25,26], and psychiatric disorders[27-29], as well as complicated (including hemorrhage and perforation) and uncomplicated PUs[8-11], for which the highest incidences are in winter. This seasonal fluctuation of PU may be affected by several factors, including climatic factors[30], increased intake of NSAIDs in winter[31], significantly increased number of *H. pylori* infections in winter[32], and variations in alcohol consumption[33]. The importance of the diurnal and weekly rhythms in

Table 3 Emergency room admissions by weekday of patients with peptic ulcers diagnosed with *Helicobacter pylori* infection

Variable	Mean	SD	Days	Minimum	Maximum
Monday	3.35	4.56	149	0	16
Tuesday	2.54	4.00	153	0	14
Wednesday	2.57	4.06	152	0	14
Thursday	3.05	4.41	154	0	15
Friday	2.90	4.38	155	0	16
Saturday	3.33	4.88	150	0	18
Sunday	3.63	5.40	152	0	16
Holiday	5.07	7.57	30	0	21

Table 4 Emergency room admissions by weekday of patients with peptic ulcers receiving non-steroidal anti-inflammatory drugs for > 30 d

Variable	Mean	SD	Days	Minimum	Maximum
Monday	3.97	4.83	149	0	16
Tuesday	2.71	4.23	153	0	15
Wednesday	3.81	4.80	152	0	15
Thursdays	3.23	4.51	154	0	15
Friday	3.15	4.46	155	0	17
Saturday	3.61	5.16	150	0	18
Sunday	5.22	5.69	152	0	17
Holiday	9.67	6.89	30	0	22

people's lives has been emphasized for many years and the associated physiologic variables have recently been identified[34].

The discrepancy of our results with previous studies may be due to several factors. First, all of the preceding studies focused on the onset of perforated PU, whereas the present study included both complicated and uncomplicated PUs. Second, the studies in Canada and Israel used data from selected hospitals, preventing the generalizability of their conclusions[12,13]. Third, most of the studies were conducted before *H. pylori* was identified. In recent years, the management of PU has improved and the prevalence of *H. pylori* infection has decreased[35]. Thus, the current fluctuations in PU onset may be different from those reported three decades ago. However, our results of increased PU admissions on weekends and on holidays (defined as off-hour in Taiwan) are in agreement with a recent study in Norway reporting that off-hour admission rates (Saturdays and Sundays and/or during evening and nighttime) of perforated PU are higher than during regular office hours (weekdays and/or daytime)[36].

There are different patterns of weekly variations in PU admission by age in this study. In contrast to the significantly higher PU incidence on weekends and holidays in those 18-64 years of age, the frequency of PU admission is not different between workdays and weekends/holidays in those ⩾ 65 years-old. This phenomenon may be due to dissimilar lifestyles of patients in different age groups. In Taiwan, the retirement age for workers is approximately 65 years-old. Moreover, the lifestyle of people aged ⩾ 65 years is more consistent across the whole week than in the working population. Thus, the weekly variation in PU admission is not observed in the elderly group.

The strengths of this study include the use of a nationwide population-based dataset, which has a single-payer system and covers 99.6% of the population (23 million) in Taiwan[37]. These features can avoid selection bias. This study also uses the diagnostic codes and medical information from the National Health Insurance database rather than the memory of PU patients, preventing recall bias. However, some limitations should also be noted. First, there is no clinical information or records regarding cigarette smoking, alcohol consumption, or emotional stress, which may all be risk factors for PU onset. Second, some PU patients with mild symptoms may not be admitted to the ER, and thus, will not be recorded in the database. This condition may underestimate the onset of PU in this study. It is difficult for patients with severe PUs to endure the pain and associated complications, such as hemorrhage and perforation. Moreover, it is easy for patients to access medical care in Taiwan due to the National Health Insurance system. In addition, most patients will be admitted to ERs immediately when severe PUs occur. ERs are the only medical care sources available on Saturday afternoons, Sundays, and holidays, which can result in an increase number of ER visits on such days. Nonetheless, ER admission is provided on all days of the week, even at night. Therefore, the working population could receive ER health service after getting off work. Furthermore, patients must have a serious symptom in order to be admitted to the ER for observation in Taiwan. In order to mitigate the potential effect of this issue, the weekly variation for only complicated PUs (including hemorrhage and perforation) was investigated, and its fluctuation is similar to that found for both complicated and uncomplicated PUs.

In conclusion, daily fluctuations over the week are observed in PU ER admission. There is a higher incidence of PU admission on weekends than on weekdays for the total population and working populations. The findings may provide some information for clinicians and government officials. Public health authorities can tailor their health promotion events to include information on how to prepare for high-risk PU days and provide strategies to reduce the risk of PU on weekends and holidays.

COMMENTS

Background

A peptic ulcer (PU) is a common gastrointestinal tract disorder that is mainly located in the stomach and proximal duodenum. Many studies have reported monthly or seasonal variations in PU-related disorders within various regions or countries. However, very few studies have focused on the weekly pattern concerning PU onset.

Research frontiers

The importance of the diurnal and weekly rhythms in people's lives has been

emphasized in recent years, and the associated physiologic variables have been identified. However, relevant studies on the weekly fluctuation of PU onset are still limited.

Innovations and breakthroughs

This study demonstrates a statistically significant variation over the days of the week for PU emergency room (ER) admissions. The highest ER admissions for PU with or without complications occur on Sundays and holidays. Similar patterns are also observed for ER admissions of PU with hemorrhage and perforation. Moreover, with further stratification by age, a significant weekly variation of PU admission is observed in the working population.

Applications

Daily fluctuations over the week are observed in PU ER admission. There is a higher incidence of PU admission on weekends than on weekdays for the total and working populations. The findings may provide some information for clinicians and government officials. Public health authorities can tailor their health promotion events to include information on how to prepare for high-risk PU days, and to provide strategies to reduce the risk of PU on weekends and holidays.

Peer-review

This is a good population-based study in which the authors observed the fluctuation of PU ER admissions over the week. The results of this study showed that higher incidence of PU admission occurs on weekends than on weekdays for the total and working populations.

REFERENCES

1 **Najm WI**. Peptic ulcer disease. *Prim Care* 2011; **38**: 383-394, vii [PMID: 21872087 DOI: 10.1016/j.pop.2011.05.001]

2 **Ramakrishnan K**, Salinas RC. Peptic ulcer disease. *Am Fam Physician* 2007; **76**: 1005-1012 [PMID: 17956071]

3 **Malfertheiner P**, Chan FK, McColl KE. Peptic ulcer disease. *Lancet* 2009; **374**: 1449-1461 [PMID: 19683340 DOI: 10.1016/s0140-6736(09)60938-7]

4 **Aro P**, Storskrubb T, Ronkainen J, Bolling-Sternevald E, Engstrand L, Vieth M, Stolte M, Talley NJ, Agréus L. Peptic ulcer disease in a general adult population: the Kalixanda study: a random population-based study. *Am J Epidemiol* 2006; **163**: 1025-1034 [PMID: 16554343 DOI: 10.1093/aje/kwj129]

5 **Garrow D**, Delegge MH. Risk factors for gastrointestinal ulcer disease in the US population. *Dig Dis Sci* 2010; **55**: 66-72 [PMID: 19160043 DOI: 10.1007/s10620-008-0708-x]

6 **Li Z**, Zou D, Ma X, Chen J, Shi X, Gong Y, Man X, Gao L, Zhao Y, Wang R, Yan X, Dent J, Sung JJ, Wernersson B, Johansson S, Liu W, He J. Epidemiology of peptic ulcer disease: endoscopic results of the systematic investigation of gastrointestinal disease in China. *Am J Gastroenterol* 2010; **105**: 2570-2577 [PMID: 20736940 DOI: 10.1038/ajg.2010.324]

7 **Manfredini R**, Manfredini F, Boari B, Bergami E, Mari E, Gamberini S, Salmi R, Gallerani M. Seasonal and weekly patterns of hospital admissions for nonfatal and fatal myocardial infarction. *Am J Emerg Med* 2009; **27**: 1097-1103 [PMID: 19931757 DOI: 10.1016/j.ajem.2008.08.009]

8 **Du T**, Lewin MR, Wang H, Ji X, Bohn HH, Xu T, Xu L, Zhang Y, Li Y. Circadian and seasonal rhythms of acute upper gastrointestinal bleeding in Beijing. *Emerg Med J* 2010; **27**: 504-507 [PMID: 20515908 DOI: 10.1136/emj.2009.075820]

9 **Chen J**, Li D, Xu S, Sun Z, Wang B, Deng C. Influence of meteorological factors on the seasonal onset of esophagogastric variceal bleeding. *O J Gas* 2013; 3: 134-137 [DOI: 10.4236/ojgas.2013.32022]

10 **Nomura T**, Ohkusa T, Araki A, Chuganji Y, Momoi M, Takashimizu I, Watanabe M. Influence of climatic factors in the incidence of upper gastrointestinal bleeding. *J Gastroenterol Hepatol* 2001; **16**: 619-623 [PMID: 11422613]

11 **Kocer B**, Surmeli S, Solak C, Unal B, Bozkurt B, Yildirim O, Dolapci M, Cengiz O. Factors affecting mortality and morbidity in patients with peptic ulcer perforation. *J Gastroenterol Hepatol* 2007; **22**: 565-570 [PMID: 17376052 DOI: 10.1111/j.1440-1746.2006.04500.x]

12 **Cohen MM**. Perforated peptic ulcer in the Vancouver area: a survey of 852 cases. *Can Med Assoc J* 1971; **104**: 201-205 [PMID: 5539727]

13 **Lazarus S**. Perforated peptic ulcer in israel. *Gut* 1964; **5**: 590-596 [PMID: 14244037]

14 **Jamieson RA**. Acute perforated peptic ulcer; frequency and incidence in the West of Scotland. *Br Med J* 1955; **2**: 222-227 [PMID: 14389732]

15 **Svanes C**, Sothern RB, Sørbye H. Rhythmic patterns in incidence of peptic ulcer perforation over 5.5 decades in Norway. *Chronobiol Int* 1998; **15**: 241-264 [PMID: 9653578]

16 **Kang JH**, Chen YH, Lin HC. Comorbidity profiles among patients with ankylosing spondylitis: a nationwide population-based study. *Ann Rheum Dis* 2010; **69**: 1165-1168 [PMID: 20375121 DOI: 10.1136/ard.2009.116178]

17 **Cheng CL**, Kao YH, Lin SJ, Lee CH, Lai ML. Validation of the National Health Insurance Research Database with ischemic stroke cases in Taiwan. *Pharmacoepidemiol Drug Saf* 2011; **20**: 236-242 [PMID: 21351304 DOI: 10.1002/pds.2087]

18 **Rosenstock S**, Jørgensen T, Bonnevie O, Andersen L. Risk factors for peptic ulcer disease: a population based prospective cohort study comprising 2416 Danish adults. *Gut* 2003; **52**: 186-193 [PMID: 12524398]

19 **Huang JQ**, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002; **359**: 14-22 [PMID: 11809181 DOI: 10.1016/s0140-6736(02)07273-2]

20 **von Elm E**, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008; **61**: 344-349 [PMID: 18313558 DOI: 10.1016/j.jclinepi.2007.11.008]

21 **Bytzer P**, Howell S, Leemon M, Young LJ, Jones MP, Talley NJ. Low socioeconomic class is a risk factor for upper and lower gastrointestinal symptoms: a population based study in 15 000 Australian adults. *Gut* 2001; **49**: 66-72 [PMID: 11413112]

22 **Herlitz J**, Eek M, Holmberg M, Holmberg S. Diurnal, weekly and seasonal rhythm of out of hospital cardiac arrest in Sweden. *Resuscitation* 2002; **54**: 133-138 [PMID: 12161292]

23 **Eng H**, Mercer JB. Seasonal variations in mortality caused by cardiovascular diseases in Norway and Ireland. *J Cardiovasc Risk* 1998; **5**: 89-95 [PMID: 9821061]

24 **Arntz HR**, Willich SN, Schreiber C, Brüggemann T, Stern R, Schultheiss HP. Diurnal, weekly and seasonal variation of sudden death. Population-based analysis of 24,061 consecutive cases. *Eur Heart J* 2000; **21**: 315-320 [PMID: 10653679 DOI: 10.1053/euhj.1999.1739]

25 **Manfredini R**, Casetta I, Paolino E, la Cecilia O, Boari B, Fallica E, Granieri E. Monday preference in onset of ischemic stroke. *Am J Med* 2001; **111**: 401-403 [PMID: 11583644]

26 **Wang H**, Sekine M, Chen X, Kagamimori S. A study of weekly and seasonal variation of stroke onset. *Int J Biometeorol* 2002; **47**: 13-20 [PMID: 12461606 DOI: 10.1007/s00484-002-0147-x]

27 **Marriott PF**, Greenwood KM, Armstrong SM. Seasonality in panic disorder. *J Affect Disord* 1994; **31**: 75-80 [PMID: 8071478]

28 **Bulbena A**, Pailhez G, Aceña R, Cunillera J, Rius A, Garcia-Ribera C, Gutiérrez J, Rojo C. Panic anxiety, under the weather? *Int J Biometeorol* 2005; **49**: 238-243 [PMID: 15726446 DOI: 10.1007/s00484-004-0236-0]

29 **Geoffroy PA**, Bellivier F, Scott J, Etain B. Seasonality and bipolar disorder: a systematic review, from admission rates to seasonality of symptoms. *J Affect Disord* 2014; **168**: 210-223 [PMID: 25063960 DOI: 10.1016/j.jad.2014.07.002]

30 **Liu DY**, Gao AN, Tang GD, Yang WY, Qin J, Wu XG, Zhu DC, Wang GN, Liu JJ, Liang ZH. Relationship between onset of peptic ulcer and meteorological factors. *World J Gastroenterol* 2006; **12**: 1463-1467 [PMID: 16552822]

31 **Langman MI**. The seasonal incidence of bleeding from the upper gastrointestinal tract. *Gut* 1964; **5**: 142-144 [PMID: 14159402]

32 **Moshkowitz M**, Konikoff FM, Arber N, Peled Y, Santo M,

Bujanover Y, Gilat T. Seasonal variation in the frequency of Helicobacter pylori infection: a possible cause of the seasonal occurrence of peptic ulcer disease. *Am J Gastroenterol* 1994; **89**: 731-733 [PMID: 8172147]

33 **Manfredini R**, De Giorgio R, Smolensky MH, Boari B, Salmi R, Fabbri D, Contato E, Serra M, Barbara G, Stanghellini V, Corinaldesi R, Gallerani M. Seasonal pattern of peptic ulcer hospitalizations: analysis of the hospital discharge data of the Emilia-Romagna region of Italy. *BMC Gastroenterol* 2010; **10**: 37 [PMID: 20398297 DOI: 10.1186/1471-230x-10-37]

34 **Tuomisto MT**, Terho T, Korhonen I, Lappalainen R, Tuomisto T, Laippala P, Turjanmaa V. Diurnal and weekly rhythms of health-related variables in home recordings for two months. *Physiol Behav* 2006; **87**: 650-658 [PMID: 16500686 DOI: 10.1016/j.physbeh.2005.12.012]

35 **Sung JJ**, Kuipers EJ, El-Serag HB. Systematic review: the global incidence and prevalence of peptic ulcer disease. *Aliment Pharmacol Ther* 2009; **29**: 938-946 [PMID: 19220208 DOI: 10.1111/j.1365-2036.2009.03960.x]

36 **Thorsen K**, Søreide JA, Kvaløy JT, Glomsaker T, Søreide K. Epidemiology of perforated peptic ulcer: age- and gender-adjusted analysis of incidence and mortality. *World J Gastroenterol* 2013; **19**: 347-354 [PMID: 23372356 DOI: 10.3748/wjg.v19.i3.347]

37 **Bureau of National Health Insurance**. Universal Health Coverage in Taiwan, May 2012. Available from: URL: http://www.nhi.gov.tw/Resource/webdata/21717_1_20120808UniversalHealthCoverage.pdf

P- Reviewer: Matsuda A, Tripathi D, Xu JM **S- Editor**: Qi Y
L- Editor: AmEditor **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3351

World J Gastroenterol 2015 March 21; 21(11): 3351-3360
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

META-ANALYSIS

Diagnostic value of magnetic resonance cholangiopancreatography in choledocholithiasis

Wen Chen, Jing-Jia Mo, Li Lin, Chao-Qun Li, Jian-Feng Zhang

Wen Chen, Department of Educational Administration, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China
Jing-Jia Mo, Li Lin, Chao-Qun Li, Jian-Feng Zhang, Department of Emergency, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China
Author contributions: Mo JJ formulated the research questions; Zhang JF and Chen W designed the study; Zhang JF, Chen W, and Mo JJ searched the databases; Lin L and Li CQ designed the data abstraction form and served as second reviewers for data extraction; Zhang JF extracted the data; Zhang JF and Mo JJ analyzed the data; Zhang JF and Chen W wrote the manuscript; Chen W revised the manuscript; all authors have read and approved the final manuscript.

Correspondence to: Dr. Jian-Feng Zhang, MD, Department of Emergency, The First Affiliated Hospital of Guangxi Medical University, No. 22, Shuangyong Road, Guangxi Zhuang Autonomous Region, Nanning 530021, China. zfj@188.com
Telephone: +86-771-5356501
Fax: +86-771-5356585
Received: July 5, 2014
Peer-review started: July 7, 2014
First decision: August 15, 2014
Revised: August 26, 2014
Accepted: October 15, 2014
Article in press: October 15, 2014
Published online: March 21, 2015

Abstract

AIM: To evaluate the diagnostic accuracy of magnetic resonance cholangiopancreatography (MRCP) in patients with choledocholithiasis.

METHODS: We systematically searched MEDLINE, EMBASE, Web of Science, and Cochrane databases for studies reporting on the sensitivity, specificity and other accuracy measures of diagnostic effectiveness of MRCP for detection of common bile duct (CBD) stones. Pooled analysis was performed using random effects models, and receiver operating characteristic curves were generated to summarize overall test performance. Two reviewers independently assessed the methodological quality of studies using standards for reporting diagnostic accuracy and quality assessment for studies of diagnostic accuracy tools.

RESULTS: A total of 25 studies involving 2310 patients with suspected choledocholithiasis and 738 patients with CBD stones met the inclusion criteria. The average inter-rater agreement on the methodological quality checklists was 0.96. Pooled analysis of the ability of MRCP to detect CBD stones showed the following effect estimates: sensitivity, 0.90 (95%CI: 0.88-0.92, χ^2 = 65.80; P < 0.001); specificity, 0.95 (95%CI: 0.93-1.0, χ^2 = 110.51; P < 0.001); positive likelihood ratio, 13.28 (95%CI: 8.85-19.94, χ^2 = 78.95; P < 0.001); negative likelihood ratio, 0.13 (95%CI: 0.09-0.18, χ^2 = 6.27; P < 0.001); and diagnostic odds ratio, 143.82 (95%CI: 82.42-250.95, χ^2 = 44.19; P < 0.001). The area under the receiver operating characteristic curve was 0.97. Significant publication bias was not detected (P = 0.266).

CONCLUSION: MRCP has high diagnostic accuracy for the detection of choledocholithiasis. MRCP should be the method of choice for suspected cases of CBD stones.

Key words: Choledocholithiasis; Diagnosis; Magnetic resonance cholangiopancreatography; Common bile duct; Meta-analysis

Core tip: Unlike endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography (MRCP) is noninvasive, can be performed rapidly and has demonstrated good results for the detection of common bile duct stones. Moreover, MRCP does not expose patients to ionizing radiation or iodinated contrast media, which is useful for evaluating biliopancreatic disease. However, the selective use of MRCP in clinically equivocal situations has not been explored until now. The goal of this study was to evaluate the effectiveness of MRCP for the detection of common bile duct stones in patients with suspected choledocholithiasis.

Chen W, Mo JJ, Lin L, Li CQ, Zhang JF. Diagnostic value of magnetic resonance cholangiopancreatography in choledocholithiasis. *World J Gastroenterol* 2015; 21(11): 3351-3360 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3351.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3351

INTRODUCTION

The incidence of choledocholithiasis in patients with the common disorder, cholelithiasis, varies between 7% and 20%, of which 5% are asymptomatic[1,2]. Although common bile duct (CBD) stones may be silent, the development of complications such as cholangitis and acute pancreatitis is associated with major morbidity and mortality. Therefore, the detection and treatment of CBD stones is mandatory.

Usually, the diagnosis of choledocholithiasis is based on a combination of clinical suspicion (biliary colic, jaundice and cholangitis), biochemical analysis (raised conjugated bilirubin and alkaline phosphatase levels) and imaging findings. Individually, these indicators have varying levels of diagnostic accuracy and none represent a completely reliable method for identifying bile duct stones[3]. Intraoperative cholangiography (IOC) is standard procedure during open cholecystectomy that can detect CBD stones with a sensitivity of 100% and specificity of 98%[4]. It is an invasive investigation with intraoperative and postoperative morbidity of 6.3% and 15.9%, respectively. Its routine use is associated with increased cost and operating time[5].

Endoscopic retrograde cholangiopancreatography (ERCP) is the gold standard for both diagnosis and treatment of CBD stones. It also allows direct visualization of duct anatomy. However, the procedure is associated with an overall complication rate of 5%-10% and mortality rate of 0.02%-0.50%[6-8]. Ductal cannulation is difficult or impossible in patients who have undergone previous surgery, which includes Billroth type Ⅱ gastrectomy and hepaticoenterostomy. Early ERCP and stone extraction after endoscopic sphincterotomy decrease morbidity in patients with severe biliary pancreatitis. However, ERCP and endoscopic sphincterotomy are invasive procedures that may cause serious complications[7,9] and can potentially exacerbate acute pancreatitis[6]. Therefore, an accurate, safe, and efficacious method is needed to diagnose CBD stones in a definitive manner.

The diagnostic accuracy of endoscopic ultrasonography (EUS) for biliary tract stone disease is > 95%, which is less invasive than ERCP and is reliable at identifying bile duct stones[10-13]. However, its results are highly dependent on the operator, and the procedure is not widely available in clinical practice. In addition, visualization of all segments of the biliary tract may be incomplete or unsuccessful during EUS[11].

In many institutions, magnetic resonance cholangiopancreatography (MRCP) is replacing ERCP as a diagnostic procedure for the investigation of benign biliary obstructions and chronic pancreatitis, in part due to its comparable accuracy[14]. MRCP has an advantage because of its technical versatility, multiplanar capability, superior soft tissue resolution, and the potential to evaluate choledocholithiasis accurately in the preoperative acute calculous cholecystitis setting. Unlike ERCP, MRCP is noninvasive, can be performed rapidly, does not expose the patients to ionizing radiation or iodinated contrast materials[15], which is useful for evaluating biliopancreatic disease, and has good results for detecting CBD stones[16]. All segments of the biliary tree can be visualized using MRCP. Although ERCP is considered the standard for diagnosis of bile duct stones, small bile duct stones can be overlooked[17]. However, the selective use of MRCP in clinically equivocal situations has not been explored until now. The goal of this study was, therefore, to rigorously evaluate the effectiveness of MRCP for detection of CBD stones in patients with suspected choledocholithiasis *via* systematic review and meta-analysis.

MATERIALS AND METHODS

Search strategy

In March 2014, we searched MEDLINE (1980-2014), EMBASE (1980-2014), Web of Science (1990-2014) and Cochrane databases to identify studies. Although no language restrictions were imposed initially, only English-language articles were included for the full-text review and final analysis. Additional articles were searched using the "Related articles" function in PubMed and by manually searching reference lists of identified articles and review articles. The following search terms were used: "magnetic resonance cholangiopancreatography" or "MRCP" and "common bile duct" or "choledocholithiasis" and "diagnosis" and "sensitivity" and "specificity." We contacted experts in the field to ask about studies that we may have missed in the databases. Conference abstracts and letters to the editor were excluded because of the limited data they contained.

Study inclusion criteria

A study was included when it provided both the sensitivity (true-positive rate) and specificity (false-positive rate) of using MRCP for detection of CBD stones in patients of any age with suspected choledocholithiasis. Studies were also included if they reported the values of MRCP effectiveness in a scatter plot format that allowed patient data to be extracted. Studies were excluded if they involved fewer than ten patients with suspected choledocholithiasis to reduce selection bias due to small numbers of participants. Patients had to be diagnosed with choledocholithiasis based on ERCP and/or IOC. Two reviewers (Mo JJ, Lin L) independently determined study eligibility, and disagreements were resolved by consensus.

Data extraction and *quality assessment*

Two reviewers (Mo JJ, Lin L) independently confirmed the eligibility of the final set of studies and extracted the following data: first author, publication year, participant characteristics, assay methods, sensitivity and specificity data, and methodological quality. The values of MRCP effectiveness provided in scatter plots were extracted by placing scalar grids over the plots. A receiver operating characteristic (ROC) curve was calculated for each study (IBM Inc., Armonk, NY, United States).

To enable us to assess the methodological quality of the included studies, we extracted data on the following study design characteristics: (1) cross-sectional or case-control design; (2) consecutive or random sampling of patients; (3) blinded (single or double) or non-blinded interpretation of experimental and reference measurements; and (4) prospective or retrospective data collection. The two reviewers (Mo JJ, Lin L) independently assessed the methodological quality of studies using the standards for reporting diagnostic accuracy (STARD) guidelines[18], which provide for a maximum score of 25, and quality assessment for studies of diagnostic accuracy (QUADAS) guidelines[19], which provide for a maximum score of 14. Average inter-rater agreement on the methodological quality checklists was 0.96. If primary studies did not report information needed to assess methodological quality, we contacted the authors in an effort to obtain the data. If the authors did not respond, we changed the response for the relevant items from "not reported" to "no" on the assessment instruments.

Statistical analysis

Standard methods recommended for meta-analyses of diagnostic test evaluations were used[20]. Analyses were performed using professional statistical software program (Meta-DiSc for Windows; XI Cochrane Colloquium; Barcelona, Spain) and Stata version 12.0 (Stata Corporation, College Station, TX, United States). The following measures of test accuracy were analyzed for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR). A summary ROC (SROC) curve[21] was generated for each study based on a single test threshold for sensitivity and specificity[20,22]. A random effects model was adopted to calculate the average sensitivity, specificity, and other measures across studies[23,24].

To assess the effects of STARD and QUADAS scores on the diagnostic power of MRCP, we included them as covariates in a univariate, inverse variance-weighted meta-regression. We also analyzed the effects of other covariates on DOR, such as cross-sectional design, consecutive or random sampling of patients, single- or double-blinded interpretation of experimental and reference measurements, and prospective or retrospective data collection. The relative DOR (RDOR) was calculated to analyze the change in diagnostic precision in each study per unit increase in the covariate[25,26].

The heterogeneity, or variability, across studies was assessed for statistical significance using the χ^2 and Fisher's exact tests. Publication bias can pose problems for meta-analyses of diagnostic studies, therefore, we tested for the potential presence of this bias with funnel plots and the Egger's test[27].

RESULTS

Selection and summary of studies

We identified 292 citations *via* electronic searches, and 40 were retrieved for detailed analysis (Figure 1). Six studies were excluded for failing to satisfy the inclusion criteria[28-33], and another three were excluded because they failed to provide sufficient information[34-36]. Two articles were meta-analyses[37,38]. One paper was excluded because it was a Chinese study[39]. One study was a duplicate publication[3]. One study was excluded for being a reply letter[40] and one paper was excluded for involving fewer than 10 participants[41]. In the end, 25 publications were included in the analysis[42-66], involving 2310 patients with suspected choledocholithiasis and 738 with CBD stones. The average sample size of the studies was 69 patients (range: 27-278). Table 1 summarizes the clinical characteristics of participants in each study; numbers of true positives, false positives, false negatives and true negatives; and STARD and QUADAS scores.

Methodological quality of the included studies

Of the 25 studies in the meta-analysis, 23 had STARD scores $\geqslant$ 13, and 21 had QUADAS scores $\geqslant$ 10. All studies collected data from consecutive patients. There were nine randomized, prospective, blinded trials according to the corresponding reference measurements (Table 2).

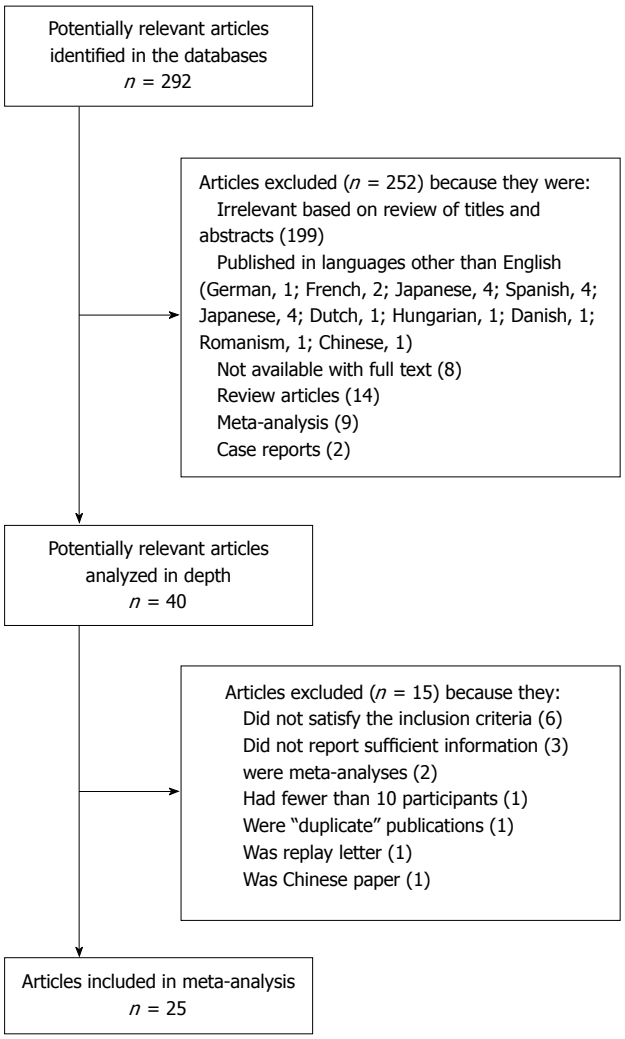


Figure 1 Flow chart of study selection.

Diagnostic accuracy

A Forest plot of MRCP values in all 25 included studies showed that the sensitivity of MRCP in detection of CBD stones ranged from 0.38 to 1.0 (mean 0.90, 95%CI: 0.88-0.92, χ^2 = 65.80; P < 0.001), while the specificity ranged from 0.19 to 1.0 (mean 0.95, 95%CI: 0.93-1.00, χ^2 = 110.51; P < 0.001) (Figure 2). The PLR was 13.28 (95%CI: 8.85-19.94, χ^2 = 78.95; P < 0.001), NLR was 0.13 (95%CI: 0.09-0.18, χ^2 = 66.27; P < 0.001) and DOR was 143.82 (95%CI: 82.42-250.95, χ^2 = 44.19; P < 0.001). These χ^2 and associated P values indicate significant heterogeneity among studies. The ten randomized controlled trials (RCTs) showed that the sensitivity, specificity, PLR, NLR and DOR of MRCP in detection of CBD stones was 0.91, 0.95, 10.83, 0.13 and 136.32, respectively.

Unlike the traditional ROC plot for assessing diagnostic power, an SROC plot reveals the effect of varying thresholds on sensitivity and specificity in a single study. Different studies appear as different data points in an SROC plot. In this way, SROC curves provide a global summary of test performance and illustrate the trade-off between sensitivity and specificity. Figure 3 shows an SROC curve for rates of true and false positives from individual studies of MRCP detection. Using this plot, we determined the Q value, defined as the point of intersection of the SROC curve with a diagonal line extending from the left upper corner to the right lower corner of the plot. The Q value indicates the highest identical value of sensitivity and specificity, thereby serving as an overall measure of the discriminatory power of a test. Our SROC curve was desirably positioned near the upper left corner, and the maximum joint sensitivity and specificity was 0.92. The area under the curve was 0.97, indicating high overall accuracy.

Multiple regression analysis and publication bias

Quality scores based on the STARD[18] and QUADAS[19] guidelines were generated for every study on the basis of the title and introduction, methods, results and discussion (Table 1). These scores were used in a meta-regression to assess the effect of study quality on the RDOR of MRCP in the diagnosis of CBD stones. As shown in Table 3, studies of higher quality (STARD score ⩾ 13; QUADAS score ⩾ 10) produced RDOR values similar to those of lower-quality studies. In addition, RDOR values did not differ significantly as a function of blinding, cross-sectional or case-control design, consecutive or random sampling, prospective or retrospective design (all P > 0.05). These results suggest that study design did not significantly affect diagnostic accuracy and that the risk of detection bias was lower. The Egger's test showed no evidence of significant publication bias in the reporting of MRCP detection as a method for diagnosis of CBD stones (P = 0.266).

DISCUSSION

Although MRCP can provide an accurate diagnosis of CBD stones, only a few investigators have evaluated its utility in the preoperative evaluation of symptomatic gallstones. Accordingly, the precise role of MRCP in this regard has yet to be determined. Some authors recommend MRCP for patients with a moderate risk of CBD stones and ERCP before any other imaging examination for patients who are at high risk[67,68]. Others recommend MRCP for patients with a high or moderate risk for CBD stones and ERCP for patients in whom stones have been depicted by other imaging modalities[69].

MRCP has recently been developed as a noninvasive, yet highly sensitive, method for diagnosing diseases of the biliary tract. One meta-analysis that included 15 studies concluded that the sensitivity of MRCP for diagnosis of choledocholithiasis ranged from 0.5 to 1.0, while specificity ranged from 0.83 to 1.0[37]. Another systematic review including five RCTs showed that the aggregated sensitivity and specificity of MRCP for the detection of choledocholithiasis were 0.85 and

Table 1 Summarized details of magnetic resonance cholangiopancreatography detections and overall methodological quality of included studies

Ref.	Year	Patients, *n*	Assay method	Assay system	Assay results				Quality score	
					TP	FP	FN	TN	STARD	QUADAS
Hochwald *et al*[42]	1998	48	MRCP, ERCP	1.5 T machine	19	3	1	25	15	11
Boraschi *et al*[43]	1999	278	MRCP, ERCP	1.5 T MR unit	71	5	5	197	16	11
de Lédinghen *et al*[44]	1999	32	EUS, MRCP, ERCP	1 T system	10	6	0	16	20	13
Lomas *et al*[45]	1999	69	MRCP, ERCP	1.5 T MR system	9	2	0	58	13	9
Varghese *et al*[46]	1999	100	MRCP, ERCP	1.5 GE unit	28	1	2	69	17	12
Stiris *et al*[47]	2000	50	MRCP, ERCP	1.0 T	28	1	4	17	17	12
Taylor *et al*[48]	2002	129	MRCP, ERCP	1.5 T MR system	45	9	1	74	18	12
Topal *et al*[49]	2003	69	MRCP, ERCP	1.5 T MR system	18	0	1	50	14	10
Kejriwal *et al*[50]	2004	81	MRCP, ERCP	Vision 1.5T MRI	20	1	2	58	13	10
Simone *et al*[51]	2004	65	MRCP, ERCP, IOC	1.0 T gyroscan NT	13	6	8	38	13	9
Dalton *et al*[52]	2005	69	MRCP, ERCP, IOC	1.5 T MR unit	16	2	1	50	11	7
Hallal *et al*[53]	2005	27	MRCP, ERCP, IOC	Unknown	4	2	0	21	14	10
Kondo *et al*[54]	2005	28	EUS, MRCP, HCT-C	1.5 T MR system	21	1	3	3	18	13
Moon *et al*[55]	2005	29	IDUS, MRCP, ERCP	1.5T MR system	16	1	4	8	17	11
Okada *et al*[56]	2005	40	CTCh, MRCP	1.5 T system	12	3	3	22	13	9
Shanmugam *et al*[57]	2005	221	MRCP, ERCP, EUS	0.5 T MRI	97	19	2	103	18	14
De Waele *et al*[58]	2007	104	MRCP, ERCP, EUS	1.5 T unit	19	2	4	79	16	11
Schmidt *et al*[59]	2007	57	MRCP, ERCP, EUS	1 T magnet	17	2	5	33	15	10
Hekimoglu *et al*[60]	2008	269	MRCP, ERCP	1.5 T unit	16	0	2	251	19	14
Nandalur *et al*[61]	2008	95	MRCP, ERCP	1.5 T system	21	1	7	66	18	13
Norero *et al*[62]	2008	125	MRCP, ERCP, CT	1.5 T MR system	83	10	3	29	15	11
Srinivasa *et al*[63]	2010	117	MRCP, ERCP, IOC	Siemens Vision 1.5 T	15	2	8	102	16	12
Bilgin *et al*[64]	2012	108	MRCP, ERCP, IOC	1.5 T MR scanner	28	3	6	71	16	11
Zhang *et al*[65]	2012	70	MRCP, MDCT	1.5 T MR system	19	2	1	48	18	13
Mandelia *et al*[66]	2013	30	MRCP, USG	1.5 T MR system	19	1	1	9	17	12

CT: Computed tomography; CTCh: Cholangiography computed tomography; ERCP: Endoscopic retrograde cholangiopancreatography; EUS: Endoscopic ultrasonography; FN: False-negative; FP: False-positive; HCT-C: Helical-computed-tomographic cholangiography; IDUS: Intraductal ultrasonography; IOC: Intraoperative cholangiography; MDCT: Multidetector-row computed tomography; MR: Magnetic resonance; MRCP: Magnetic resonance cholangiopancreatography; QUADAS: Quality assessment for studies of diagnostic accuracy; STARD: Standards for reporting diagnostic accuracy; TN: True-negative; TP: True-positive; USG: Ultrasonography.

Table 2 Additional characteristics of patients and methodologies in the included studies

Ref.	Year	Country	CBD/N-CBD, *n*	Reference standard	Cross-sectional design	Consecutive or random sampling	Blinded design	Prospective design
Hochwald *et al*[42]	1998	United States	20/28	ERCP	No	Yes	No	No
Boraschi *et al*[43]	1999	Italy	76/202	ERCP, PTC, IOC	No	Yes	No	No
de Lédinghen *et al*[44]	1999	France	10/-22	ERCP, IOC	Yes	Yes	Yes	Yes
Lomas *et al*[45]	1999	United Kingdom	9/60	ERCP	No	Yes	No	Yes
Varghese *et al*[46]	1999	Ireland	30/70	ERCP	No	Yes	Yes	Yes
Stiris *et al*[47]	2000	Norway	32/18	ERCP	Yes	Yes	Yes	Yes
Taylor *et al*[48]	2002	Australia	46/83	ERCP	Yes	Yes	Yes	Yes
Topal *et al*[49]	2003	Belgium	19/50	ERCP, IOC	No	Yes	No	No
Kejriwal *et al*[50]	2004	New Zealand	22/59	ERCP	No	Yes	No	No
Simone *et al*[51]	2004	France	21/44	ERCP, IOC	No	Yes	Yes	Yes
Dalton *et al*[52]	2005	United Kingdom	17/52	ERCP, IOC	No	Yes	No	Yes
Hallal *et al*[53]	2005	United States	4/-23	IOC	Yes	Yes	Yes	Yes
Kondo *et al*[54]	2005	Japan	24/-4	ERCP	Yes	Yes	Yes	Yes
Moon *et al*[55]	2005	South Korea	20/-9	ERCP, IDUS	No	Yes	Yes	Yes
Okada *et al*[56]	2005	Japan	15/25	IOC	No	Yes	Yes	No
Shanmugam *et al*[57]	2005	United Kingdom	99/122	ERCP, IOC	Yes	Yes	No	No
De Waele *et al*[58]	2007	Belgium	23/81	ERCP, IOC	No	Yes	No	Yes
Schmidt *et al*[59]	2007	Switzerland	22/35	EUS, ERCP	No	Yes	No	Yes
Hekimoglu *et al*[60]	2008	Turkey	18/251	ERCP	No	Yes	Yes	Yes
Nandalur *et al*[61]	2008	United States	28/67	ERCP, PTC	Yes	Yes	No	No
Norero *et al*[62]	2008	Chile	86/39	ERCP	No	Yes	No	No
Srinivasa *et al*[63]	2010	Australia	23/104	ERCP, IOC	No	Yes	No	No
Bilgin *et al*[64]	2012	Turkey, Germany	34/74	ERCP, PTC	No	Yes	No	No
Zhang *et al*[65]	2012	China	20/50	MDCT	No	Yes	Yes	No
Mandelia *et al*[66]	2013	India	20/-10	ERCP	No	Yes	No	Yes

CBD: Common bile duct; ERCP: Endoscopic retrograde cholangiopancreatography; EUS: Endoscopic ultrasonography; IDUS: Intraductal ultrasonography; IOC: Intraoperative cholangiography; MDCT: Multidetector-row computed-tomography; PTC: Percutaneous transhepatic cholangiography.

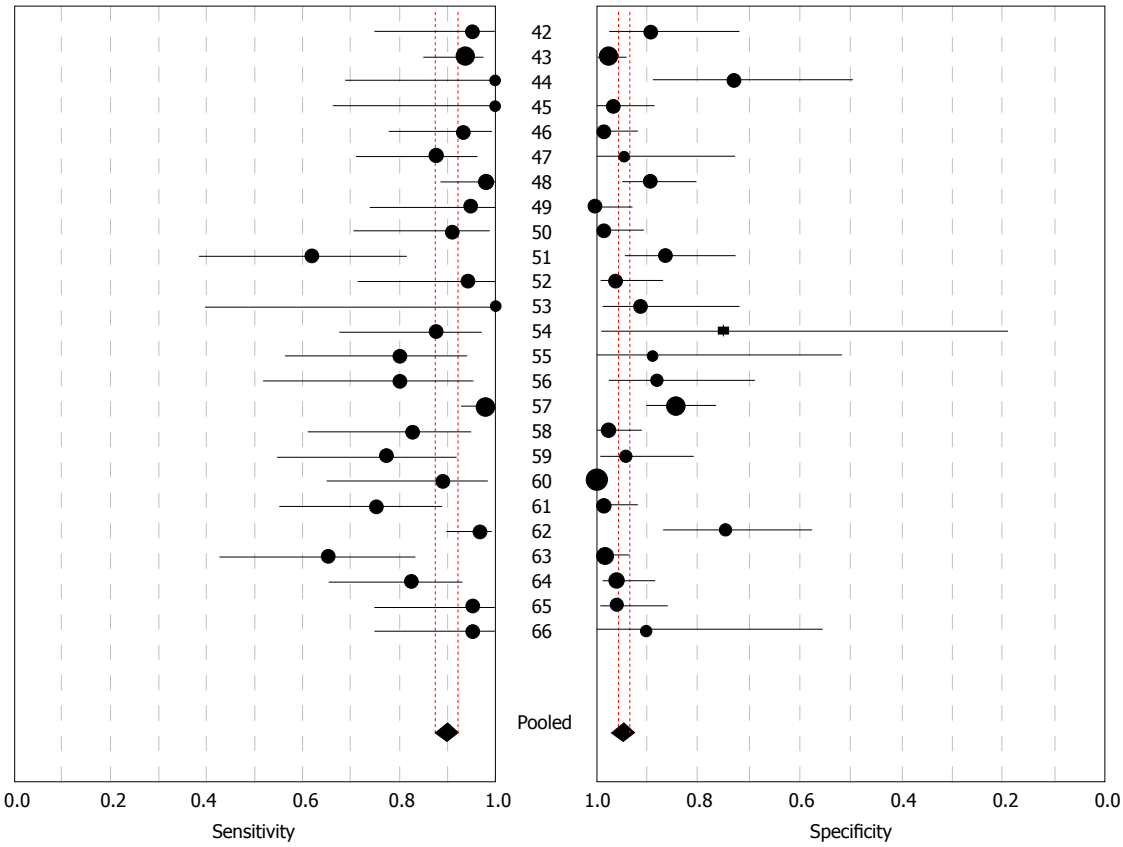


Figure 2 Forest plot showing sensitivity and specificity of magnetic resonance cholangiopancreatography in the diagnosis of choledocholithiasis. The point estimates of sensitivity and specificity from each study are shown as solid circles. Horizontal error bars indicate 95%CIs. Numbers between the plots refer to references. Pooled estimates for the magnetic resonance cholangiopancreatography detections were 0.90 for sensitivity (95%CI: 0.88-0.92) and 0.95 for specificity (95%CI: 0.93-1.0).

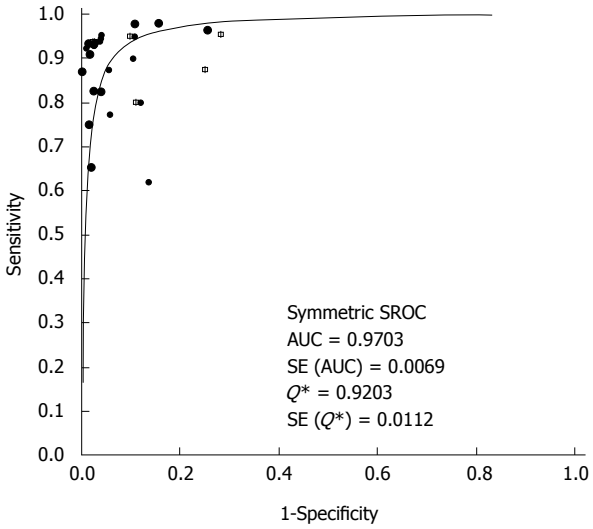


Figure 3 Summary receiver operating characteristic curves for magnetic resonance cholangiopancreatography detection. Solid circles represent each study included in the meta-analysis. The size of each study is indicated by the size of the solid circle. Summary receiver operating characteristic (SROC) curves summarize the overall diagnostic accuracy; AUC: Area under the curve.

0.93, respectively[38]. In this review, we provide high-quality systematic evidence for MRCP as a predictor of choledocholithiasis, demonstrating high sensitivity and specificity for predicting CBD stones with high overall accuracy.

DOR is an indicator of test accuracy that combines sensitivity and specificity data into a single number[70]. The DOR is the ratio of the odds of positive test results in patients with disease relative to the odds of positive test results in patients without disease. The value of a DOR ranges from 0 to infinity, with higher values indicating better discriminatory test performance (higher accuracy). A DOR of 1.0 indicates that a test does not discriminate between patients with the disorder and those without it. Thus, higher DOR values indicate better discriminatory test performance. The mean DOR in our study was 143.82, indicating a high level of overall accuracy.

The SROC curve and DOR are difficult to interpret and relate to clinical practice, whereas likelihood ratios are more clinically meaningful[71], therefore, we also calculated PLRs and NLRs to assess diagnostic accuracy. Likelihood ratios of > 10.0 or < 0.1 indicate high accuracy. The overall PLR value in our meta-analysis indicates that patients with CBD stones have an approximately 13-fold higher chance of being positive for MRCP detection compared with patients without choledocholithiasis. This high probability is considered sufficient to begin or continue ERCP/IOC

Table 3 Weighted meta-regression for the effects of design, methods and quality of studies on diagnostic accuracy of magnetic resonance cholangiopancreatography detections

Covariate	Studies (*n*)	Coefficient	RDOR (95%CI)	*P* value
QUADAS ⩾ 10	21	0.0830	1.09 (0.14-8.50)	0.9334
STARD ⩾ 13	23	1.5100	4.53 (0.51-40.21)	0.1637
Prospective design	14	0.1260	1.13 (0.27-4.82)	0.8564
Cross-sectional design	7	0.0980	1.10 (0.24-5.06)	0.8936
Blinded design	11	-0.6850	0.50 (0.13-2.02)	0.3130
Consecutive/ random sampling	25	-	-	-

RDOR: Relative diagnostic odds ratio; STARD: Standards for reporting diagnostic accuracy; QUADAS: Quality assessment for studies of diagnostic accuracy.

treatment of choledocholithiasis patients. In contrast, the NLR value in our meta-analysis indicates that a patient without choledocholithiasis would still have a 13% chance of having CBD stones, which is insufficient to rule out choledocholithiasis. These findings suggest that a negative MRCP detection result should not be used alone as a justification to deny or discontinue CBD stone therapy. A better approach may be a combined diagnostic strategy drawing on clinical information as well as findings from clinical symptoms, ERCP, EUS, and/or serum bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase levels.

An exploration of the reasons for heterogeneity rather than the computation of a single summary measure is an important goal of meta-analyses[72]. In our study, both STARD and QUADAS scores were used in the meta-regression analysis to assess the effect of study quality on RDOR. Most of the studies were high quality (STARD score ⩾ 13 or QUADAS score ⩾ 10). We found that there was no statistical heterogeneity for sensitivity, specificity, PLR, NLR, or DOR among the studies, which indicates that the differences for studies with or without blinded, cross-sectional, consecutive/random and prospective designs did not reach statistical significance, and the study design did not substantially affect diagnostic accuracy.

The present meta-analysis had several limitations. First, the exclusion of conference abstracts, letters to editors, and non-English-language studies may have led to publication bias, although our bias analysis suggests that this was not a significant problem. Second, nonrandom misclassification bias may have occurred due to the fact that different studies used various approaches to diagnose choledocholithiasis, including ERCP, IOC and/or EUS. Third, we did not identify multicenter and large, blinded RCTs that satisfied our inclusion criteria.

In conclusion, MRCP is a noninvasive investigation with fewer complications and it has high sensitivity, specificity and positive and negative predictive values for detection of CBD stones. We propose MRCP as the best method of choice for suspected cases of CBD stones, instead of ERCP, IOC and EUS, because of its high diagnostic accuracy and excellent features with technical versatility, multiplanar capability, and noninvasive nature.

COMMENTS

Background

Endoscopic retrograde cholangiopancreatography (ERCP) is applied both as a diagnostic and therapeutic tool. However, ERCP has significant morbidity of l%-7% and mortality of 0.2%-1.0%. Unlike ERCP, magnetic resonance cholangiopancreatography (MRCP) is noninvasive, can be performed rapidly, and does not expose the patients to ionizing radiation or iodinated contrast materials, which is useful for evaluating biliopancreatic disease. Moreover, MRCP has demonstrated good results for detecting common bile duct (CBD) stones. However, the selective use of MRCP in clinically equivocal situations has not been explored until now.

Research frontiers

MRCP is a noninvasive method for diagnosing choledocholithiasis. The selective use of MRCP in clinically equivocal situations has not been explored until now.

Innovations and breakthroughs

This study is believed to be the first rigorous evaluation of the effectiveness of MRCP for detection of CBD stones in patients with suspected choledocholithiasis, using a meta-analysis.

Applications

MRCP should be the method of choice for suspected cases of CBD stones because of its technical versatility, multiplanar capability, and noninvasive nature.

Peer-review

This is a very interesting and useful paper. The manuscript is well written and the method for statistical evaluation is properly used. In the clinical situation, it is sometimes difficult to correctly detect small stones or sludge as well as multiple stones by MRCP.

REFERENCES

1 **Mitchell SA**, Jacyna MR, Chadwick S. Common bile duct stones: a controversy revisited. *Br J Surg* 1993; **80**: 759-760 [PMID: 8330169]

2 **Del Santo P**, Kazarian KK, Rogers JF, Bevins PA, Hall JR. Prediction of operative cholangiography in patients undergoing elective cholecystectomy with routine liver function chemistries. *Surgery* 1985; **98**: 7-11 [PMID: 4012608]

3 **Varghese JC**, Liddell RP, Farrell MA, Murray FE, Osborne H, Lee MJ. The diagnostic accuracy of magnetic resonance cholangiopancreatography and ultrasound compared with direct cholangiography in the detection of choledocholithiasis. *Clin Radiol* 1999; **54**: 604-614 [PMID: 10505997]

4 **Montariol T**, Msika S, Charlier A, Rey C, Bataille N, Hay JM, Lacaine F, Fingerhut A. Diagnosis of asymptomatic common bile duct stones: preoperative endoscopic ultrasonography versus intraoperative cholangiography--a multicenter, prospective controlled study. French Associations for Surgical Research. *Surgery* 1998; **124**: 6-13 [PMID: 9663245]

5 **Demartines N**, Eisner L, Schnabel K, Fried R, Zuber M, Harder F. Evaluation of magnetic resonance cholangiography in the management of bile duct stones. *Arch Surg* 2000; **135**: 148-152 [PMID: 10668871]

6 **Loperfido S**, Angelini G, Benedetti G, Chilovi F, Costan F, De Berardinis F, De Bernardin M, Ederle A, Fina P, Fratton A. Major early complications from diagnostic and therapeutic ERCP: a prospective multicenter study. *Gastrointest Endosc* 1998; **48**: 1-10 [PMID: 9684657]

7 **Freeman ML**, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ, Moore JP, Fennerty MB, Ryan ME, Shaw MJ,

Lande JD, Pheley AM. Complications of endoscopic biliary sphincterotomy. *N Engl J Med* 1996; **335**: 909-918 [PMID: 8782497 DOI: 10.1056/NEJM199609263351301]
8 **Masci E**, Toti G, Mariani A, Curioni S, Lomazzi A, Dinelli M, Minoli G, Crosta C, Comin U, Fertitta A, Prada A, Passoni GR, Testoni PA. Complications of diagnostic and therapeutic ERCP: a prospective multicenter study. *Am J Gastroenterol* 2001; **96**: 417-423 [PMID: 11232684 DOI: 10.1111/j.1572-0241.2001.03594.x]
9 **Sherman S**, Ruffolo TA, Hawes RH, Lehman GA. Complications of endoscopic sphincterotomy. A prospective series with emphasis on the increased risk associated with sphincter of Oddi dysfunction and nondilated bile ducts. *Gastroenterology* 1991; **101**: 1068-1075 [PMID: 1889699]
10 **Amouyal P**, Amouyal G, Lévy P, Tuzet S, Palazzo L, Vilgrain V, Gayet B, Belghiti J, Fékété F, Bernades P. Diagnosis of choledocholithiasis by endoscopic ultrasonography. *Gastroenterology* 1994; **106**: 1062-1067 [PMID: 8143973]
11 **Palazzo L**, Girollet PP, Salmeron M, Silvain C, Roseau G, Canard JM, Chaussade S, Couturier D, Paolaggi JA. Value of endoscopic ultrasonography in the diagnosis of common bile duct stones: comparison with surgical exploration and ERCP. *Gastrointest Endosc* 1995; **42**: 225-231 [PMID: 7498687]
12 **Shim CS**, Joo JH, Park CW, Kim YS, Lee JS, Lee MS, Hwang SG. Effectiveness of endoscopic ultrasonography in the diagnosis of choledocholithiasis prior to laparoscopic cholecystectomy. *Endoscopy* 1995; **27**: 428-432 [PMID: 8549439 DOI: 10.1055/s-2007-1005735]
13 **Sugiyama M**, Atomi Y. Endoscopic ultrasonography for diagnosing choledocholithiasis: a prospective comparative study with ultrasonography and computed tomography. *Gastrointest Endosc* 1997; **45**: 143-146 [PMID: 9040999]
14 **Brisbois D**, Plomteux O, Nchimi A, Hock D, Dupont P, Delforge M, Bastens B, Weerts J, Magotteaux P. [Value of MRCP for detection of choledocholithiasis in symptomatic patients: one-year experience with a standardized high resolution breath-hold technique]. *JBR-BTR* 2001; **84**: 258-261 [PMID: 11822367]
15 **Sperlongano P**, Pisaniello D, Del Viscovo L, De Falco M, Parmeggiani D, Piatto A, Parmeggiani U. Efficacy of magnetic resonance cholangiopancreatography in detecting common bile duct lithiasis: our experience. *Chir Ital* 2005; **57**: 635-640 [PMID: 16241096]
16 **Chan YL**, Chan AC, Lam WW, Lee DW, Chung SS, Sung JJ, Cheung HS, Li AK, Metreweli C. Choledocholithiasis: comparison of MR cholangiography and endoscopic retrograde cholangiography. *Radiology* 1996; **200**: 85-89 [PMID: 8657949 DOI: 10.1148/radiology.200.1.8657949]
17 **Prat F**, Amouyal G, Amouyal P, Pelletier G, Fritsch J, Choury AD, Buffet C, Etienne JP. Prospective controlled study of endoscopic ultrasonography and endoscopic retrograde cholangiography in patients with suspected common-bileduct lithiasis. *Lancet* 1996; **347**: 75-79 [PMID: 8538344]
18 **Bossuyt PM**, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, Lijmer JG, Moher D, Rennie D, de Vet HC. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. The Standards for Reporting of Diagnostic Accuracy Group. *Croat Med J* 2003; **44**: 635-638 [PMID: 14515428]
19 **Whiting P**, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003; **3**: 25 [PMID: 14606960 DOI: 10.1186/1471-2288-3-25]
20 **Devillé WL**, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, Bezemer PD. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002; **2**: 9 [PMID: 12097142]
21 **Lau J**, Ioannidis JP, Balk EM, Milch C, Terrin N, Chew PW, Salem D. Diagnosing acute cardiac ischemia in the emergency department: a systematic review of the accuracy and clinical effect of current technologies. *Ann Emerg Med* 2001; **37**: 453-460 [PMID: 11326181 DOI: 10.1067/mem.2001.114903]
22 **Moses LE**, Shapiro D, Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: data-analytic approaches and some additional considerations. *Stat Med* 1993; **12**: 1293-1316 [PMID: 8210827]
23 **Irwig L**, Tosteson AN, Gatsonis C, Lau J, Colditz G, Chalmers TC, Mosteller F. Guidelines for meta-analyses evaluating diagnostic tests. *Ann Intern Med* 1994; **120**: 667-676 [PMID: 8135452]
24 **Vamvakas EC**. Meta-analyses of studies of the diagnostic accuracy of laboratory tests: a review of the concepts and methods. *Arch Pathol Lab Med* 1998; **122**: 675-686 [PMID: 9701328]
25 **Suzuki S**, Moro-oka T, Choudhry NK. The conditional relative odds ratio provided less biased results for comparing diagnostic test accuracy in meta-analyses. *J Clin Epidemiol* 2004; **57**: 461-469 [PMID: 15196616 DOI: 10.1016/j.jclinepi.2003.09.017]
26 **Westwood ME**, Whiting PF, Kleijnen J. How does study quality affect the results of a diagnostic meta-analysis? *BMC Med Res Methodol* 2005; **5**: 20 [PMID: 15943861 DOI: 10.1186/1471-2288-5-20]
27 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563]
28 **Shamiyeh A**, Lindner E, Danis J, Schwarzenlander K, Wayand W. Short- versus long-sequence MRI cholangiography for the preoperative imaging of the common bile duct in patients with cholecystolithiasis. *Surg Endosc* 2005; **19**: 1130-1134 [PMID: 16021379 DOI: 10.1007/s00464-004-2167-6]
29 **Anderson SW**, Rho E, Soto JA. Detection of biliary duct narrowing and choledocholithiasis: accuracy of portal venous phase multidetector CT. *Radiology* 2008; **247**: 418-427 [PMID: 18372450 DOI: 10.1148/radiol.2472070473]
30 **McMahon CJ**. The relative roles of magnetic resonance cholangiopancreatography (MRCP) and endoscopic ultrasound in diagnosis of common bile duct calculi: a critically appraised topic. *Abdom Imaging* 2008; **33**: 6-9 [PMID: 17874159 DOI: 10.1007/s00261-007-9304-3]
31 **Chang JH**, Lee IS, Lim YS, Jung SH, Paik CN, Kim HK, Kim TH, Kim CW, Han SW, Choi MG, Jung IS. Role of magnetic resonance cholangiopancreatography for choledocholithiasis: analysis of patients with negative MRCP. *Scand J Gastroenterol* 2012; **47**: 217-224 [PMID: 22149906 DOI: 10.3109/00365521.2011.638394]
32 **Epelboym I**, Winner M, Allendorf JD. MRCP is not a cost-effective strategy in the management of silent common bile duct stones. *J Gastrointest Surg* 2013; **17**: 863-871 [PMID: 23515912 DOI: 10.1007/s11605-013-2179-4]
33 **Richard F**, Boustany M, Britt LD. Accuracy of magnetic resonance cholangiopancreatography for diagnosing stones in the common bile duct in patients with abnormal intraoperative cholangiograms. *Am J Surg* 2013; **205**: 371-373 [PMID: 23518180 DOI: 10.1016/j.amjsurg.2012.07.033]
34 **Kim HJ**, Park DI, Park JH, Cho YK, Sohn CI, Jeon WK, Kim BI, Kim SK. Multidetector computed tomography cholangiography with multiplanar reformation for the assessment of patients with biliary obstruction. *J Gastroenterol Hepatol* 2007; **22**: 400-405 [PMID: 17295774 DOI: 10.1111/j.1440-1746.2006.04503.x]
35 **Wong HP**, Chiu YL, Shiu BH, Ho LC. Preoperative MRCP to detect choledocholithiasis in acute calculous cholecystitis. *J Hepatobiliary Pancreat Sci* 2012; **19**: 458-464 [PMID: 21983892 DOI: 10.1007/s00534-011-0456-8]
36 **Bhat M**, Romagnuolo J, da Silveira E, Reinhold C, Valois E, Martel M, Barkun JS, Barkun AN. Randomised clinical trial: MRCP-first vs. ERCP-first approach in patients with suspected biliary obstruction due to bile duct stones. *Aliment Pharmacol Ther* 2013; **38**: 1045-1053 [PMID: 24024705 DOI: 10.1111/apt.12481]
37 **Kaltenthaler EC**, Walters SJ, Chilcott J, Blakeborough A, Vergel YB, Thomas S. MRCP compared to diagnostic ERCP for diagnosis when biliary obstruction is suspected: a systematic review. *BMC Med Imaging* 2006; **6**: 9 [PMID: 16907974 DOI: 10.1186/1471-2342-6-9]

38 **Verma D**, Kapadia A, Eisen GM, Adler DG. EUS vs MRCP for detection of choledocholithiasis. *Gastrointest Endosc* 2006; **64**: 248-254 [PMID: 16860077 DOI: 10.1016/j.gie.2005.12.038]

39 **Chen Y**. Diagnosis of common bile duct stones before ERCP: An analysis of 238 cases. *Shijie Huaren Xiaohua Zazhi* 2013; **21**: 1811 [DOI: 10.11569/wcjd.v21.i19.1811]

40 **Lindsell DR**. The diagnostic accuracy of magnetic resonance cholangiopancreatography (MRCP) and ultrasound compared with direct cholangiography in the detection of choledocholithiasis. *Clin Radiol* 2000; **55**: 579 [PMID: 10924386 DOI: 10.1053/crad.1999.0426]

41 **Scheiman JM**, Carlos RC, Barnett JL, Elta GH, Nostrant TT, Chey WD, Francis IR, Nandi PS. Can endoscopic ultrasound or magnetic resonance cholangiopancreatography replace ERCP in patients with suspected biliary disease? A prospective trial and cost analysis. *Am J Gastroenterol* 2001; **96**: 2900-2904 [PMID: 11693324 DOI: 10.1111/j.1572-0241.2001.04245.x]

42 **Hochwald SN**, Dobryansky M BA, Rofsky NM, Naik KS, Shamamian P, Coppa G, Marcus SG. Magnetic resonance cholangiopancreatography accurately predicts the presence or absence of choledocholithiasis. *J Gastrointest Surg* 1998; **2**: 573-579 [PMID: 10457316]

43 **Boraschi P**, Neri E, Braccini G, Gigoni R, Caramella D, Perri G, Bartolozzi C. Choledocolithiasis: diagnostic accuracy of MR cholangiopancreatography. Three-year experience. *Magn Reson Imaging* 1999; **17**: 1245-1253 [PMID: 10576709]

44 **de Lédinghen V**, Lecesne R, Raymond JM, Gense V, Amouretti M, Drouillard J, Couzigou P, Silvain C. Diagnosis of choledocholithiasis: EUS or magnetic resonance cholangiography? A prospective controlled study. *Gastrointest Endosc* 1999; **49**: 26-31 [PMID: 9869719]

45 **Lomas DJ**, Bearcroft PW, Gimson AE. MR cholangiopancreatography: prospective comparison of a breath-hold 2D projection technique with diagnostic ERCP. *Eur Radiol* 1999; **9**: 1411-1417 [PMID: 10460385]

46 **Varghese JC**, Farrell MA, Courtney G, Osborne H, Murray FE, Lee MJ. A prospective comparison of magnetic resonance cholangiopancreatography with endoscopic retrograde cholangiopancreatography in the evaluation of patients with suspected biliary tract disease. *Clin Radiol* 1999; **54**: 513-520 [PMID: 10484218]

47 **Stiris MG**, Tennøe B, Aadland E, Lunde OC. MR cholangiopancreaticography and endoscopic retrograde cholangiopancreaticography in patients with suspected common bile duct stones. *Acta Radiol* 2000; **41**: 269-272 [PMID: 10866083]

48 **Taylor AC**, Little AF, Hennessy OF, Banting SW, Smith PJ, Desmond PV. Prospective assessment of magnetic resonance cholangiopancreatography for noninvasive imaging of the biliary tree. *Gastrointest Endosc* 2002; **55**: 17-22 [PMID: 11756908 DOI: 10.1067/mge.2002.120324]

49 **Topal B**, Van de Moortel M, Fieuws S, Vanbeckevoort D, Van Steenbergen W, Aerts R, Penninckx F. The value of magnetic resonance cholangiopancreatography in predicting common bile duct stones in patients with gallstone disease. *Br J Surg* 2003; **90**: 42-47 [PMID: 12520573 DOI: 10.1002/bjs.4025]

50 **Kejriwal R**, Liang J, Anderson G, Hill A. Magnetic resonance imaging of the common bile duct to exclude choledocholithiasis. *ANZ J Surg* 2004; **74**: 619-621 [PMID: 15315557 DOI: 10.1111/j.1445-1433.2004.03114.x]

51 **Simone M**, Mutter D, Rubino F, Dutson E, Roy C, Soler L, Marescaux J. Three-dimensional virtual cholangioscopy: a reliable tool for the diagnosis of common bile duct stones. *Ann Surg* 2004; **240**: 82-88 [PMID: 15213622]

52 **Dalton SJ**, Balupuri S, Guest J. Routine magnetic resonance cholangiopancreatography and intra-operative cholangiogram in the evaluation of common bile duct stones. *Ann R Coll Surg Engl* 2005; **87**: 469-470 [PMID: 16263021 DOI: 10.1308/003588405x51137]

53 **Hallal AH**, Amortegui JD, Jeroukhimov IM, Casillas J, Schulman CI, Manning RJ, Habib FA, Lopez PP, Cohn SM, Sleeman D. Magnetic resonance cholangiopancreatography accurately detects common bile duct stones in resolving gallstone pancreatitis. *J Am Coll Surg* 2005; **200**: 869-875 [PMID: 15922197 DOI: 10.1016/j.jamcollsurg.2005.02.028]

54 **Kondo S**, Isayama H, Akahane M, Toda N, Sasahira N, Nakai Y, Yamamoto N, Hirano K, Komatsu Y, Tada M, Yoshida H, Kawabe T, Ohtomo K, Omata M. Detection of common bile duct stones: comparison between endoscopic ultrasonography, magnetic resonance cholangiography, and helical-computed-tomographic cholangiography. *Eur J Radiol* 2005; **54**: 271-275 [PMID: 15837409 DOI: 10.1016/j.ejrad.2004.07.007]

55 **Moon JH**, Cho YD, Cha SW, Cheon YK, Ahn HC, Kim YS, Kim YS, Lee JS, Lee MS, Lee HK, Shim CS, Kim BS. The detection of bile duct stones in suspected biliary pancreatitis: comparison of MRCP, ERCP, and intraductal US. *Am J Gastroenterol* 2005; **100**: 1051-1057 [PMID: 15842578 DOI: 10.1111/j.1572-0241.2005.41057.x]

56 **Okada M**, Fukada J, Toya K, Ito R, Ohashi T, Yorozu A. The value of drip infusion cholangiography using multidetector-row helical CT in patients with choledocholithiasis. *Eur Radiol* 2005; **15**: 2140-2145 [PMID: 15968515 DOI: 10.1007/s00330-005-2820-z]

57 **Shanmugam V**, Beattie GC, Yule SR, Reid W, Loudon MA. Is magnetic resonance cholangiopancreatography the new gold standard in biliary imaging? *Br J Radiol* 2005; **78**: 888-893 [PMID: 16177010 DOI: 10.1259/bjr/51075444]

58 **De Waele E**, Op de Beeck B, De Waele B, Delvaux G. Magnetic resonance cholangiopancreatography in the preoperative assessment of patients with biliary pancreatitis. *Pancreatology* 2007; **7**: 347-351 [PMID: 17703081 DOI: 10.1159/000107269]

59 **Schmidt S**, Chevallier P, Novellas S, Gelsi E, Vanbiervliet G, Tran A, Schnyder P, Bruneton JN. Choledocholithiasis: repetitive thick-slab single-shot projection magnetic resonance cholangiopancreaticography versus endoscopic ultrasonography. *Eur Radiol* 2007; **17**: 241-250 [PMID: 16941091 DOI: 10.1007/s00330-006-0380-5]

60 **Hekimoglu K**, Ustundag Y, Dusak A, Erdem Z, Karademir B, Aydemir S, Gundogdu S. MRCP vs. ERCP in the evaluation of biliary pathologies: review of current literature. *J Dig Dis* 2008; **9**: 162-169 [PMID: 18956595 DOI: 10.1111/j.1751-2980.2008.00339.x]

61 **Nandalur KR**, Hussain HK, Weadock WJ, Wamsteker EJ, Johnson TD, Khan AS, D'Amico AR, Ford MK, Nandalur SR, Chenevert TL. Possible biliary disease: diagnostic performance of high-spatial-resolution isotropic 3D T2-weighted MRCP. *Radiology* 2008; **249**: 883-890 [PMID: 18941164 DOI: 10.1148/radiol.2493080389]

62 **Norero E**, Norero B, Huete A, Pimentel F, Cruz F, Ibáñez L, Martínez J, Jarufe N. [Accuracy of magnetic resonance cholangiopancreatography for the diagnosis of common bile duct stones]. *Rev Med Chil* 2008; **136**: 600-605 [PMID: 18769807]

63 **Srinivasa S**, Sammour T, McEntee B, Davis N, Hill AG. Selective use of magnetic resonance cholangiopancreatography in clinical practice may miss choledocholithiasis in gallstone pancreatitis. *Can J Surg* 2010; **53**: 403-407 [PMID: 21092433]

64 **Bilgin M**, Toprak H, Burgazli M, Bilgin SS, Chasan R, Erdogan A, Balcı C. Diagnostic value of dynamic contrast-enhanced magnetic resonance imaging in the evaluation of the biliary obstruction. *ScientificWorldJournal* 2012; **2012**: 731089 [PMID: 22489200 DOI: 10.1100/2012/731089]

65 **Zhang ZY**, Wang D, Ni JM, Yu XR, Zhang L, Wu WJ, Gong L, Hu MH. Comparison of three-dimensional negative-contrast CT cholangiopancreatography with three-dimensional MR cholangiopancreatography for the diagnosis of obstructive biliary diseases. *Eur J Radiol* 2012; **81**: 830-837 [PMID: 21377820 DOI: 10.1016/j.ejrad.2011.02.036]

66 **Mandelia A**, Gupta AK, Verma DK, Sharma S. The Value of Magnetic Resonance Cholangio-Pancreatography (MRCP) in the Detection of Choledocholithiasis. *J Clin Diagn Res* 2013; **7**: 1941-1945 [PMID: 24179904 DOI: 10.7860/jcdr/2013/6158.3365]

67 **Dwerryhouse SJ**, Brown E, Vipond MN. Prospective evaluation of magnetic resonance cholangiography to detect common

bile duct stones before laparoscopic cholecystectomy. *Br J Surg* 1998; **85**: 1364-1366 [PMID: 9782014 DOI: 10.1046/j.1365-2168.1998.00957.x]

68 **Liu TH**, Consorti ET, Kawashima A, Ernst RD, Black CT, Greger PH, Fischer RP, Mercer DW. The efficacy of magnetic resonance cholangiography for the evaluation of patients with suspected choledocholithiasis before laparoscopic cholecystectomy. *Am J Surg* 1999; **178**: 480-484 [PMID: 10670857]

69 **Zidi SH**, Prat F, Le Guen O, Rondeau Y, Rocher L, Fritsch J, Choury AD, Pelletier G. Use of magnetic resonance cholangiography in the diagnosis of choledocholithiasis: prospective comparison with a reference imaging method. *Gut* 1999; **44**: 118-122 [PMID: 9862837]

70 **Glas AS**, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. *J Clin Epidemiol* 2003; **56**: 1129-1135 [PMID: 14615004]

71 **Deeks JJ**. Systematic reviews in health care: Systematic reviews of evaluations of diagnostic and screening tests. *BMJ* 2001; **323**: 157-162 [PMID: 11463691]

72 **Petitti DB**. Approaches to heterogeneity in meta-analysis. *Stat Med* 2001; **20**: 3625-3633 [PMID: 11746342]

P- Reviewer: Cardinale V, Kikuyama M, Tan HJ, Yagi H
S- Editor: Gou SX **L- Editor**: AmEditor **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3361

World J Gastroenterol 2015 March 21; 21(11): 3361-3366
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

New therapeutic option for irritable bowel syndrome: Serum-derived bovine immunoglobulin

Larry Good, Roxanne Rosario, Raymond Panas

Larry Good, Department of Gastroenterology, South Nassau Communities Hospital, Oceanside, NY 11572, United States
Roxanne Rosario, Department of Research, Good Pharmaceutical Development Company, LLC, Lynbrook, NY 11563, United States
Raymond Panas, Department of Medical Affairs, Entera Health, Inc., Cary, NC 27518, United States
Author contributions: Good L and Rosario R had full access to review and pull available data from the medical records; Good L and Panas R consolidated and analyzed the collected data; Good L, Rosario R, and Panas R contributed to the organization and writing of the manuscript.

Correspondence to: Larry Good, MD, Department of Gastroenterology, South Nassau Communities Hospital, No. 444 Merrick Road, Lynbrook, NY 11572, United States. good1b@optonline.com
Telephone: +1-516-766-0300
Fax: +1-516-766-2444
Received: August 19, 2014
Peer-review started: August 20, 2014
First decision: September 27, 2014
Revised: October 17, 2014
Accepted: December 16, 2014
Article in press: December 16, 2014
Published online: March 21, 2015

Abstract

Oral prescription medical foods have long been used in hospital settings but are also appropriate therapies for gastrointestinal disorders in outpatient medical practice. Oral serum-derived bovine immunoglobulin/protein isolate (SBI) has been shown in clinical studies to reduce loose stools and improve stool consistency as well as other symptoms (*i.e.*, abdominal pain, bloating, and urgency) in patients with irritable bowel syndrome with diarrhea (IBS-D) and human immunodeficiency virus-associated enteropathy. This case series reports the outcomes of 14 IBS patients who received SBI as an addition to standard of care at an individual physician's clinical practice. The patients: 2 IBS with constipation (IBS-C), 7 IBS-D, 2 mixed diarrhea and constipation IBS (IBS-M) and 3 undefined IBS (IBS-U; also described by some physicians as IBS-Bloating), ranged in age from 22-87 years. SBI (5 g or 10 g daily dose) was added to the patient's current standard care and followed for several weeks to determine if symptoms were improved with the addition of SBI. Overall, 12 of the 14 patients indicated some level of improvement through direct questioning of the patients regarding changes from the prior visit. One IBS-Bloating patient had a resolution of symptoms and two patients (1 IBS-Bloating and 1 IBS-C) discontinued therapy because of insufficient relief. The 12 patients who continued on therapy reported an overall improvement in symptoms with better stool consistency, decreased frequency as well as reductions in abdominal pain, bloating, distention, and incontinence. In most cases, therapeutic effects of SBI were seen within the first four weeks of therapy with continued improvements at subsequent visits. SBI has a multifaceted mechanism of action and may help to manage IBS by providing a distinct protein source required to normalize bowel function, gastrointestinal microbiota, and nutritionally enhance tight junction protein expression between intestinal epithelial cells. SBI as a medical food provides a safe option for patients with IBS-D but may have application in other forms of IBS.

Key words: Irritable bowel syndrome; Diarrhea; Immunoglobulin; Bovine; Serum-derived; Gastrointestinal disease; Medical food

Core tip: Oral prescription medical foods are becoming part of the outpatient medical practice and are finding new uses as a therapeutic option for gastrointestinal disorders. This case series investigates the use of oral serum-derived bovine immunoglobulin/protein isolate (SBI) in the management of differing forms of irritable bowel syndrome (IBS). Because of the multifaceted mechanism of action, SBI provides a distinct protein source to normalize bowel function, gastrointestinal microbiota, and nutritionally enhance tight junction protein expression. As such, there may be potential use for patients with other forms of IBS besides IBS-D. Additional research is needed to explore this use.

Good L, Rosario R, Panas R. New therapeutic option for irritable bowel syndrome: Serum-derived bovine immunoglobulin. *World J Gastroenterol* 2015; 21(11): 3361-3366 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3361.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3361

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder that is defined based upon the presence of abdominal pain and a change in bowel habit[1,2]. It is further categorized based upon stool consistency leading to a diagnosis of IBS with either constipation (IBS-C), diarrhea (IBS-D), mixed with alternating constipation and diarrhea (IBS-M) or undefined (IBS-U), which may have symptoms of bloating and distention. Population-based studies have found IBS to be a common disorder affecting from 9%-22% of the population[3,4].

IBS is the most commonly diagnosed gastrointestinal (GI) disorder and has both a detrimental impact on patient quality of life as well as affecting work productivity [2,5,6]. When compared to another GI disorder like gastroesophageal reflux disease (GERD), IBS patients had significantly greater impairment in the ability to carry out daily activities of living and basic work activities, which led to a greater loss of work[5,6]. IBS patients often suffer from other comorbidities such as anxiety, depression, fibromyalgia, migraine headaches, interstitial cystitis and temporomandibular joint syndrome[2]. The impact from changes in quality of life and activities of daily living results in an estimated annual economic burden of $25-50 billion[2,5,6].

Successful management of IBS is dependent upon symptom relief but options tend to be limited. For those with IBS-D, the primary goal is to manage bowel symptoms (reduce stool frequency, urgency, and bloating; improve stool consistency), while managing abdominal symptoms (pain and discomfort). There is no single accepted therapy for IBS. While there are some limited evidence-based recommendations and guidelines, there is no general consensus among clinicians for specific treatment options. Alosetron, a 5HT3 antagonist, was originally approved for women with severe IBS-D because serotonin has been shown to affect motility and pain; however the safety profile has limited its use[7,8]. Rifixamin is an oral antibiotic that has shown potential benefits for some IBS-D patients, and has been shown to reduce abdominal pain in patients with IBS[9,10]. Other options include tricyclic antidepressants which can cause constipation, but can be of benefit improving stool consistency as well as addressing pain[11]. Anti-diarrheals, like loperamide and diphenoxylate hydrochloride/atropine, can increase transit time thereby allowing for increased water absorption[12]. Bulking agents such as methylcellulose and psyllium fiber also help with stool consistency[13]. Low FODMAP (fermentable oligo-, di-, and monosaccharides and polyol sugars) diets are yet another option to help address discomfort, bloating and flatulence by minimizing the ingestion of certain sugars and vegetables[14]. Despite the potential benefit of these various approaches, they all tend to provide limited improvements in patient symptoms leaving many patients unsatisfied with the overall effectiveness. As such, patients continue to seek other therapy options.

SBI (EnteraGam™) is a prescription medical food product intended for the clinical dietary management of intestinal disorders in patients with chronic loose and frequent stools who have a limited or impaired capacity to ingest, digest, absorb, or metabolize certain nutrients; it is used under physician supervision[15]. SBI is a specially-formulated protein source consisting of > 90% protein, of which > 50% is immunoglobulin G (IgG)[15]. Studies have demonstrated that SBI is safe and improves gastrointestinal symptoms (*e.g.*, chronic loose and frequent stools, abdominal discomfort, bloating, and urgency) in patients with ISB-D[16] or HIV-associated enteropathy[17]. Approximately 25%-50% of orally administered IgG survives digestion in the stomach and small intestine[18]. The mechanism of action of SBI is postulated to involve binding to microbial components, maintaining immune balance in the gastrointestinal tract, managing gut barrier function including increasing expression of the tight junction proteins zonala occludens-1 (ZO1) and occludin, and improving nutrient uptake[18]. As such, SBI may provide distinct nutrition in the form of immunoglobulins and other proteins for patients and physicians when conventional therapies fail to adequately manage IBS-D.

CASE REPORT

IBS has different clinical expressions based upon stool consistency or frequency, as well as other associated gastrointestinal symptoms. This retrospective chart analysis explores the use of SBI in the management of 14 IBS patients with differing forms: 7 IBS-D, 2 IBS-C, 2 IBS-M, and 3 IBS-Bloating (IBS-U) through clinical observations, physician questioning and patient

Table 1 Presentation of irritable bowel syndrome patients with diarrhea

Patient No./age (yr)/gender	Primary symptoms	Comorbidity	Other GI therapy	SBI therapy/ duration	Outcome
IBS-D 1/24/M	Diarrhea, frequency urgency, ABD pain	Chronic urethritis ulcerative proctitis	Low FODMAP diet, mesalamine	Ongoing 32 wk	Complete resolution of symptoms
IBS-D 2/36/F	Diarrhea urgency incontinence	Hypothyroidism anxiety, depression	None	Ongoing 18 wk	Marked improvement in urgency and diarrhea
IBS-D 3/63/M	Diarrhea Flatulence, ABD cramps, urgency	Eosinophilic esophagitis, RIH, BPH	Pantoprazole	Ongoing 27 wk	Complete resolution of symptoms
IBS-D 4/86/M	Loose stools, urgency, cramping	COPD, lung cancer	Domperidone	Ongoing 12 wk	Marked improvement of urgency and diarrhea
IBS-D 5/36/F	Diarrhea, severe ABD pain	Ulcerative colitis	Low FODMAP diet, mesalamine	Ongoing 12 wk	No ABD pain, Loose stools/diarrhea improved
IBS-D 6/87/F	Diarrhea, ABD pain, distention, urgency	Osteoporosis, GERD, anxiety	Perphenazine/amitriptyline, omeprazole	Ongoing 17 wk	Dramatic reduction in symptoms
IBS-D 7/66/M	Diarrhea, urgency, incontinenc, ABD pain	Hypertension, benign prostatic hyperplasia	Tramadol	Ongoing 16 wk	Marked reduction in pain and urgency, Formed bowel movements

ABD: Abdominal; IBS-D: Irritable bowel syndrome with diarrhea; SBI: Serum-derived bovine immunoglobulin; GI: Gastrointestinal; GERD: Gastroesophageal reflux disease.

Table 2 Presentation of irritable bowel syndrome patients with constipation

Patient No./age (yr)/ gender	Primary symptoms	Comorbidity	Other GI therapy	SBI therapy/duration	Outcome
IBS-C 1/22/F	Constipation, bloating, distension	None	Low FODMAP diet, linaclotide, lubiprostone	Discontinued after 11 wk	Ineffective
IBS-C 2/55/F	Bloating, distension, nausea, obstipation	Non-erosive reflux disease	lubiprostone	Ongoing 14 wk	Reduced bloating and distension, obstipation unchanged

IBS-C: Irritable bowel syndrome with constipation; SBI: Serum-derived bovine immunoglobulin; GI: Gastrointestinal.

reporting.

The first group investigated were those patients with IBS-D (Table 1). Overall these patients, ranging in from 24-87 years of age, responded well to SBI therapy and all continued usage. The key complaints among these 7 patients were diarrhea (6), urgency (6), abdominal pain (6), frequency (5), and incontinence but symptoms of flatulence, distension, and cramping were also noted by some patients. While symptoms varied among these patients, the general response indicated a consistent improvement in abdominal and bowel symptoms with a marked reduction in abdominal pain, diarrhea, urgency and an improved stool consistency. There was also a noted resolution of incontinence. The duration of SBI therapy ranged from 17-32 wk and all patients continue their SBI therapy for management of their IBS-D symptoms.

The second group investigated consisted of two patients diagnosed with IBS-C (Table 2). While SBI is specifically intended for use in IBS-D rather than IBS-C, the potential mechanism of action regarding barrier restoration may provide some symptom management in IBS-C. For one patient, a 22 year old female, SBI was ineffective in managing the patient's overall IBS-C symptoms. In a second 55 year female patient, SBI improved the patient's bloating, distension and nausea. However, the patient had no improvement in her obstipation (severe constipation resulting from an intestinal obstruction). The benefits perceived by the patient, however, were subjectively sufficient during the 14 wk of SBI therapy that the patient has elected to continue the therapy.

The third group was two patients experiencing alternating diarrhea and constipation symptoms noted as IBS-M (Table 3). For the 33 year old female patient, there was an overall improvement in IBS-M

Table 3 Presentation of irritable bowel syndrome patients with mixed with alternating constipation and diarrhea

Patient No./age (yr)/gender	Primary Symptoms	Comorbidity	Other GI Therapy	SBI Therapy/ Duration	Outcome
IBS-M 1/33/F	Alternating diarrhea and constipation, bloating, distension, ABD pain	Morbid obesity	Low FODMAP diet	Ongoing 15 wk	Overall improvement, mild obstipation, reduced bloating
IBS-M 2/66/F	Alternating diarrhea and constipatio, bloating, distension	Osteoporosis	Low FODMAP diet	Ongoing 14 wk	No bloating or distension, improved bowel movements

IBS-M: Irritable bowel syndrome with mixed with alternating constipation and diarrhea; SBI: Serum-derived bovine immunoglobulin; GI: Gastrointestinal.

Table 4 Presentation of patients with irritable bowel syndrome -bloating

Patient No./age (yr)/gender	Primary symptoms	Comorbidity	Other GI therapy	SBI therapy/duration	Outcome
IBS-U 1/50/M	Gas, Bloating, ABD Pain	GERD, osteoarthritis	polycarbophil, saccharomyces boulardii lyo, polyethylene glycol 3350, prn	Ongoing 35 wk	Resolution of symptoms
IBS-U 2/82/F	Severe ABD pain, bloating, distension	Hypertension, atherosclerotic cardiovascular disease	Antidiarrheals	Discontinued after 6 wk	Unimproved
IBS-U 3/62/F	Bloating, distension, ABD pain, flatulence	Osteoporosis	denosumab, rifaximin, Low FODMAP diet	Completed after 8 wk	Resolution of symptoms

IBS-Bloating: Irritable bowel syndrome with bloating; SBI: Serum-derived bovine immunoglobulin; GI: Gastrointestinal; GERD: Gastroesophageal reflux disease.

and a reduction in bloating during the 15 wk of SBI therapy although mild obstipation was noted. In a second patient, 66 years old female, there was an elimination of the patient's bloating and distension and improvement in the bowel movements during the 14 wk of SBI therapy. Both patients are on low FODMAP diets and continue their SBI therapy.

The final group of IBS patients was those with no specific bowel symptoms associated with stool consistency, but who indicated that their primary IBS symptom was bloating (Table 4). For this group of patients, an 82 year female patient discontinued SBI therapy after 6 wk indicating there was insufficient relief of symptoms. A second patient, a 62 years old female, completed 8 wk of SBI therapy and indicated a resolution of her gastrointestinal symptoms (bloating, distention, flatulence, and abdominal pain) but given the cyclic nature of IBS, it is possible that these symptoms may recur. A third patient, a 50 years old male whose primary symptoms included gas, bloating and abdominal pain, reported a resolution of symptoms. He has been managed with SBI therapy for 35 wk and continues on therapy.

No adverse effects have been noted due to SBI therapy in any of the IBS patient populations being managed with the product.

DISCUSSION

For patients with IBS-D observed in this physician's clinical practice, SBI has been highly effective in managing chronic loose and frequent stools in IBS. For patients with IBS-C, the results are inconclusive due to small sample size but seem less effective in this patient population. For patients with IBS-M, there is some potential for efficacy during bouts of diarrhea and reduction in bloating but the full extent of benefit suggests some mixed results. Because of the alternating nature of symptoms in IBS-M patients, this population is often difficult to manage. Further investigation is warranted to determine the potential timing and management of dosing in this patient population. Similarly for patients with IBS-bloating, there appears to be some alleviation in bloating symptoms but the full extent of the benefit is mixed. Additional study may help determine the extent of benefit that is possible in this population. Despite these findings, the results and conclusions drawn from these patients must be tempered by the small sample sizes.

While the findings in patients with IBS-D were expected based upon prior clinical evidence[16], the elements of benefit for patients with other types of IBS, particularly for IBS-M and IBS-bloating, merit more study. Such investigations will provide for more thorough analysis of SBI-mediated outcomes in these types of IBS patients. While patients with IBS-C share some common symptoms with other IBS patients, the results in patients with IBS-C were inconclusive and more data is needed to draw any final conclusions.

SBI is intended specifically for the management of intestinal disorders in patients with chronic loose and frequent stools, such conditions as IBS-D and HIV-

associated enteropathy under physician supervision[15]. These findings suggest improvements in symptoms that affect patients with IBS-D and other types of IBS without any adverse effects. This further supports the SBI designation as Generally Recognized As Safe (GRAS) or food-like safety, an FDA requirement for this category of therapeutics[15]. As such, SBI as a medical food would appear to have safe and practical applications in the management of IBS (particularly patients with IBS-D) and further investigation is needed to determine the extent of benefits that SBI holds for patients with other forms of IBS.

COMMENTS

Case characteristics

This case series reports the outcomes of 14 irritable bowel syndrome (IBS) patients (2 IBS-C, 7 IBS-D, 2 IBS-M and 3 IBS-Bloating), ages 22-87 years, who received serum-derived bovine immunoglobulin/protein isolate (SBI) as an addition to standard of care in a clinical practice setting.

Clinical diagnosis

General diagnosis consisted of abdominal pain with altered bowel habits associated with diarrhea and/or constipation or bloating.

Differential diagnosis

Irritable bowel syndrome with diarrhea, constipation, mixed (diarrhea and constipation) or bloating.

Laboratory diagnosis

Individual laboratory testing was not provided as patients had an established diagnosis of IBS.

Imaging diagnosis

Imaging such as a colonoscopy was not provided as patients had an established diagnosis of IBS.

Pathological diagnosis

Pathological diagnosis was not provided as patients had an established diagnosis of IBS.

Treatment

Serum-derived bovine (SBI) immunoglobulin/protein isolate (5 g or 10 g/d) was added to the patients' current standard care and followed for several weeks to determine if symptoms improved.

Related reports

Despite some limited evidence based recommendations for treatment of IBS, there is no clear consensus on therapeutic options for IBS and patients are often dissatisfied with their current therapeutic options.

Term explanation

IBS-bloating or IBS-U refers to patients without any distinctive stool consistency patterns for diagnosis as IBS-D, IBS-C or IBS-M but show symptoms of IBS such as abdominal pain with a chief complaint of bloating rather than stool consistency.

Experiences and lessons

Overall, 12 of the 14 IBS patients using SBI indicated some level of improvement with onset within the first four weeks of therapy and 11 of the 14 are continuing therapy, but two patients discontinued therapy because of insufficient relief.

Peer-review

The article describes 14 cases of IBS where SBI was added to current standard of care and found improvement in 12 cases. The article highlights the potential benefits that can come from a medical food like SBI in a clinical practice and the data suggest the need for further study to confirm these practice findings.

REFERENCES

1 **Longstreth GF**, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; **130**: 1480-1491 [PMID: 16678561 DOI: 10.1053/j.gastro.2005.11.061]

2 **Brandt LJ**, Chey WD, Foxx-Orenstein AE, Schiller LR, Schoenfeld PS, Spiegel BM, Talley NJ, Quigley EM. An evidence-based position statement on the management of irritable bowel syndrome. *Am J Gastroenterol* 2009; **104** Suppl 1: S1-S35 [PMID: 19521341 DOI: 10.1038/ajg.2008.122]

3 **Saito YA**, Schoenfeld P, Locke GR. The epidemiology of irritable bowel syndrome in North America: a systematic review. *Am J Gastroenterol* 2002; **97**: 1910-1915 [PMID: 12190153 DOI: 10.1111/j.1572-0241.2002.05913.x]

4 **Andrews EB**, Eaton SC, Hollis KA, Hopkins JS, Ameen V, Hamm LR, Cook SF, Tennis P, Mangel AW. Prevalence and demographics of irritable bowel syndrome: results from a large web-based survey. *Aliment Pharmacol Ther* 2005; **22**: 935-942 [PMID: 16268967 DOI: 10.1111/j.1365-2036.2005.02671.x]

5 **Drossman DA**, Li Z, Andruzzi E, Temple RD, Talley NJ, Thompson WG, Whitehead WE, Janssens J, Funch-Jensen P, Corazziari E. U.S. householder survey of functional gastrointestinal disorders. Prevalence, sociodemography, and health impact. *Dig Dis Sci* 1993; **38**: 1569-1580 [PMID: 8359066 DOI: 10.1007/BF01303162]

6 **Locke GR**, Clark S, Cerulli A, Marehbian J, Kahler KH, Shetzline MA. Work productivity is more impaired in functional gastrointestinal disorders compared to GERD: six-month data from PROGRESS [abstract 1052]. *Am J Gastroenterol* 2007; **102**: S504 [DOI: 10.1111/j.1572-0241.2007.01491_10.x]

7 **Krause R**, Ameen V, Gordon SH, West M, Heath AT, Perschy T, Carter EG. A randomized, double-blind, placebo-controlled study to assess efficacy and safety of 0.5 mg and 1 mg alosetron in women with severe diarrhea-predominant IBS. *Am J Gastroenterol* 2007; **102**: 1709-1719 [PMID: 17509028 DOI: 10.1111/j.1572-0241.2007.01282.x]

8 **Chey WD**, Chey WY, Heath AT, Dukes GE, Carter EG, Northcutt A, Ameen VZ. Long-term safety and efficacy of alosetron in women with severe diarrhea-predominant irritable bowel syndrome. *Am J Gastroenterol* 2004; **99**: 2195-2203 [PMID: 15555002 DOI: 10.1111/j.1572-0241.2004.30509.x]

9 **Lembo A**, Zakko SF, Ferreira NC. T1390 rifaximin for the treatment of diarrhea associated irritable bowel syndrome: short-term treatment lending to long- term sustained response. *Gastroenterology* 2008; **134**: A545 [DOI: 10.1016/S0016-5085(08)62544-5]

10 **Pimentel M**, Park S, Mirocha J, Kane SV, Kong Y. The effect of a nonabsorbed oral antibiotic (rifaximin) on the symptoms of the irritable bowel syndrome: a randomized trial. *Ann Intern Med* 2006; **145**: 557-563 [PMID: 17043337]

11 **Clouse RE**. Managing functional bowel disorders from the top down: lessons from a well-designed treatment trial. *Gastroenterology* 2003; **125**: 249-253 [PMID: 12851889 DOI: 10.1016/S0016-5085(03)00808-4]

12 **Omar MI**, Alexander CE. Drug treatment for faecal incontinence in adults. *Cochrane Database Syst Rev* 2013; **6**: CD002116 [PMID: 23757096 DOI: 10.1002/14651858.CD002116.pub2]

13 **Bliss DZ**, Jung HJ, Savik K, Lowry A, LeMoine M, Jensen L, Werner C, Schaffer K. Supplementation with dietary fiber improves fecal incontinence. *Nurs Res* 2001; **50**: 203-213 [PMID: 11480529]

14 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]

15 Entera Health, Inc. (2014/04). EnteraGam Prescribing Information.

16 **Wilson D**, Evans M, Weaver E, Shaw AL, Klein GL. Evaluation of serum-derived bovine immunoglobulin protein isolate in subjects with diarrhea-predominant irritable bowel syndrome. *Clin Med Insights Gastroenterol* 2013; **6**: 49-60 [PMID: 24833942 DOI: 10.4137/CGast.S13200]

17 **Asmuth DM**, Ma ZM, Albanese A, Sandler NG, Devaraj S, Knight TH, Flynn NM, Yotter T, Garcia JC, Tsuchida E, Wu TT, Douek DC, Miller CJ. Oral serum-derived bovine immunoglobulin improves duodenal immune reconstitution and absorption function in patients with HIV enteropathy. *AIDS* 2013; **27**: 2207-2217

[PMID: 23660579 DOI: 10.1097/QAD.0b013e328362e54c]
18 **Petschow BW**, Burnett B, Shaw AL, Weaver EM, Klein GL. Serum-derived bovine immunoglobulin/protein isolate: postulated mechanism of action for management of enteropathy. *Clin Exp Gastroenterol* 2014; **7**: 181-190 [PMID: 24904221 DOI: 10.2147/CEG.S62823]

P- Reviewer: Guo YM, Grundmann O **S- Editor**: Yu J **L- Editor**: A
E- Editor: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3367

World J Gastroenterol 2015 March 21; 21(11): 3367-3375
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Strongyloidiasis: A case with acute pancreatitis and a literature review

Jasbir Makker, Bhavna Balar, Masooma Niazi, Myrta Daniel

Jasbir Makker, Bhavna Balar, Myrta Daniel, Division of Gastroenterology, Bronx Lebanon Hospital Center, Albert Einstein College of Medicine of Yeshiva University, New York, NY 10457, United States
Jasbir Makker, Bhavna Balar, Myrta Daniel, Department of Medicine, Bronx Lebanon Hospital Center, Albert Einstein College of Medicine of Yeshiva University, New York, NY 10457, United States
Masooma Niazi, Department of Pathology, Bronx Lebanon Hospital Center, Albert Einstein College of Medicine of Yeshiva University, New York, NY 10457, United States
Author contributions: Makker J wrote the manuscript; Niazi M provided the pathology images; Balar B and Daniel M edited the final version of manuscript.
Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.
Conflict-of-interest: Authors have no conflict of interest to disclose.

Correspondence to: Jasbir Makker, MD, Division of Gastroenterology, Bronx Lebanon Hospital Center, Albert Einstein College of Medicine of Yeshiva University, 1650 Grand Concourse, Bronx, New York, NY 10457, United States. makkerjs@gmail.com
Telephone: +1-347322-8242
Fax: +1-718518-5111
Received: October 13, 2014
Peer-review started: October 14, 2014
First decision: November 14, 2014
Revised: November 24, 2014
Accepted: January 16, 2015
Article in press: January 16, 2015
Published online: March 21, 2015

Abstract

Strongyloides stercoralis, a soil transmitted helminth infection, affects millions with varying prevalence worldwide. A large number of affected hosts are asymptomatic. Symptoms pertaining to pulmonary and gastrointestinal involvement may be present. Manifestations of involvement beyond lung and intestine can be seen with dissemination of infection and lethal hyperinfection. Immunosuppression secondary to use of steroids or other immunosuppressants and coexistence of human T-lymphotropic virus type-1 are the known risk factors for dissemination and hyperinfection. Diagnostic modalities comprise stool examination, serology and molecular testing. Stool tests are inexpensive but are limited by low sensitivity, whereas serologic and molecular tests are more precise but at the expense of higher cost. Treatment with Ivermectin or Albendazole as an alternative is safe and efficacious. We present a rare case of acute pancreatitis secondary to Strongyloides. High index of suspicion in patients specifically from endemic countries of origin and lack of other common etiologies of acute pancreatitis may help in early diagnosis and prompt treatment of this potentially fatal infection.

Key words: Strongyloides; Pancreatitis; Autoinfection; Helminth; Eosinophilia

Core tip: Strongyloides affects millions of people worldwide. Large numbers of infected hosts are asymptomatic or have non-specific gastrointestinal and/or pulmonary symptoms. Infected hosts, especially in the setting of human T-lymphotropic virus type-1 infection and immunosuppressant or steroid use, may develop overwhelming infection in the form of

dissemination or hyperinfection. Peripheral eosinophilia may be the only non-specific finding. Diagnostic methods range from simple stool examination to serologic tests and molecular techniques based on nucleic acid amplification. Endoscopic examination may be needed which may provide evidence of infection on pathological exam. Treatment options are both safe and efficacious with oral Ivermectin being superior to Albendazole.

Makker J, Balar B, Niazi M, Daniel M. Strongyloidiasis: A case with acute pancreatitis and a literature review. *World J Gastroenterol* 2015; 21(11): 3367-3375 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3367.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3367

INTRODUCTION

Strongyloidiasis, an infection caused by *Strongyloides stercoralis*, is endemic in tropical and sub-tropical regions. Poor sanitary conditions favor its propagation. Infection with Strongyloides is usually asymptomatic which leads to underestimated figures of its prevalence. The most commonly affected organs are lung and intestines with mild non-specific symptoms like abdominal pain, vomiting, diarrhea, tracheal irritation and cough. Hyperinfection or disseminated infection due to unique feature of autoinfection may occur. We present a rare case of Strongyloides associated acute pancreatitis and a review of four reported cases.

CASE REPORT

A 48-year-old man presented to Bronx Lebanon Hospital Center with abdominal pain after he had been admitted to another hospital for epigastric pain of ten days duration. He described the pain as intermittent, non-radiating and worsening with food intake. The patient denied nausea, vomiting, fever or dysphagia. An upper endoscopic exam had reportedly revealed normal esophagus, gastric erythema and normal duodenum. On the 4th day of hospitalization, the patient reported slight improvement in his symptoms and he was discharged home on omeprazole 20 mg oral daily.

Two days after discharge from the other hospital, on 16th day of illness he presented to Bronx Lebanon Hospital Center due to persistent epigastric pain.

He had hypertension, and he was taking losartan 50 mg daily. He had had surgery for kidney stones. He had no known drug or food allergies. He did not smoke, drink alcohol, or use illicit drugs. He was married and had children. He had immigrated to United States from the Dominican Republic 5 years ago, lived in Pennsylvania and worked in a New York factory that produced cleaning liquids. His brother had diabetes mellitus; his parents and children were healthy.

Table 1 Results of blood tests

Blood tests	Labs on first admission Day 16 of illness	Labs on second admission Day 22 of illness
Hemoglobin (g/dL)	16.4	15.9
Hematocrit (%)	48.3	46.0
Platelet count (/μL)	237000	286000
White blood cell count (/mm^3)	18700	23300
Differential count (%)		
Neutrophils	35.2	33.5
Lymphocytes	18.4	16.5
Eosinophils	42.2	46.5
INR	1.1	1.0
Blood urea nitrogen (mg/dL)	10	10
Creatinine (mg/dL)	1.0	0.9
Albumin (g/dL)	3.4	3.8
Alanine aminotransferase (U/L)	26	65
Aspartate aminotransferase (U/L)	31	39
Alkaline phosphatase (U/L)	144	284
Total bilirubin (mg/dL)	0.2	0.3

On examination, temperature was 97.9°F, blood pressure 134/95 mmHg, pulse 68 beats per minute and regular, weight 81.6 kg with a body-mass index of 30. The patient looked stable but was uncomfortable due to pain. The abdomen was non-distended and had normal bowel sounds on auscultation; but it was soft with moderate tenderness in the epigastrium region. The rest of the examination was normal.

The white blood cell count was 18700 per mm^3 with an eosinophil percentage of 42.2 and absolute eosinophil count of 7900 per mm^3. Serum amylase and lipase levels were 78 units per liter and 83 units per liter respectively. Other test results were unremarkable as shown in Table 1. Computed tomography (CT) of the abdomen and pelvis, performed after the administration of intravenous and oral contrast material was normal. An upper gastrointestinal endoscopy was performed which showed normal appearing esophagus and multiple erosions in the stomach and duodenum (Figure 1A, B). Pathological exam of random esophageal biopsy specimen revealed hyperplastic squamous mucosa with mucosal congestion. Gastric biopsy specimen showed increased eosinophils in lamina propria, intraepithelial eosinophils, and eosinophilic cryptitis; and duodenal specimen showed increased eosinophils in lamina propria (more than 20 per high power field), intraepithelial eosinophilic infiltrates, eosinophilic cryptitis and no shortening of villi or crypt distortion. A diagnosis of eosinophilic gastroenteritis was entertained. Omeprazole was administered, test for stool ova and parasite was ordered before considering steroid treatment for eosinophilic gastroenteritis while awaiting results of endoscopic biopsies. The patient was discharged home after three days of hospitalization with instructions to

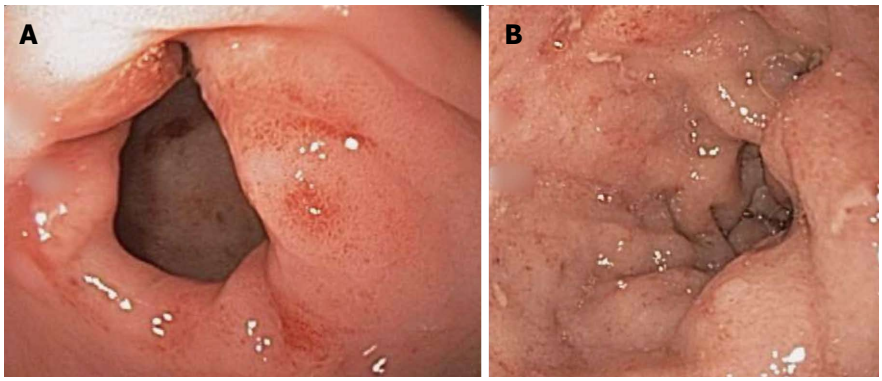


Figure 1 Endoscopic images of gastric antrum and duodenal bulb erosions (A and B).

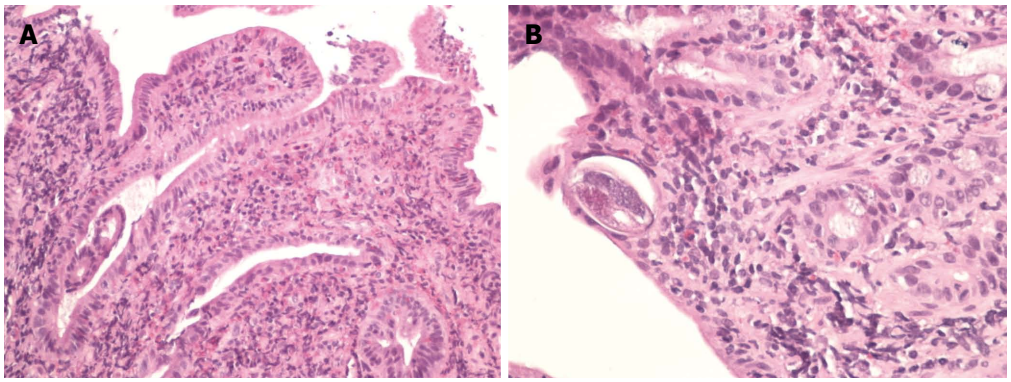


Figure 2 Hematoxylin and Eosin staining of the biopsy specimen. A: Hematoxylin and eosin (HE) stained section of small bowel biopsy showing prominent eosinophilia associated with Strongyloides infection; B: HE stained longitudinal cross section of a small bowel biopsy showing Strongyloides worm lying within the crypt.

follow up in outpatient clinic in 1 wk.

Three days later, on the 22nd day of his illness, he returned to this hospital with worsening epigastric pain that he rated at 10 out of 10. It was constant, radiating to his back and associated with one episode of non-bilious vomiting. He also reported about 11 pounds of unintentional weight loss over last three weeks. On examination he was anxious and uncomfortable. His temperature was 97.0°F, blood pressure 166/107 mmHg, pulse 60 beats per minute and respiratory rate 15 per minute. His abdomen was non-distended with normal bowel sounds, it was soft but there was severe tenderness in the epigastrium region. The remainder of the examination was normal. The white blood cell count level was again found to be elevated at 23300 per mm^3 with an eosinophil percentage of 46.5 and absolute eosinophil count of 10900 per mm^3. Serum levels of amylase and lipase were 263 and 476 units per liter. The other blood test results are shown in Table 1. Radiography of the abdomen was normal. CT of the abdomen and pelvis following the administration of oral and intravenous contrast was normal. Ultrasonography of abdomen revealed gall bladder sludge, a normal common bile duct and no gallstone.

A diagnosis of acute pancreatitis was made. The patient was managed conservatively with aggressive intravenous fluid administration. Several possibilities were entertained in the search for an etiology. Etiologies of acute pancreatitis, like gallstones and hypertriglyceridemia, were excluded based on the normal abdominal sonogram and serum triglyceride level. Pancreatitis secondary to alcohol and drug were also excluded based on history. The differential diagnosis, especially in the presence of eosinophilia, also included aggressive systemic mastocytosis with lymphadenopathy and eosinophilia, hypereosinophilic syndrome, occult parasitic infection and hypoadrenalism. Hematology consult was obtained and bone marrow biopsy was performed.

Bone marrow biopsy revealed normocellular marrow with trilineage maturation but increase in both mature and immature eosinophils to about 30 percent of nucleated hematopoietic cells. Serum levels of aldosterone, tryptase and immunoglobulin E level were all normal. Stool for ova and parasite was reported negative on multiple occasions. Strongyloides immunoglobulin G (IgG) tested by Enzyme Linked Immunosorbent Assay (ELISA) level was reported positive. Careful review of the endoscopic biopsy specimens again revealed a *Strongyloides stercoralis* larva in the duodenal biopsy specimen (Figure 2A, B).

A single dose of Ivermectin 200 microgram per kilogram bodyweight was administered orally and 24 h later the patient reported complete resolution of his symptoms. A repeat dose of Ivermectin 200 microgram per kilogram bodyweight was administered

orally two weeks later during an outpatient follow up visit.

DISCUSSION

Background

Strongyloides are soil-transmitted helminths. They are vertebrate parasites and the genus comprises about fifty species[1]. They infect different hosts like amphibians, birds and mammals including humans. The latter can be infected by three different species of *Strongyloides*, namely *Strongyloides stercoralis*, *Strongyloides fuelleborni fuelleborni* and *Strongyloides fuelleborni kelleyi*[2]. *Strongyloides stercoralis* is the most prevalent species worldwide, whereas Strongyloides fuelleborni fuelleborni is prevalent in Africa and Strongyloides fuelleborni kelleyi in Papua New Guinea.

Epidemiology

Strongyloides was first discovered by Louis Normand in 1876 in the stools of French soldiers returning from Indochina[3]. The two different forms of Strongyloides larvae were initially considered as two different species and were named as *Anguillula stercoralis* and *Anguillula intestinalis*. Later they were classified under genus Strongyloides and the worm was renamed as *Strongyloides stercoralis*. Since its discovery more than a century ago, it has been found in all parts of world except Antarctica.

Strongyloides stercoralis is most prevalent in tropical and sub-tropical regions but also occurs in temperate regions when conditions are favorable. It is endemic in Southeast Asia, Sub Saharan Africa and Latin America. Due to the dearth of epidemiologic studies, worldwide prevalence is unknown but about 30 to 100 million people are estimated to be infected[4].

A recent literature review aimed at estimating prevalence of Strongyloides in different countries of the world exhibited the diversity in its worldwide prevalence. On the African continent, prevalence as high as 99% in Namibia, 92% in Gabon and 80% in Kenya was reported. In Latin America, prevalence was found to be 98% in the Dominican Republic and 75% in Peru. Prevalence in New Papua Guinea was reported as 99%[5].

Historically, in the United States, *Strongyloides stercoralis* has been considered endemic in southern states and Appalachia. With the last high quality study from the United States having been published three decades ago[6], information about most recent prevalence trends of *Strongyloides stercoralis* is lacking. Due to improvement in sanitary conditions and hygienic practices, these prior studies had shown marginal decline in prevalence from 3.8% to 3%[7]. In studies among veterans returning from endemic regions and immigrants, respective prevalence as high as 37%[8] and 40%[5] has been recorded. Since the initiative from the Centers for Disease Control and prevention to presumptively treat parasitic infections in immigrants prior to their arrival in the United States, there has been a significant decline in *Strongyloides stercoralis* prevalence among immigrants[9].

Life cycle

The life cycle for *Strongyloides* is unique and features like parthenogenesis (development of larvae from unfertilized eggs without sexual reproduction), release of larvae in feces and not eggs, and autoinfection make it stand out from the rest. *Strongyloides* life cycle comprises two forms - free-living form and parasitic form. Rhabditiform larvae upon their release in the feces of an infected host can molt to form either free living adult worms or the infective filariform larvae. The infective filariform larvae can be transmitted to another host through exposure to contaminated soil. After penetrating skin of the new host, these infective filariform larvae are carried by blood to the lungs where they break out of lung capillaries into alveoli and then ascend up the tracheobronchial tree to be swallowed eventually into the gastrointestinal tract. In the gastrointestinal tract, larvae molt into adult female worms that settle down in the lamina propria of the small intestine and release eggs without involvement of male for reproduction (parthenogenesis). Rhabditiform larvae hatch out of these eggs and are eventually passed in feces. These rhabditiform larvae, instead of being released into feces can also re-penetrate the gastrointestinal mucosa or the perianal skin (autoinfection)[10].

Immunology

Overall understanding on human immune responses to *Strongyloides stercoralis* is limited. The limitation is partly from the complex nature of the Strongyloides as well as inability to reproduce the pathophysiology of the *Strongyloides stercoralis* human infection in the currently available rat model studies. Nevertheless, these rat models have demonstrated an armamentarium of immune responses that interplay to fight against Strongyloides. Innate immune responses begin by recruitment of neutrophils and eosinophils. Neutrophils need myeloperoxidase and toll like receptors to kill the larvae. On the other hand, eosinophils mediate larval killing by supplying cytokines needed for immunoglobulin IgM production. Eosinophils, as antigen presenting cells, also play a key role in activating adaptive immune responses. Once adaptive immunity is activated, helper T cells activate B cells through interleukin-5 and initiate production of IgM and IgG. Immunoglobulin IgM and IgG not only recognize different antigens of *Strongyloides stercoralis* but also work through different mechanisms. IgG needs neutrophils and complement activation for larval killing (antibody dependent cellular cytotoxicity), whereas IgM action is independent of neutrophils and cytokines[11,12].

Table 2 Reported cases of acute pancreatitis associated with *Strongyloides stercoralis*

Case	Age (yr)/ gender	Place of origin	Presentation	Eosinophil count (per mm^3)	Amylase (U/L)	Lipase (U/L)	Diagnostic method	Treatment	Ref.
1	44/F	West Indies (Caribbean)	Abdominal pain Fever Pruritus	210	Not provided	Not provided	Examination of biliary fluid obtained through percutaneous biliary drain	Thiabendazole	Delarocque Astagneau *et al*[14], 1994
2	45/F	Ecuador	Abdominal pain Nausea Vomiting	900	259	1574	Endoscopic duodenal biopsy Stool examination	Albendazole Thiabendazole Ivermectin	Núñez *et al*[15], 2003
3	81/F	Kentucky, USA	Abdominal pain Nausea Vomiting	1254	2100	>2000	Examination of biliary fluid obtained through ERCP	Albendazole	Perez-Jorge *et al*[16], 2008
4	64/M	Puerto Rico	Abdominal pain Nausea Vomiting Dyspnea Confusion	776	4367	>396	Clinical history and stool examination	Ivermectin	Jones *et al*[17], 2009
5 (our case)	48/M	Dominican Republic	Abdominal pain	10900	263	476	Endoscopic duodenal biopsy and serology	Ivermectin	Makker *et al*, 2015

ERCP: Endoscopic retrograde cholangio pancreatography.

Clinical features

Clinical manifestations of *Strongyloides stercoralis* infection vary depending on the worm burden. Most of the immunocompetent hosts infected with *Strongyloides stercoralis* are asymptomatic which is one of the reasons for the underestimation of its true prevalence. After the skin penetration by infective filariform larvae, a rash may be recognized in some but not all patients. The rash, which may last for several weeks, can present in the form of multiple wheels like urticarial reaction or a serpiginous creeping rash created by movement of larva under the skin (larva currens). In patients with autoinfection, where larvae re-enter the peri-anal skin as soon as they exit from the anal canal, the rash may be seen on the buttocks and thighs.

With involvement of respiratory system, it can manifest as cough, tracheal irritation and shortness of breath. Gastrointestinal manifestations, which develop about two weeks after the entry of the larvae, include abdominal pain, nausea, vomiting and diarrhea. Fever, malaise, anorexia and weight loss can also be seen frequently[13]. Rarely, association with acute pancreatitis as in our case has been described in the medical literature. Prior to our case, there have been only four cases of acute pancreatitis related to *Strongyloides* reported in the English and Spanish medical literature (Table 2)[14-17]. Likely mechanism of pancreatitis is involvement of duodenal ampulla and then pancreatic duct leading to intense inflammation and edema. A rare case report of Strongyloides association with cystadenocarcinoma of pancreas and pancreatic head mass has also been described[18,19].

After the establishment of *Strongyloides stercoralis* infection, factors that lead to autoinfection are unclear. The immune system constantly attempts to contain the infection, however in the presence of specific risk factors, *Strongyloides* infection supersedes and severe forms of infection known as hyperinfection and disseminated infection emerge. Well-established risk factors are treatment with steroids[20] or immunosuppressants[21] and coexistence of human T-lymphotropic virus type-1 (HTLV-1)[22]. Hyperinfection is characterized by enormous burden of parasite in the lungs and gastrointestinal tract, leading to the hallmark appearance of multiple larvae in sputum and/or feces. As the parasitic burden increases with autoinfection, these larvae can go beyond the normally infected tissues - lungs and gastrointestinal tract. Dissemination to other organs can manifest as meningitis, atrial fibrillation, hemoptysis, pneumothorax, intestinal hemorrhage, intestinal obstruction, protein losing enteropathy and intestinal ulcerations with subsequent gram-negative septicemia[23].

Diagnostic methods

Diagnosis of *Strongyloides stercoralis* infection in the absence of a gold standard remains a challenge. Various methods as discussed below are available but none seem to be ideal. Many patients with low parasite burden and non-specific symptoms may have peripheral eosinophilia as the only finding, which in itself is a non-specific indicator. Stool examination for larvae, one of the oldest methods available, in such patients may be completely negative, especially if only one sample is tested. Testing multiple samples increases the yield of stool examination[24]. Stool examination can be done by two different methods - direct stool smear examination or stool culture. Historically, different methods have been described to increase the detection rate of stool examination. These

Table 3 Various diagnostic tests available for *Strongyloides stercoralis*

Test	Sensitivity and specificity	Remarks
Stool smear	30% sensitivity for single stool sample[35]	Simple test but insensitive
Agar plate culture	90% sensitivity[28]	Sensitive and simple But needs 2 d for results Health hazard to lab workers
Serologic tests		
IFAT	IFAT: 97% sensitivity and 98% specificity[29]	Cross reactivity except with LIPS
ELISA	ELISA: 93% sensitivity and 95% specificity[30]	Inability to differentiate past and current infection
LIPS	LIPS: 97% sensitivity and 100% specificity[32]	Expensive
Molecular tests		
PCR	PCR: 99%-100% sensitivity and 15%-100% specificity[34]	Low specificity with low parasite burden Expensive
LAMP		Not widely available

ELISA: Enzyme Linked Immunosorbent Assay; IFAT: Indirect immunofluorescence antibody test; LAMP: Loop-mediated isothermal amplification; LIPS: Immunoprecipitation system; PCR: Polymerase chain reaction.

include formalin-ethyl acetate concentration, Baermann method that relies on the ability of larvae to convert to free-living stage, and Harada-Mori filter paper method that relies on water tropism of larvae[25]. Stool culture on agar plate requires stool inoculation and incubation for at least 2-3 d, following which larvae can be seen on the agar plate with the help of a microscope. Even if larvae are not seen, specific track marks on agar plate created by larval movement can be seen and also be differentiated from hookworm larvae tracks[26,27]. Agar plate culture has a high sensitivity of 90% but is time consuming, expensive and a health hazard to laboratory workers[28].

The next available diagnostic studies are serological tests (Table 3), which depend on the detection of the larval antigen or antibody generated in response to their antigens. Indirect immunofluorescence antibody test for *Strongyloides stercoralis* has been shown to have a sensitivity of 97% and a specificity of 98%[29]. ELISA to detect antibody against crude *Strongyloides* larva antigen has also been widely used, with sensitivity and specificity of 93% and 95% respectively[30]. The methods using ELISA have been hampered by the constant need for crude antigen, which must be prepared from feces of heavily infected patients or experimental animals. Hence interest in recombinant antigens was born, leading to the emergence of recombinant antigen NIE based ELISA test[31]. More recently a newer technique called luciferase immunoprecipitation system has been utilized to detect IgG against recombinant antigen NIE with sensitivity equal to that of NIE-ELISA test and a specificity of 100%[32]. Detection of antibodies does not differentiate between past and current infection, as these antibodies may last for years after the infection has cleared. Moreover, there may be cross-reactivity between Strongyloides antibodies and other helminths.

Molecular tests based on nucleic acid amplification by polymerase chain reaction (PCR) have also been used. Real time PCR technique using the 18S ribosomal DNA has been shown to have a specificity of 99%-100% but varying sensitivity based on the parasitic burden. With moderate to high burden, a sensitivity of 100% was found, however with low parasite burden it drastically dropped to only 15%[33]. A newer technique of nucleic acid amplification named Loop-mediated isothermal amplification has been introduced but needs further validation[34]. Table 3 above summarizes the sensitivity and specificity of these various diagnostic tests available[28-30,32,34,35].

Invasive methods like endoscopy with duodenal aspirate or biopsy can also be used in patients with strong clinical suspicion of infection. Upper endoscopic exam may show normal looking mucosa with eosinophilic infiltration on pathological examination or it may show abnormal mucosa in the form of erosions and ulcerations with pathological examination characterized by cryptitis, crypt abscess and eosinophilic infiltration[36,37]. Similarly the colon, particularly right-sided colon, may get involved in the presence of overwhelming parasite burden. Colonoscopy may show colonic mucosa inflammation or nodular mucosa with pathological examination revealing larvae, eosinophilic infiltration or granulomas[38].

Treatment

Ideally, the improvement of sanitary conditions and provision of better hygienic conditions should be targeted to control or eliminate *Strongyloides* infection. However, in the real world huge economic investments especially in developing nations are needed to achieve this goal. In the absence of such perfect sanitary conditions, reliance on anti-helminthic drugs is a reasonable option. The treatment of *Strongyloides* has come a long way since the days when intravenous gentian violet was introduced in 1950[39]. Currently, three drugs have been approved for the treatment of Strongyloides: Albendazole, Mebendazole and Ivermectin. Albendazole and Mebendazole belong to a group of benzimidazole antihelminthic drugs,

Table 4 Treatment of Strongyloidiasis

Drug	Dose	Pregnancy class
Ivermectin (preferred drug of treatment)	200 μg/kg of bodyweight orally repeated on two days consecutively or after 2 wk	C
Albendazole	400 mg orally two times a day for 3-7 d	C
Hyperinfection syndrome and disseminated Strongyloides infection 200 μg/kg of bodyweight orally until stool and/or sputum tests are negative for two wk (duration of auto-infective cycle)		

which were originally developed as plant fungicides[40]. Ivermectin, a macrocyclic lactone is derived from avermectins, which is produced by the *bacterium Streptomyces avermitilis*.

Albendazole acts by interfering with the microtubular system of the parasite. The usual dose of oral Albendazole is 400 mg twice a day for three to seven days[41]. In a large review of its efficacy, a cure rate of 62.2% was seen with a 400 mg daily dosing[40]. In one study its side effect profile was similar to that of a placebo[42]. It is a pregnancy class C drug but neither the reports from its inadvertent use during the first trimester nor a large randomized controlled trial have shown any congenital defects associated with its use[43,44].

Mebendazole, another member of the benzimidazole group, also acts by interfering with the microtubular system of the parasite. Since October 2011, it has not been available in the Unites States. Mebendazole was used in doses of 100 mg twice a day for five days followed by repeated doses at weeks 1, 3 and 4. Is has been reported to achieve cure rates of 87% after 15 mo of treatment completion[45].

Ivermectin, a semi synthetic derivative of macrocyclic lactone, mediates parasite paralysis and killing through glutamate activated chloride channels[46]. In a comparison of single dose Ivermectin (200 microgram per kilogram of bodyweight) orally with three-day regimen of Albendazole 400 mg orally daily, cure rates of 83% with Ivermectin were seen as compared to 45% with Albendazole[47]. Single dose of Ivermectin (200 microgram per kilogram of bodyweight) orally was also shown to have superior cure rate of 97% *vs* 63% with high dose Albendazole 800mg orally daily for seven days[41]. Currently, oral Ivermectin in the dose of 200 microgram per kilogram of bodyweight repeated on two consecutive days or after 2 wk is the preferred drug for treatment of *Strongyloides stercoralis* (Table 4)[48,49]. In patients who cannot tolerate it orally, alternate routes like subcutaneous administration of Ivermectin has been advised[50]. Ivermectin is usually well tolerated unless the patient has concomitant *Loa loa* infection. Loa associated encephalopathy, especially with high Loa microfilaremia, has been observed after mass treatment with Ivermectin[51]. Ivermectin is a pregnancy class C drug, but in a randomized controlled trial involving more than 800 pregnant patients in their second trimester, it did not show any significant effect on mean birth weight, pregnancy outcomes or congenital defects[44].

The resolution of hyperinfection syndrome and disseminated *Strongyloides* infection requires prolonged treatment. Since the autoinfection cycle of *Strongyloides* takes two weeks, it has been recommended to give daily oral Ivermectin in the doses of 200 microgram per kilogram of bodyweight until stool and/or sputum tests are negative for two weeks[21].

In conclusion, we present here an uncommon case of *Strongyloides stercoralis* infection in an immunocompetent adult male associated with acute pancreatitis. Infection was associated with eosinophilia and negative repeated stool examinations, but was eventually diagnosed on pathological examination of duodenal biopsies.

Helminth *Strongyloides* affects millions of people worldwide with endemicity in the tropical and subtropical regions. Life cycle comprises two different forms of larvae, and is unique with features like parthenogenesis and autoinfection. Large numbers of infected hosts are asymptomatic or have non-specific gastrointestinal and/or pulmonary symptoms. Both innate and adaptive immune mechanisms get activated with infection. Infected hosts, especially in the setting of HTLV-1 infection and immunosuppressant or steroid use, may develop overwhelming infection in the form of dissemination or hyperinfection. Peripheral eosinophilia may be the only non-specific finding. Diagnostic methods range from simple stool examination to serologic tests and molecular techniques based on nucleic acid amplification. Endoscopic examination may be needed which may provide evidence of infection on pathological exam. Treatment options are both safe and efficacious with oral Ivermectin being superior to Albendazole.

COMMENTS

Case characteristics

A 48-year-old man presented with peripheral eosinophilia and recurrent epigastric pain, aggravated by food intake, not associated with nausea, vomiting and fever.

Clinical diagnosis

On examination patient appeared uncomfortable due to abdominal pain and the abdominal examination revealed moderate epigastric tenderness.

Differential diagnosis

Differential diagnosis of epigastric pain: Acute pancreatitis, peptic ulcer disease; Differential diagnosis of acute pancreatitis: gallstone, alcohol, triglyceride, drugs, and parasitic infections; Differential diagnosis of eosinophilia with gastrointestinal symptoms: aggressive systemic mastocytosis with lymphadenopathy and eosinophilia, hypereosinophilic syndrome, occult parasitic infection and hypoadrenalism.

Laboratory diagnosis

Patient had elevated lipase level as well as peripheral eosinophilia. Stool ova and parasite testing was negative repeatedly. *Strongyloides* immunoglobulin G tested by Enzyme Linked Immunosorbent Assay was positive.

Imaging diagnosis

Computed tomography and ultrasound abdomen were unremarkable. Upper

endoscopic exam showed multiple erosions in the stomach and duodenum.

Pathological diagnosis

Endoscopic biopsy revealed a *Strongyloides stercoralis* larva in the duodenum.

Treatment

Two doses of Ivermectin 200 microgram per kilogram bodyweight were administered orally at two weeks interval.

Related reports

Very few cases related to acute pancreatitis secondary to Strongyloidiasis infection have been reported in literature. All the cases were associated with peripheral eosinophilia.

Term explanation

Strongyloidiasis is a soil transmitted helminth infection caused by *Strongyloides stercoralis* that initiates after infective larvae penetrate the host skin.

Experiences and lessons

This case report presents the rare association of Strongyloidiasis and acute pancreatitis. In the absence of other common etiologies of acute pancreatitis, the authors recommend considering this parasitic infection as one of the etiologies, especially in patients from endemic regions and who demonstrate peripheral eosinophilia.

Peer-review

The authors have described a case of acute pancreatitis secondary to *Strongyloides stercoralis* infection. The article highlights the diagnostic methods and treatment options available for this parasitic infection.

REFERENCES

1 **Viney ME**, Lok JB. Strongyloides spp. *WormBook* 2007: 1-15 [PMID: 18050491 DOI: 10.1895/wormbook.1.141.1]
2 **Dorris M**, Viney ME, Blaxter ML. Molecular phylogenetic analysis of the genus Strongyloides and related nematodes. *Int J Parasitol* 2002; **32**: 1507-1517 [PMID: 12392916]
3 **Grove DI**. Who discovered that intestinal worm infections could be diagnosed by finding eggs in the faeces? *J R Soc Med* 1986; **79**: 670-673 [PMID: 3540299]
4 **Keiser J**, Utzinger J. The drugs we have and the drugs we need against major helminth infections. *Adv Parasitol* 2010; **73**: 197-230 [PMID: 20627144 DOI: 10.1016/s0065-308x(10)73008-6]
5 **Schär F**, Trostdorf U, Giardina F, Khieu V, Muth S, Marti H, Vounatsou P, Odermatt P. Strongyloides stercoralis: Global Distribution and Risk Factors. *PLoS Negl Trop Dis* 2013; **7**: e2288 [PMID: 23875033 DOI: 10.1371/journal.pntd.0002288]
6 **Walzer PD**, Milder JE, Banwell JG, Kilgore G, Klein M, Parker R. Epidemiologic features of Strongyloides stercoralis infection in an endemic area of the United States. *Am J Trop Med Hyg* 1982; **31**: 313-319 [PMID: 7072896]
7 **Starr MC**, Montgomery SP. Soil-transmitted Helminthiasis in the United States: a systematic review--1940-2010. *Am J Trop Med Hyg* 2011; **85**: 680-684 [PMID: 21976572 DOI: 10.4269/ajtmh.2011.11-0214]
8 **Pelletier LL**. Chronic strongyloidiasis in World War II Far East ex-prisoners of war. *Am J Trop Med Hyg* 1984; **33**: 55-61 [PMID: 6696184]
9 **Swanson SJ**, Phares CR, Mamo B, Smith KE, Cetron MS, Stauffer WM. Albendazole therapy and enteric parasites in United States-bound refugees. *N Engl J Med* 2012; **366**: 1498-1507 [PMID: 22512482 DOI: 10.1056/NEJMoa1103360]
10 **Mahmoud AA**. Strongyloidiasis. *Clin Infect Dis* 1996; **23**: 949-952; quiz 953 [PMID: 8922784]
11 **Bonne-Année S**, Hess JA, Abraham D. Innate and adaptive immunity to the nematode Strongyloides stercoralis in a mouse model. *Immunol Res* 2011; **51**: 205-214 [PMID: 22101674 DOI: 10.1007/s12026-011-8258-2]
12 **Iriemenam NC**, Sanyaolu AO, Oyibo WA, Fagbenro-Beyioku AF. Strongyloides stercoralis and the immune response. *Parasitol Int* 2010; **59**: 9-14 [PMID: 19892034 DOI: 10.1016/j.parint.2009.10.009]
13 **Grove DI**. Human strongyloidiasis. *Adv Parasitol* 1996; **38**: 251-309 [PMID: 8701797]
14 **Delarocque Astagneau E**, Hadengue A, Degott C, Vilgrain V, Erlinger S, Benhamou JP. Biliary obstruction resulting from Strongyloides stercoralis infection. Report of a case. *Gut* 1994; **35**: 705-706 [PMID: 8200571]
15 **Núñez E**, Montero J, García-Picazo L, Ramón y Cajal S. [Recurrent pancreatitis after cholecystectomy]. *Enferm Infecc Microbiol Clin* 2003; **21**: 461-462 [PMID: 14525711]
16 **Perez-Jorge EV**, Burdette SD. Association between acute pancreatitis and Strongyloides stercoralis. *South Med J* 2008; **101**: 771-772 [PMID: 19209121 DOI: 10.1097/SMJ.0b013e31817a8b24]
17 **Jones N**, Cocchiarella A, Faris K, Heard SO. Pancreatitis associated with Strongyloides stercoralis infection in a patient chronically treated with corticosteroids. *J Intensive Care Med* 2010; **25**: 172-174 [PMID: 20444734 DOI: 10.1177/0885066609359992]
18 **Setia U**, Bhatia G. Pancreatic cystadenocarcinoma associated with strongyloides. *Am J Med* 1984; **77**: 173-175 [PMID: 6741978]
19 **Pijls NH**, Yap SH, Rosenbusch G, Prenen H. Pancreatic mass due to Strongyloides stercoralis infection: an unusual manifestation. *Pancreas* 1986; **1**: 90-93 [PMID: 3554219]
20 **Fardet L**, Généreau T, Cabane J, Kettaneh A. Severe strongyloidiasis in corticosteroid-treated patients. *Clin Microbiol Infect* 2006; **12**: 945-947 [PMID: 16961629 DOI: 10.1111/j.1469-0691.2006.01443.x]
21 **Mejia R**, Nutman TB. Screening, prevention, and treatment for hyperinfection syndrome and disseminated infections caused by Strongyloides stercoralis. *Curr Opin Infect Dis* 2012; **25**: 458-463 [PMID: 22691685 DOI: 10.1097/QCO.0b013e3283551dbd]
22 **Carvalho EM**, Da Fonseca Porto A. Epidemiological and clinical interaction between HTLV-1 and Strongyloides stercoralis. *Parasite Immunol* 2004; **26**: 487-497 [PMID: 15771684 DOI: 10.1111/j.0141-9838.2004.00726.x]
23 **Keiser PB**, Nutman TB. Strongyloides stercoralis in the Immunocompromised Population. *Clin Microbiol Rev* 2004; **17**: 208-217 [PMID: 14726461]
24 **Nielsen PB**, Mojon M. Improved diagnosis of strongyloides stercoralis by seven consecutive stool specimens. *Zentralbl Bakteriol Mikrobiol Hyg A* 1987; **263**: 616-618 [PMID: 3604502]
25 **Requena-Méndez A**, Chiodini P, Bisoffi Z, Buonfrate D, Gotuzzo E, Muñoz J. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS Negl Trop Dis* 2013; **7**: e2002 [PMID: 23350004 DOI: 10.1371/journal.pntd.0002002]
26 **Jongwutiwes S**, Charoenkorn M, Sitthichareonchai P, Akaraborvorn P, Putaporntip C. Increased sensitivity of routine laboratory detection of Strongyloides stercoralis and hookworm by agar-plate culture. *Trans R Soc Trop Med Hyg* 1999; **93**: 398-400 [PMID: 10674087]
27 **Inês Ede J**, Souza JN, Santos RC, Souza ES, Santos FL, Silva ML, Silva MP, Teixeira MC, Soares NM. Efficacy of parasitological methods for the diagnosis of Strongyloides stercoralis and hookworm in faecal specimens. *Acta Trop* 2011; **120**: 206-210 [PMID: 21896267 DOI: 10.1016/j.actatropica.2011.08.010]
28 **Arakaki T**, Iwanaga M, Kinjo F, Saito A, Asato R, Ikeshiro T. Efficacy of agar-plate culture in detection of Strongyloides stercoralis infection. *J Parasitol* 1990; **76**: 425-428 [PMID: 2352073]
29 **Boscolo M**, Gobbo M, Mantovani W, Degani M, Anselmi M, Monteiro GB, Marocco S, Angheben A, Mistretta M, Santacatterina M, Tais S, Bisoffi Z. Evaluation of an indirect immunofluorescence assay for strongyloidiasis as a tool for diagnosis and follow-up. *Clin Vaccine Immunol* 2007; **14**: 129-133 [PMID: 17135451 DOI: 10.1128/cvi.00278-06]
30 **van Doorn HR**, Koelewijn R, Hofwegen H, Gilis H, Wetsteyn JC, Wismans PJ, Sarfati C, Vervoort T, van Gool T. Use of enzyme-linked immunosorbent assay and dipstick assay for detection of Strongyloides stercoralis infection in humans. *J Clin Microbiol* 2007; **45**: 438-442 [PMID: 17151215 DOI: 10.1128/jcm.01735-06]
31 **Ravi V**, Ramachandran S, Thompson RW, Andersen JF, Neva FA. Characterization of a recombinant immunodiagnostic antigen (NIE) from Strongyloides stercoralis L3-stage larvae. *Mol Biochem Parasitol* 2002; **125**: 73-81 [PMID: 12467975]
32 **Ramanathan R**, Burbelo PD, Groot S, Iadarola MJ, Neva FA, Nutman TB. A luciferase immunoprecipitation systems assay

enhances the sensitivity and specificity of diagnosis of Strongyloides stercoralis infection. *J Infect Dis* 2008; **198**: 444-451 [PMID: 18558872 DOI: 10.1086/589718]

33 **Sultana Y**, Jeoffreys N, Watts MR, Gilbert GL, Lee R. Real-time polymerase chain reaction for detection of Strongyloides stercoralis in stool. *Am J Trop Med Hyg* 2013; **88**: 1048-1051 [PMID: 23568289 DOI: 10.4269/ajtmh.12-0437]

34 **Watts MR**, James G, Sultana Y, Ginn AN, Outhred AC, Kong F, Verweij JJ, Iredell JR, Chen SC, Lee R. A loop-mediated isothermal amplification (LAMP) assay for Strongyloides stercoralis in stool that uses a visual detection method with SYTO-82 fluorescent dye. *Am J Trop Med Hyg* 2014; **90**: 306-311 [PMID: 24323513 DOI: 10.4269/ajtmh.13-0583]

35 **Siddiqui AA**, Berk SL. Diagnosis of Strongyloides stercoralis infection. *Clin Infect Dis* 2001; **33**: 1040-1047 [PMID: 11528578]

36 **Santos RB**, Fonseca LE, Santana AT, Silva CA, Guedes JC. Clinical, endoscopic and histopathological profiles of parasitic duodenitis cases diagnosed by upper digestive endoscopy. *Arq Gastroenterol* 2011; **48**: 225-230 [PMID: 22147125]

37 **Kakati B**, Dang S, Heif M, Caradine K, McKnight W, Aduli F. Strongyloides duodenitis: case report and review of literature. *J Natl Med Assoc* 2011; **103**: 60-63 [PMID: 21329250]

38 **Minematsu H**, Hokama A, Makishi T, Arakaki K, Kinjo F, Fujita J. Colonoscopic findings and pathologic characteristics of Strongyloides colitis: a case series. *Digestion* 2011; **83**: 210-214 [PMID: 21266818 DOI: 10.1159/000321812]

39 **BROWNE DC**, CONTACOS PG, WELCH GE, McHARDY G. Treatment of Strongyloides stercoralis infection with intravenous gentian violet. *Am J Trop Med Hyg* 1957; **6**: 1066-1067 [PMID: 13487980]

40 **Horton J**. Albendazole: a review of anthelmintic efficacy and safety in humans. *Parasitology* 2000; **121** Suppl: S113-S132 [PMID: 11386684]

41 **Suputtamongkol Y**, Premasathian N, Bhumimuang K, Waywa D, Nilganuwong S, Karuphong E, Anekthananon T, Wanachiwanawin D, Silpasakorn S. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. *PLoS Negl Trop Dis* 2011; **5**: e1044 [PMID: 21572981 DOI: 10.1371/journal.pntd.0001044]

42 **Pene P**, Mojon M, Garin JP, Coulaud JP, Rossignol JF. Albendazole: a new broad spectrum anthelmintic. Double-blind multicenter clinical trial. *Am J Trop Med Hyg* 1982; **31**: 263-266 [PMID: 7041665]

43 **Bradley M**, Horton J. Assessing the risk of benzimidazole therapy during pregnancy. *Trans R Soc Trop Med Hyg* 2001; **95**: 72-73 [PMID: 11280072]

44 **Ndyomugyenyi R**, Kabatereine N, Olsen A, Magnussen P. Efficacy of ivermectin and albendazole alone and in combination for treatment of soil-transmitted helminths in pregnancy and adverse events: a randomized open label controlled intervention trial in Masindi district, western Uganda. *Am J Trop Med Hyg* 2008; **79**: 856-863 [PMID: 19052293]

45 **Zaha O**, Hirata T, Kinjo F, Saito A. Strongyloidiasis--progress in diagnosis and treatment. *Intern Med* 2000; **39**: 695-700 [PMID: 10969899]

46 **Ikeda T**. Pharmacological effects of ivermectin, an antiparasitic agent for intestinal strongyloidiasis: its mode of action and clinical efficacy. *Nihon Yakurigaku Zasshi* 2003; **122**: 527-538 [PMID: 14639007]

47 **Marti H**, Haji HJ, Savioli L, Chwaya HM, Mgeni AF, Ameir JS, Hatz C. A comparative trial of a single-dose ivermectin versus three days of albendazole for treatment of Strongyloides stercoralis and other soil-transmitted helminth infections in children. *Am J Trop Med Hyg* 1996; **55**: 477-481 [PMID: 8940976]

48 **Igual-Adell R**, Oltra-Alcaraz C, Soler-Company E, Sánchez-Sánchez P, Matogo-Oyana J, Rodríguez-Calabuig D. Efficacy and safety of ivermectin and thiabendazole in the treatment of strongyloidiasis. *Expert Opin Pharmacother* 2004; **5**: 2615-2619 [PMID: 15571478 DOI: 10.1517/14656566.5.12.2615]

49 **Zaha O**, Hirata T, Kinjo F, Saito A, Fukuhara H. Efficacy of ivermectin for chronic strongyloidiasis: two single doses given 2 weeks apart. *J Infect Chemother* 2002; **8**: 94-98 [PMID: 11957127 DOI: 10.1007/s101560200013]

50 **Turner SA**, Maclean JD, Fleckenstein L, Greenaway C. Parenteral administration of ivermectin in a patient with disseminated strongyloidiasis. *Am J Trop Med Hyg* 2005; **73**: 911-914 [PMID: 16282302]

51 **Boussinesq M**, Gardon J, Gardon-Wendel N, Chippaux JP. Clinical picture, epidemiology and outcome of Loa-associated serious adverse events related to mass ivermectin treatment of onchocerciasis in Cameroon. *Filaria J* 2003; **2** Suppl 1: S4 [PMID: 14975061 DOI: 10.1186/1475-2883-2-S1-S4]

P- Reviewer: Luo HS, Zambudio N **S- Editor**: Yu J **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3376

World J Gastroenterol 2015 March 21; 21(11): 3376-3379
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Proposed case of mesalazine-induced cardiomyopathy in severe ulcerative colitis

Kathryn Fleming, Andrew Ashcroft, Christopher Alexakis, Demitrios Tzias, Christopher Groves, Andrew Poullis

Kathryn Fleming, Andrew Ashcroft, Christopher Alexakis, Demitrios Tzias, Christopher Groves, Andrew Poullis, Department of Gastroenterology, St Georges Hospital, SW17 0QT London, United Kingdom

Author contributions: Fleming K and Alexakis C wrote the case presentation and acquired clinical data; Ashcroft A and Alexakis C wrote and referenced the case discussion; Tzias D wrote radiology section and selected appropriate image; Groves C and Poullis A contributed to the writing of all sections as well as the design of the report.

Supported by grants from NHS St Georges Hospital.

Correspondence to: Kathryn Fleming, MD, Department of Gastroenterology, St Georges Hospital, Blackshaw Road, SW17 0QT London, United Kingdom. katym.fleming@gmail.com
Telephone: +44-20 86721255
Fax: +44-20-87253855

Received: July 25, 2014
Peer-review started: July 26, 2014
First decision: October 14, 2014
Revised: October 25, 2014
Accepted: December 1, 2014
Article in press: December 1, 2014
Published online: March 21, 2015

Abstract

Five-amino salicylic acids are recommended for use in the management of inflammatory bowel disease, cardiac complications are a rare although recognised phenomenon. This report aims to highlight this serious but rare adverse reaction. We report here a case of a young man presenting with cardiogenic shock in the context of recent mesalazine treatment in severe ulcerative colitis.

Key words: Ulcerative colitis; Mesalazine; Cardiomyopathy; Adverse reactions

Core tip: A rare but serious occurrence in ulcerative colitis is myocarditis which can often be life-threatening. Whether 5-amino salicylic acids induced (as proposed here), or an autoimmune phenomenon in acute disease flare-ups, prompt recognition and treatment will be of benefit in the clinical setting.

Fleming K, Ashcroft A, Alexakis C, Tzias D, Groves C, Poullis A. Proposed case of mesalazine-induced cardiomyopathy in severe ulcerative colitis. *World J Gastroenterol* 2015; 21(11): 3376-3379 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3376.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3376

INTRODUCTION

Five-amino salicylic acids (5-ASA) are recommended for use in the management of inflammatory bowel disease and are commonly prescribed by both gastroenterologists and general physicians[1]. Serious reactions are rare, but recognised side effects include pancreatitis, interstitial nephritis, blood dyscrasias and Stevens Johnson syndrome. Cardiac side effects from 5-ASA agents are extremely uncommon.

CASE REPORT

A previously fit and well, non-smoking 31 year old

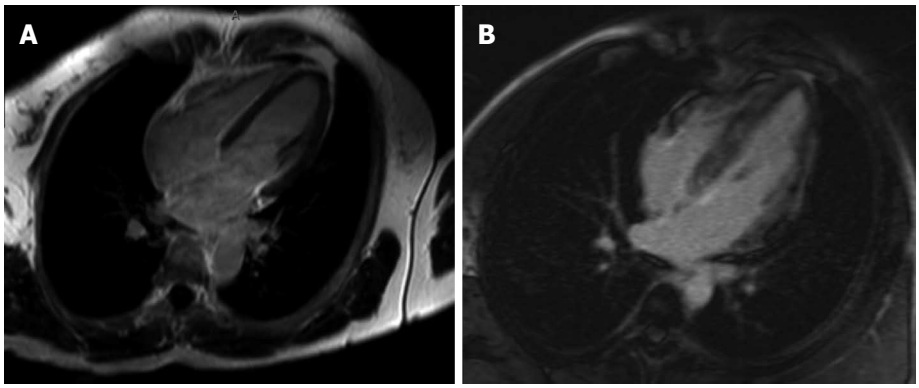


Figure 1 In the "normal" image (A) and "abnormal" image (B) in the late phase of gadolinium enhanced study. A: No enhancement in noted in the myocardium; B: There is diffuse, heterogeneous enhancement of the left and right ventricular myocardium as well as the interventricular septum in the late phase of gadolinium enhancement study.

male, presented with a 6 wk history of progressive diarrhoea, rectal bleeding and abdominal pain. Flexible sigmoidoscopy revealed severe confluent inflammation from the rectum to the point of insertion in the mid-sigmoid. Microscopy and stool culture were negative. Colonic biopsies showed moderate active chronic colitis, consistent with the endoscopic suspicion of ulcerative colitis, and so he was started on high dose oral mesalazine (2.4 g twice daily) with a view to a prompt outpatient follow up within the next few days.

Three days later he presented to the emergency department with chest pain, raised inflammatory markers and an elevated troponin. Initial ECG showed ST depression and biphasic T waves in leads V4-5 with no reciprocal changes. Mesalazine therapy was stopped at this stage. His condition deteriorated with cardiogenic shock developing, which required increasing inotropic support and subsequent insertion of an intra-aortic balloon pump. Coronary angiography was normal. Subsequent cardiac magnetic resonance imaging (MRI) revealed concentric hypertrophy and mildly impaired ejection fraction but no pericardial abnormalities. STIR2 imaging was suggestive of an acute inflammatory cardiac process. Gadolinium contrast studies excluded thrombi but showed diffuse heterogeneous enhancement of the myocardium suggestive of an atypical myocarditis, although other infiltrative cardiac pathologies (including sarcoid) were postulated (Figure 1). Serum virology showed no acute viral illness (previous parvovirus and previous Epstein-Barr virus positive but negative human immunodeficiency virus, Hepatitis B, Hepatitis C, influenza viruses A and B, coxsackie, CMV, adenovirus and enterovirus). An abdomen ultrasound scan was also unremarkable.

A diagnosis of myocarditis was highly suspected on clinical grounds and on day 2 of admission he underwent a cardiac biopsy. This showed several capillaries distended with microthrombi, myocyte vacuolation, interstitial oedema and focal haemorrhage. There was no evidence of vasculitis, fibrosis or amyloidosis. Similarly, there was no evidence of acute inflammatory infiltrate. On day 3 he suffered a cerebrovascular event manifest as acute right hemiplegia, right facial droop and dysarthria and he was treated with a heparin infusion. CT and subsequent MRI showed several acute and deep white matter infarcts highly suggestive of a cardiac source and bubble echo showed no evidence of intracardiac shunting.

Day 6 of admission he was weaned from inotropic and mechanical support. He was stepped-down from level 3 care. By day 10 repeat echo revealed significant improvement of left ventricular function and normal dimensions.

Throughout this period his ulcerative colitis progressed. Due to persistent bloody stool and resulting transfusion requirement his heparin infusion was discontinued and he was started on parenteral steroid therapy. Despite this, his clinical and biochemical parameters did not improve and he was commenced on a ciclosporin infusion as a bridging agent to long term azathioprine therapy. His colitis responded rapidly to the ciclosporin and he was converted to oral preparation. Following further neurological physiotherapy input with regards to his mild-moderate expressive language difficulty he was discharged home 27 d after his admission.

DISCUSSION

5-ASA have been shown to be efficacious in active Crohn's and ulcerative colitis, for inducing remission in ulcerative colitis[2,3] and are generally regarded as safe and well tolerated medications. Whilst the rate of reported common side effects including headache, dyspepsia or nausea can be high, one systematic review showed that the rate of adverse events or withdrawal from treatment is actually comparable to placebo[4]. Serious reactions are far rarer, but recognised side effects include pancreatitis, interstitial nephritis, blood dyscrasias and Stevens Johnson syndrome. Cardiac side effects from 5-ASA agents are extremely uncommon.

Acute cardiac complications in inflammatory bowel disease are also uncommon[5]. The most commonly reported complication is pericarditis, and in many cases this has been linked to the medications used

Table 1 Demonstrating cases of myocarditis presenting in ulcerative colitis and Crohn's disease not thought to be related to viral aetiology, inflammatory bowel disease treatment, selenium deficiency or TPN

Case report	Brief summary
Ulcerative colitis	
[7]	Fatal giant cell myocarditis after colectomy for ulcerative colitis
[8]	Idiopathic giant-cell myocarditis-natural history and treatment. Describes two cases of myocarditis in ulcerative colitis
[9]	Giant cell myocarditis monocytic immunophenotype of giant cells in a case associated with ulcerative colitis
[10]	Ulcerative colitis complicated by myopericarditis and complete artioventricular block
[11]	Myopericarditis complicating ulcerative colitis
[12]	Acute peri-myocarditis in ulcerative colitis
[13]	Transient myocarditis associated with fulminant colitis
[14]	Giant cell myocarditis (subacute congestive heart failure in context of colitis)
[15]	Fatal giant-cell myocarditis complicated with ulcerative colitis
Crohn's Disease	
[16]	A Case of Acute Myocarditis as the Initial Presentation of Crohn's Disease
[8]	Idiopathic giant-cell myocarditis-natural history and treatment. Describes one case of myocarditis in crohn's disease
[17]	Transmural inflammation consisting of lymphocytic infiltration in context of severe malnutrition leading to sudden death in Crohn´s Disease
[18]	Myocarditis and subcutaneous granulomas in a patient with Cohn's disease of the colon
[19]	Myocarditis in children with inflammatory bowel disease

to manage the disease[6]. In rare circumstance myocarditis has been described as a manifestation of inflammatory bowel disease (Table 1) however the temporal relationship with the onset of our patients' symptoms and the start of his medication would indicate a causal relationship.

Cardiac hypersensitivity to 5-ASA therapy has been reported in a small number of case studies. It was first suggested in the Lancet in 1989, where a possible myocarditis was proposed[20], and the first reported death from myocarditis associated with mesalazine was in 1990[21]. The exact mechanism by which mesalazine causes a myocarditis has not been clearly determined, but a hypersensitivity reaction with eosinophilic infiltration has been proposed[22-24].

The temporal relationship between this otherwise fit and well young patient developing rapidly progressive and severe cardiomyopathy with resultant cardiogenic shock soon after starting on mesalazine strongly suggests a link between the disease process or treatment. The onset of cardiac symptoms and impaired function correlate with the starting of 5-ASA treatment and the subsequent improvement shortly after cessation, despite a relative worsening in his ulcerative colitis over the same period would strengthen the case for medication being the culprit. Whilst the cardiac MRI and biopsy do not show definitive evidence of myocarditis and the rapidity of cardiac deterioration contrasting with previous reports suggesting a typical period of two weeks following commencement of treatment prior to presentation, with some even up to years after starting 5-ASAs, there are limited numbers of cases reported so far and the rapid improvement on cessation of therapy, as in this case, is more typical[25].

5-ASA agents are both efficacious and generally safe in the management of inflammatory bowel disease, and whilst serious and potentially life-threatening complications may occur, these are very rare and should not necessarily discourage their use. This case aims to highlight and raise awareness of one such potential complication to ensure its consideration, prompt recognition and diagnosis in the future.

COMMENTS

Case characteristics

Acute myocarditis post mesalamine treatment leading to cardiogenic shock in severe ulcerative colitis.

Clinical diagnosis

Chest pain very short followed by cardiogenic shock requiring inotropic support.

Differential diagnosis

Viral myocarditis, autoimmune myocarditis.

Laboratory diagnosis

Raised troponin and inflammatory markers at diagnosis with a negative acute virology screen only revealing past EBV and parvovirus infection.

Imaging diagnosis

Cardiac magnetic resonance imaging showing diffuse, heterogeneous enhancement of the left and right ventricular myocardium.

Pathological diagnosis

Cardiac biopsy showing several capillaries distended with microthrombi, myocyte vacuolation, interstitial oedema and focal haemorrhage with Colonic biopsy showing moderate active chronic colitis.

Treatment

This gentleman underwent a very short course of high dose mesalamine therapy.

Related reports

There are a small number of five-amino salicylic acids (5-ASA) induced myocarditis cases in inflammatory bowel disease, however the mechanism is not well understood and it remains a serious but rare occurrence.

Term explanation

STIR2 imaging - Short tau inversion recovery, a fat suppression technique to better distinguish tissue components.

Experiences and lessons

This case report highlights a rare but potentially life threatening adverse effect of 5-ASA treatment for ulcerative colitis.

Peer-review

This is an interesting case of a rare side effect of a drug which is usually thought to be a "benign" agent. It is well written.

REFERENCES

1 **Carter MJ**, Lobo AJ, Travis SP. Guidelines for the management of inflammatory bowel disease in adults. *Gut* 2004; **53** Suppl 5: V1-V16 [PMID: 15306569 DOI: 10.1136/gut.2004.043372]

2 **Hanauer SB**, Strömberg U. Oral Pentasa in the treatment of active Crohn's disease: A meta-analysis of double-blind, placebo-controlled trials. *Clin Gastroenterol Hepatol* 2004; **2**: 379-388 [PMID: 15118975 DOI: 10.1016/S1542-3565(04)00122-3]

3 **Sutherland L**, MacDonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2003; (**3**): CD000543 [PMID: 12917894 DOI: 10.1002/14651858.CD000544.pub3]

4 **Loftus EV**, Kane SV, Bjorkman D. Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 2004; **19**: 179-189 [PMID: 14723609]

5 **Tsianos EV**, Katsanos KH. The heart in inflammatory bowel disease. *Ann Gastroentol* 2002; **15**: 124-133

6 **Dubowitz M**, Gorard DA. Cardiomyopathy and pericardial tamponade in ulcerative colitis. *Eur J Gastroenterol Hepatol* 2001; **13**: 1255-1258 [PMID: 11711786 DOI: 10.1097/00042737-200110000-00022]

7 **McKeon J**, Haagsma B, Bett JH, Boyle CM. Fatal giant cell myocarditis after colectomy for ulcerative colitis. *Am Heart J* 1986; **111**: 1208-1209 [PMID: 3716997 DOI: 10.1016/0002-8703(86)90031-1]

8 **Cooper LT**, Berry GJ, Shabetai R. Idiopathic giant-cell myocarditis--natural history and treatment. Multicenter Giant Cell Myocarditis Study Group Investigators. *N Engl J Med* 1997; **336**: 1860-1866 [PMID: 9197214 DOI: 10.1056/NEJM199706263362603]

9 **Ariza A**, López MD, Mate JL, Curós A, Villagrasa M, Navas-Palacios JJ. Giant cell myocarditis: monocytic immunophenotype of giant cells in a case associated with ulcerative colitis. *Hum Pathol* 1995; **26**: 121-123 [PMID: 7821909 DOI: 10.1016/0046-8177(95)90124-8]

10 **Thuesen L**, Sørensen J. [Ulcerative colitis complicated by myopericarditis and complete atrioventricular block]. *Ugeskr Laeger* 1979; **141**: 2760-2761 [PMID: 531970]

11 **Mowat NA**, Bennett PN, Finlayson JK, Brunt PW, Lancaster WM. Myopericarditis complicating ulcerative colitis. *Br Heart J* 1974; **36**: 724-727 [PMID: 4414769 DOI: 10.1136/hrt.36.7.724]

12 **Seitz R**, Wehr M. [Acute peri-myocarditis in ulcerative colitis]. *Internist* (Berl) 1980; **21**: 760-763 [PMID: 6110643]

13 **Williamson JM**, Dalton RS. Transient myocarditis associated with fulminant colitis. *ISRN Surg* 2011; **2011**: 652798 [PMID: 22084770 DOI: 10.5402/2011/652798]

14 **Mohite PN**, Zych B, Popov AF, Banner NR, Simon AR. Successful treatment of ulcerative colitis-related fulminant myocarditis using extracorporeal life support. *Heart Surg Forum* 2013; **16**: E208-E209 [PMID: 23958533 DOI: 10.1532/HSF98.20121141]

15 **Nakamura F**, Nakashima Y, Takeuchi T, Tomida T, Naruse K, Ohno O, Okamura S, Maeda M. [Fatal giant-cell myocarditis complicated with ulcerative colitis]. *Nihon Naika Gakkai Zasshi* 2006; **95**: 1112-1114 [PMID: 16846062 DOI: 10.2169/naika.95.1112]

16 **Oh IS**, Choi CH, Park JH, Kim JW, Cha BK, Do JH, Chang SK, Kwon GY. A case of acute myocarditis as the initial presentation of Crohn's disease. *Gut Liver* 2012; **6**: 512-515 [PMID: 23170159 DOI: 10.5009/gnl.2012.6.4.512]

17 **Hitosugi M**, Kitamura O, Takatsu A. Sudden death of a patient with Crohn's disease. *Nihon Hoigaku Zasshi* 1998; **52**: 211-214 [PMID: 9780667]

18 **Weiss N**, Rademacher A, Zoller WG, Schlöndorff D. Myocarditis and subcutaneous granulomas in a patient with Crohn's disease of the colon. *Am J Med* 1995; **99**: 434-436 [PMID: 7573101 DOI: 10.1016/S0002-9343(99)80194-6]

19 **Frid C**, Bjarke B, Eriksson M. Myocarditis in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1986; **5**: 964-965 [PMID: 3794918 DOI: 10.1097/00005176-198611000-00025]

20 **Agnholt J**, Sørensen HT, Rasmussen SN, Gøtzsche CO, Halkier P. Cardiac hypersensitivity to 5-aminosalicylic acid. *Lancet* 1989; **1**: 1135 [PMID: 2566070 DOI: 10.1016/S0140-6736(89)92407-0]

21 **Kristensen KS**, Høegholm A, Bohr L, Friis S. Fatal myocarditis associated with mesalazine. *Lancet* 1990; **335**: 605 [PMID: 1968595 DOI: 10.1016/0140-6736(90)90387-K]

22 **Galvão Braga C**, Martins J, Arantes C, Ramos V, Vieira C, Salgado A, Magalhães S, Correia A. Mesalamine-induced myocarditis following diagnosis of Crohn's disease: a case report. *Rev Port Cardiol* 2013; **32**: 717-720 [PMID: 23993290 DOI: 10.1016/j.repc.2012.12.018]

23 **Robertson E**, Austin D, Jamieson N, Hogg KJ. Balsalazide-induced myocarditis. *Int J Cardiol* 2008; **130**: e121-e122 [PMID: 17889383 DOI: 10.1016/j.ijcard.2007.07.033]

24 **García-Ferrer L**, Estornell J, Palanca V. Myocarditis by mesalazine with cardiac magnetic resonance imaging. *Eur Heart J* 2009; **30**: 1015 [PMID: 19168869 DOI: 10.1093/eurheartj/ehn615]

25 **Merceron O**, Bailly C, Khalil A, Pontnau F, Hammoudi N, Dorent R, Michel PL. Mesalamine-induced myocarditis. *Cardiol Res Pract* 2010; **2010**: [PMID: 20871830 DOI: 10.4061/2010/930190]

P- Reviewer: Ahluwalia NK, Desai DC, Meucci G, Shussman N
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3380

World J Gastroenterol 2015 March 21; 21(11): 3380-3387
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Colonic sarcoidosis: Unusual onset of a systemic disease

Paola Erra, Sonia Crusco, Loredana Nugnes, Anna Maria Pollio, Gianni Di Pilla, Giuseppe Biondi, Giovanni Vigliardi

Paola Erra, Sonia Crusco, Gianni Di Pilla, Department of Radiology and Nuclear Medicine, ASREM, "F. Veneziale" Hospital, 86070 Isernia, Italy
Loredana Nugnes, Anna Maria Pollio, Department of Pathological Anatomy and Histology: Cytodiagnostics, ASREM, "F. Veneziale" Hospital, 86070 Isernia, Italy
Giuseppe Biondi, Giovanni Vigliardi, Department of General Surgery, ASREM, "F. Veneziale" Hospital, 86070 Isernia, Italy
Author contributions: Vigliardi G and Erra P designed the report; Crusco S and Nugnes L collected the patient's clinical data; Biondi G, Di Pilla G and Pollio AM performed the literature review; Erra P and Vigliardi G wrote the manuscript.
Ethics approval: Si comunica che in data 29/07/2014 il Comitato Eticl dell'Azienda Sanitaria Regionale del Molisc ha csaminato cd approvato I'articolo scientifico dal titolo "Unusual onset of colonic sarcoidosis a case report" redatto dai Dottori Paola Erra, Sonia Crusco, Loredana Nugnes, Anna Maria Pollio, Gianni Di Pilla, Giuseppe Biondi, Giovanni Vigliardi Dirigenti Medici in servizio presso ASREM Ospedale "F. Veneziale" di Isernia.
Informed consent: Il sottoscritto Barile Costantino, nato a Lamusei (OG), Italia, il 22.03.1955 concede il consenso al trattamento dei propri dati personali e all'utilizzo delle immagini relative alla propria patologia rilevata presso l'Ospedale "F.Veneziale" di Isernia (IS), Italia.
Conflict-of-interest: There are no conflicts of interest.

Correspondence to: Paola Erra, MD, Department of Radiology and Nuclear Medicine, ASREM, "F. Veneziale" Hospital, 1 Via Sant'Ippolito, 86070 Isernia, Italy. paola.erra@libero.it
Telephone: +11-39-0865442206
Fax: +11-39-0865442422
Received: October 5, 2014
Peer-review started: October 6, 2014
First decision: October 29, 2014
Revised: November 8, 2014
Accepted: December 16, 2014
Article in press: December 16, 2014
Published online: March 21, 2015

Abstract

Sarcoidosis is a multisystem chronic inflammatory condition of unknown etiology that has the potential to involve every tissue in the body. Sarcoidosis in the gastrointestinal system, and particularly the colon, is very rare. Here, we report the case of a 57-year-old man with no previous diagnosis of sarcoidosis who presented with new onset of abdominal pain and constipation. A colonoscopy revealed that the abdominal pain was caused by an obstructing lesion in the cecum-ascending colon and lacked a clear histologic diagnosis. Radiologic investigation revealed concentric wall thickening of the cecum-ascending colon with multiple satellite lymphadenopathies, highly suggestive of a malignancy. The patient underwent a laparotomy and a right hemicolectomy was performed. A diagnosis of colonic sarcoidosis was made after the resected specimen was examined. Additionally, a chest computed tomography scan revealed lung involvement with atypical radiologic features in the absence of respiratory symptoms. Only histologic examination of the surgical specimen can yield a diagnosis of gastrointestinal sarcoidosis due to the non-specificity of endoscopic and radiologic findings.

Key words: Colon; Sarcoidosis; Hemicolectomy; Systemic disease; Noncaseating granuloma

Core tip: Gastrointestinal tract involvement in systemic sarcoidosis is rare. This case report of a patient with gastrointestinal sarcoidosis is clinically relevant because the colonic location highlights an unusual cause of abdominal pain. This study provides an opportunity to clarify diagnostic criteria and therapeutic management for such a rare condition.

Erra P, Crusco S, Nugnes L, Pollio AM, Di Pilla G, Biondi G, Vigliardi G. Colonic sarcoidosis: Unusual onset of a systemic

disease. *World J Gastroenterol* 2015; 21(11): 3380-3387 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3380.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3380

INTRODUCTION

Sarcoidosis is a multisystem chronic inflammatory condition of unknown etiology. The characteristic histologic lesions are noncaseating granulomas that contain multinucleated giant cells in the absence of other autoimmune diseases, infectious disease or foreign agents[1]. Sarcoidosis is more common in young and middle-aged patients and is more prevalent in females[2]. It most commonly affects African-Americans, Swedes and Danes[2]. Sarcoidosis has a prevalence range of 1-40 cases per 100000 people in the general population. In the United States, the age-adjusted annual incidence of sarcoidosis among blacks is more than triple that for Caucasians (35.5 *vs* 10.9 per 100000 inhabitants)[3].

Sarcoidosis is an immune-mediated multisystem disease; it is hypothesized it affects genetically susceptible hosts after interaction with a single or multiple factors. Different potential antigenic agents for sarcoidosis have been proposed, such as infectious pathogens, environmental and occupational exposures, without any definitive conclusion.

The clinical signs and symptoms for sarcoidosis are not specific and include respiratory symptoms (cough, dyspnea, bronchial hyper-reactivity), fatigue, night sweats, weight loss, and, less commonly, fever. However, approximately 50% of the sarcoidosis cases are asymptomatic and may be detected by chance during chest radiography[4,5]. Pulmonary involvement is the most common feature, with bilateral hilar adenopathy being the primary radiologic finding after a chest X-ray. Moreover, sarcoidosis in the lungs accounts for a majority of the morbidity and mortality associated with this condition. Multiple small nodules distributed along the lymphatic vessels that run within the interstitial tissues of bronchovascular bundles and the subpleural and perilobular spaces, which are potentially associated with ground-glass opacities or air-space consolidation, are also typically observed[6]. In 50% of symptomatic sarcoidosis patients, extrathoracic involvement can be an initial manifestation. Although skin and ocular lesions are very common, any organ or gland can be involved, including the liver, spleen, lymph nodes, parotid glands, central nervous system, genitourinary system, muscles and bones. Gastrointestinal involvement is quite rare, however, with a prevalence of less than 1% and may present along with systemic disease or as an isolated finding[1,7]. Although sarcoidosis has been observed in every part of the gastrointestinal tract, it most commonly affects the stomach[8]. The colon is involved less frequently[1,9,10] and sarcoidosis in the colon is difficult to diagnose preoperatively due to nonspecific symptoms and/or endoscopic findings.

Here, we report a case of a 57-year-old man in apparent good health who presented with newly onset abdominal pain and constipation due to an obstructing lesion in the cecum-ascending colon as a result of sarcoidosis. The patient underwent a right hemicolectomy and a formal diagnosis was made upon histologic examination of the surgical specimen. Simultaneously, in the absence of respiratory symptoms, lung involvement with atypical radiologic features was discovered by chance during a chest computed tomography (CT) scan. Sarcoidosis was later confirmed after histologic examination of pulmonary tissue obtained *via* CT-guided biopsy.

CASE REPORT

A 57-year-old white Italian male patient presented at our hospital with abdominal pain and symptoms related to colonic obstruction. The patient was healthy until three to four weeks prior to presentation, when new-onset constipation developed and he began passing pencil-like stools. His primary complaints were bloating, anorexia and a slight weight loss over the previous two months.

He had no recent travel history or family members with autoimmune disease; his sister had pulmonary tuberculosis at a young age.

A physical examination of the patient revealed mild abdominal distention and tenderness of the right lower abdominal quadrant; no peritoneal signs were found. Routine hematology and biochemistry analyses revealed a white cell count of 8.6 × 10^3/mL with a low lymphocyte count (16.9% and 1.4 × 10^3/mL). Alpha-amylase levels were elevated (114 mg/dL), whereas liver tests and serum electrolytes were normal. Iron serum levels were 42 μg/dL, with normal values of ferritin (188 ng/mL) and hemoglobin (14.5 g/dL). Serum electrophoresis revealed an albumin level of 54.4%, with a peak of alpha-1 globulin (6.2%). Carcinoembryonic antigen levels were slightly elevated (3.6 ng/mL).

A colonoscopy revealed a stenotic obstructive lesion at the cecum-ascending colon transition and we were unable to advance the colonoscope beyond this area. Biopsy specimens obtained from this site showed no evidence of a neoplastic lesion, but rather an acute inflammatory infiltration of the submucosa. An abdominal CT (Figure 1) revealed marked symmetric concentric wall thickening in the cecum-ascending colon, which notably reduced the enteral lumen and was associated with a heterogeneous hyperdensity of perivisceral fat tissue. Enlargement of the appendix characterized by a thickened wall with marked enhancement was also found. We observed multiple satellite lymphadenopathies along the ileocolic vessels,

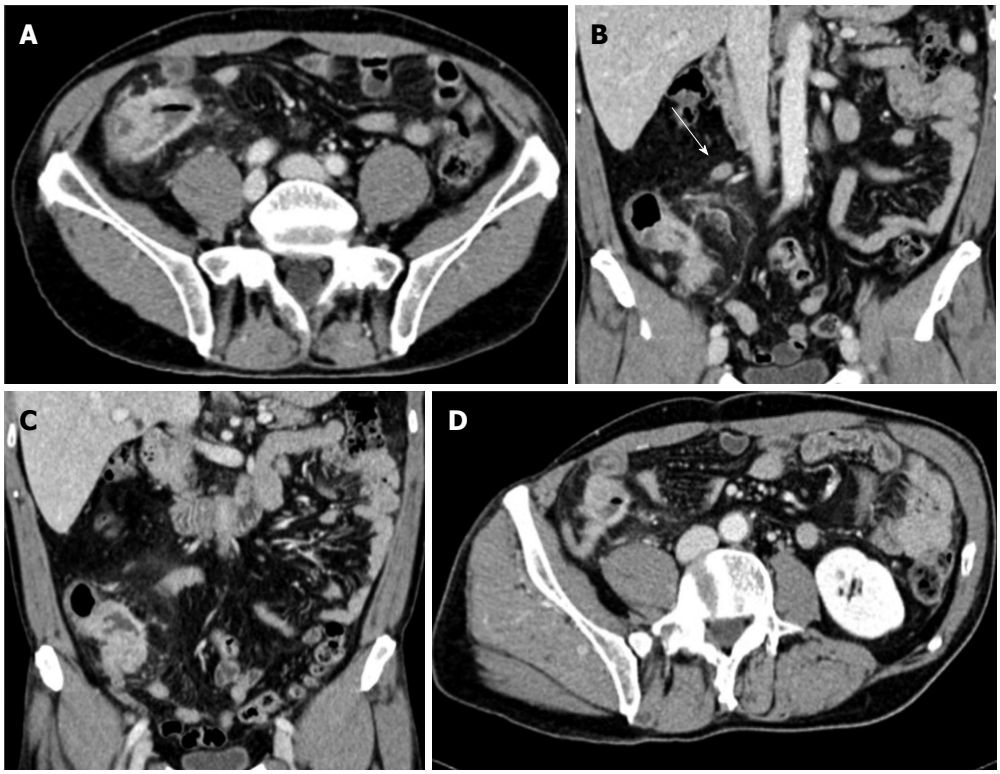


Figure 1 Abdominal computed tomography performed after colonoscopy. A: Transverse image of the cecum-ascending colon; B and C: Coronal images showing symmetric concentric wall thickening of the cecum-ascending colon mimicking a tumor lesion; heterogeneous hyperdensity of perivisceral fat tissue is also seen. Satellite lymphadenopathy (arrow) is observed along the ileocolic vessels; D: Enlarged appendix with a thickened wall and marked contrast enhancement.

with the largest measuring approximately 1.6 cm.

A chest CT (Figure 2) simultaneously performed to assess patient whole-body staging revealed asymmetric discrete airspace consolidation with air bronchograms in the right upper lobe. Numerous micronodular opacities were also found distributed in the peribronchovascular interstitium in both upper pulmonary lobes. Pathologic hilar or mediastinal adenopathies were not observed, and 1.2 cm was the maximum diameter in the right hilum. A radiologist interpreted these findings as a manifestation of right-sided pneumonia associated with bilateral small airway inflammation in relation to a mild cough and slight fever in the patient the previous week. The patient was administered four weeks of oral antibiotic therapy with quinolones.

Using the findings from the endoscopy and observations *via* radiology, a malignant colonic lesion was suspected, despite the unclear histology results. The patient underwent an exploratory laparotomy that revealed a 4 cm stenotic lesion in the cecum-ascending colon, with numerous peritoneal micronodules and adjacent lymphadenopathies (Figure 3). The appendix was enlarged with a maximum diameter of 4 cm. A carcinologic right hemicolectomy was performed.

Examination of the resected specimen revealed a stenotic ulcerated lesion in the colonic wall in proximity to the ileocecal valve and microscopic examination showed multiple noncaseous granulomas composed of a central core of epithelioid cells and multinucleated giant cells surrounded by a lymphocyte cuff (Figure 4). These features were also observed in the locoregional lymph nodes and peritoneal micronodules (Figure 5). The appendix was also pathologically involved. Ziehl-Neelsen staining did not reveal acid-fast bacilli and no other organisms or foreign bodies were identified. In addition, polymerase chain reaction analysis did not reveal *Mycobcaterium tuberculosis* or atypical mycobacterial DNA.

Following the surgery, the patient responded favorably and was discharged six days later in good condition. A chest CT performed four weeks after the antibiotic therapy revealed no change in the bilateral pulmonary manifestations indicating that the inflammatory condition was not responsive to the treatment. A CT-guided biopsy of the lung was later performed on the right upper lobe parenchymal consolidations and a final histologic diagnosis of pulmonary sarcoidosis was made.

DISCUSSION

Sarcoidosis is a multisystem disorder of unknown etiology that has the potential to affect almost every tissue in the body. Gastrointestinal involvement, however, is quite rare and accounts for less than 1% of cases. Sarcoidosis of the colon is even more rare, usually asymptomatic[11,12] and may mimic many other diseases, including colonic granulomatous disorders, such as tuberculosis, syphilis, fungal infection,

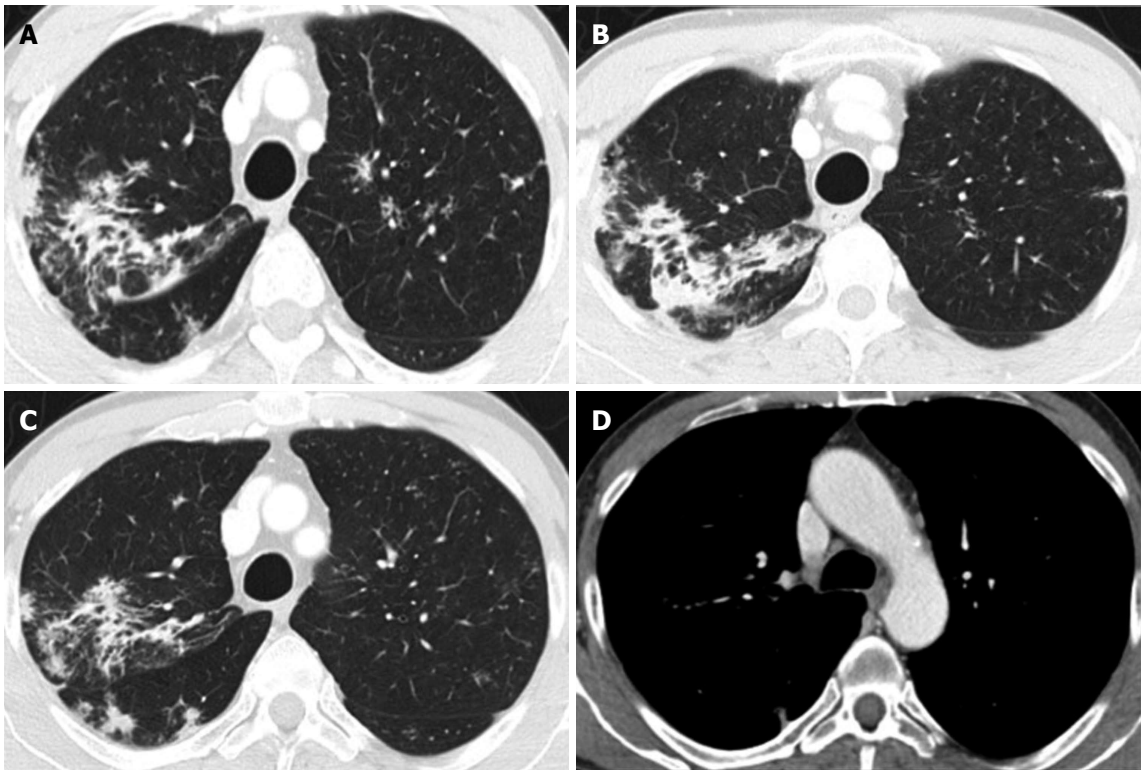


Figure 2 Chest computed tomography performed after colonoscopy for whole-body staging. A and B: Asymmetric discrete airspace consolidation with air bronchograms in the right upper lobe; C: Numerous micronodular opacities are present in both upper pulmonary lobes (also seen in panel A) with distribution in the peribronchovascular interstitium. These findings showed no significant change after four weeks of antibiotic therapy; D: No pathologic hilar or mediastinal lymph node enlargement was observed.

inflammatory bowel disease, and malignancies.

A systematic review of the literature revealed that only a few cases for this location have been reported. Beniwal and colleagues performed a literature review and found ten cases of colonic sarcoidosis from 1966 to 2003[7]. The sigmoid colon was most frequently affected.

When tubular organs are affected in sarcoidosis, it is usually associated with concomitant pulmonary disease. Hilzenrat *et al*[13] published a case report of a nonoperative diagnosis and management of colonic obstruction secondary to systemic sarcoidosis. In this case, the obstructive symptoms were resolved with a moderate dose of corticosteroid therapy, thus eliminating the need for urgent surgical resection of the colonic lesion. It is important to note that pulmonary sarcoidosis had been diagnosed in this case six years earlier and endoscopic mucosal biopsies on the colonic-obstructing lesion resulted in a final diagnosis of sarcoidosis.

Nchimi *et al*[14] reported a case of 7-year-old boy admitted for moderate weight loss and intermittent diarrhea associated with cough and exertional dyspnea. In this case, symmetric wall thickening of the terminal ileum and cecum observed on an abdominal CT were correctly interpreted as a sarcoidotic gastrointestinal location due to an association with typical features of pulmonary sarcoidosis observed by chest radiography (bilateral and diffuse parenchymal air-space and interstitial opacities with bilateral hilar node enlargement). Moreover, gallium 67 scintigraphy revealed marked bilateral and symmetric uptake in the lungs and the parotid and lacrimal gland, definitively confirming a diagnosis of sarcoidosis.

To the best of our knowledge, this is the first case in which the sarcoidosis started with symptomatic gastrointestinal involvement that was associated with atypical pulmonary features revealed by chest CT, in the absence of clear and definite respiratory symptoms. Using these findings alone, it would have been very difficult to relate the patient's abdominal manifestations to a systemic disease. The clinical presentation of gastrointestinal sarcoidosis is not specific, and when present, the symptoms generally resemble those of an obstructive colonic disease.

The case reported by Nchimi *et al*[14] showed thickening of the terminal ileum wall, which caused intermittent diarrhea. Aaronson *et al*[15] discussed a case of sigmoid colonic sarcoidosis presenting with constipation and hematochezia. Hilzenrat *et al*[13] reported a case with stricture and obstruction of the colon, resulting in abdominal pain, distention, vomiting, constipation and weight loss. Similar to these findings, our patient was symptomatic with colonic obstruction-related symptoms, such as constipation and abdominal pain with mild tenderness of the right lower abdominal quadrant. While reviewing the literature, we found only one case of rectal sarcoidosis with secondary

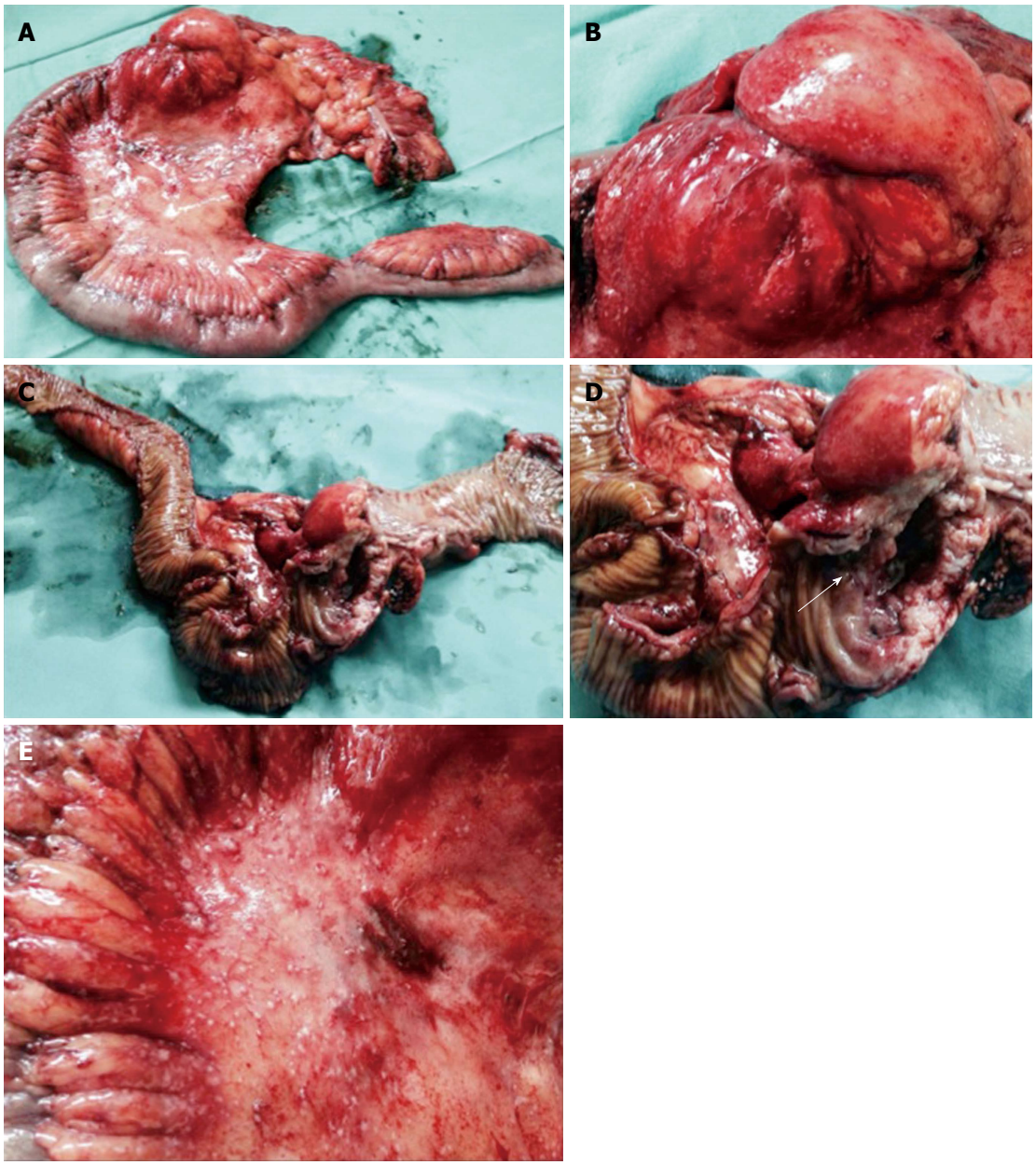


Figure 3 Resected specimen after laparotomy. A: Right hemicolectomy was performed; B-D: A voluminous stenotic ulcerated lesion of the colonic wall in proximity to the ileocecal valve (arrow in D) was observed; E: Numerous peritoneal micronodules near the colonic lesion are shown.

paralytic ileus that resembled adult-onset Hirschsprung disease[16]. In this particular case, a barium enema and rectal manometry showed rectal dilatation with weak or nearly absent colonic contraction. Neither a gastrografin small bowel study nor a colonoscopy identified an organic obstruction causing a mechanical ileus.

Imaging and endoscopic findings are not diagnostically specific for sarcoidosis and may overlap with other diseases, such as chronic inflammatory bowel disease, tuberculosis, lymphoma and carcinoma. Endoscopic appearances are extremely varied and include aphthous erosions or ulcers, friable mucosa or small punctate bleeding sites mimicking colitis, plaque-like lesions, focal nodularity or segmental narrowing[17].

Veitch *et al*[18] reported a case with asymptomatic colonic sarcoidosis presenting as colonic polyposis where the polyps were adenomatous in appearance. Ushiki *et al*[9] presented another case of colonic sarcoidosis with multiple elevated lesions mimicking submucosal tumors in the colon; these lesions were sessile, soft, had a smooth surface and a diameter of 2-5 mm. Sarcoidosis lesions may also form irregular mass lesions mimicking a carcinoma[19]. In the case presented in this study, the stenotic lesion was highly suggestive of malignancy.

Histologic examination is necessary to establish or exclude diagnosis of colonic involvement. In biopsy specimens, the presence of noncaseating granulomas is the pathologic hallmark of this disease[5,7,8]. It is necessary

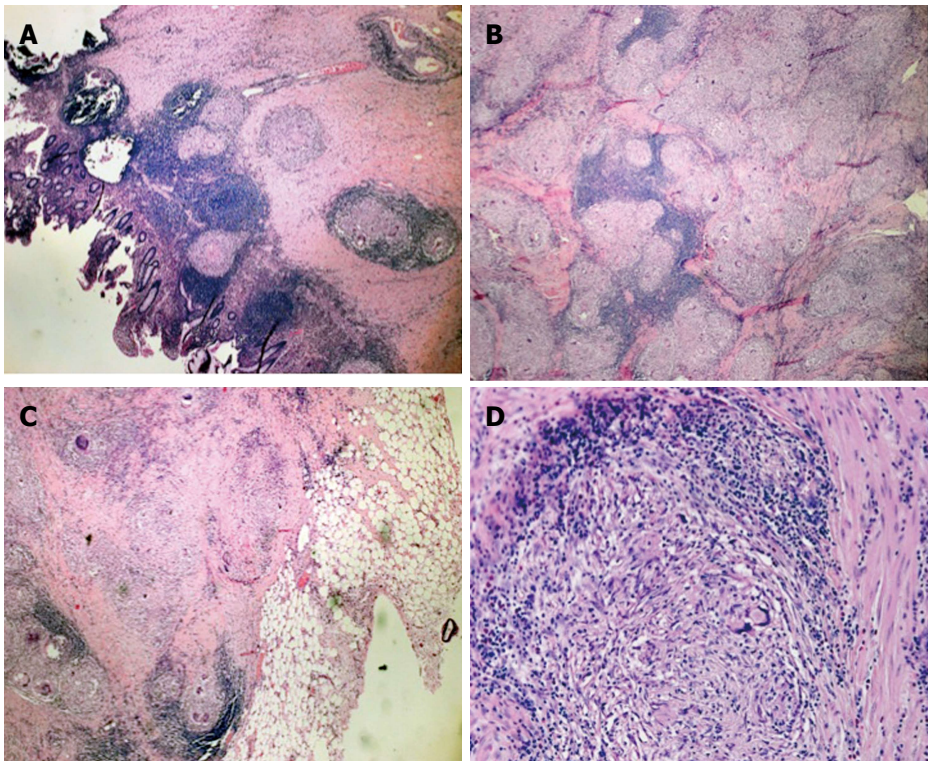


Figure 4 Histologic examination of the intraoperative specimen. Hematoxylin and eosin staining showed A: Noncaseating epithelioid granulomas in the colonic wall (magnification × 5); B: Confluent granulomata in the colonic wall (magnification × 10); C: Perivisceral involvement (magnification × 5); D: Microscopic aspects of the sarcoidotic granulomas (magnification × 20).

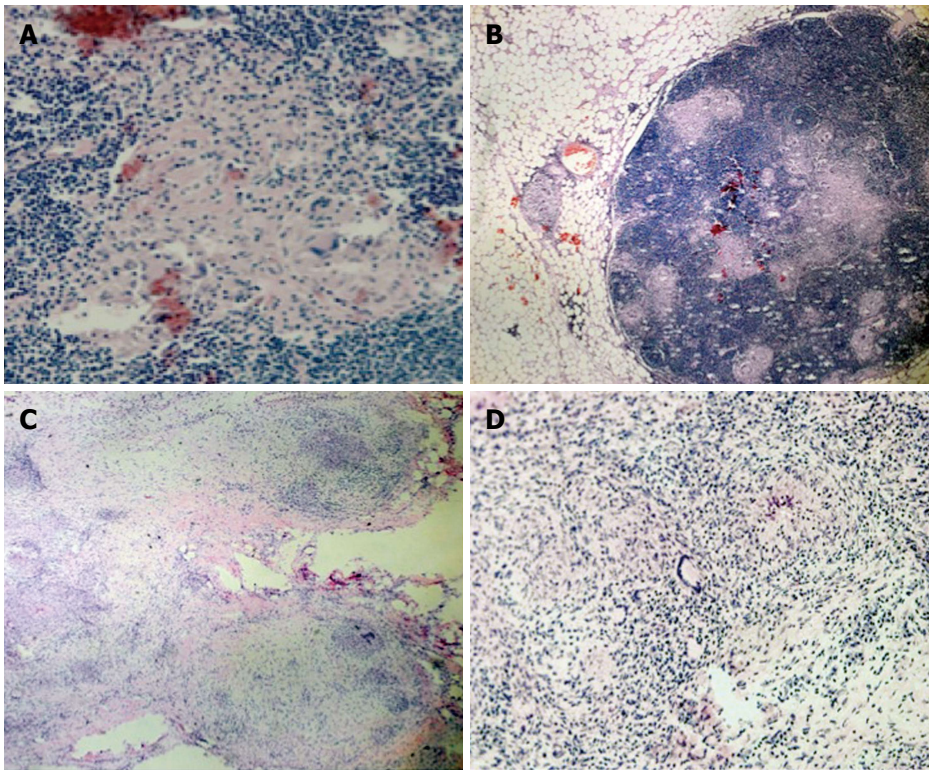


Figure 5 Extracolonic sarcoidosis involvement. Hematoxylin and eosin staining revealed: Sarcoidotic involvement of locoregional lymphadenopathy (A, B) (A: × 10; B: magnification × 40); Sarcoidotic granulomas in the parietal peritoneum adjacent to the colonic lesion (C, D) (C: magnification × 2; D: magnification × 20).

to exclude other granulomatous disorders such as tuberculosis, fungal infections, inflammatory bowel disease, malignancy and delayed-type hypersensitivity to foreign antigens. Intestinal sarcoidosis is easy to distinguish from Crohn's disease and Wegener granulomatosis.

It's noteworthy that, in contrast with our case, most of authors[9,13,16,20,21] obtained definitive diagnosis of sarcoidosis on colon biopsy. Only Daldoul *et al*[17] revealed histology failure on endoscopic biopsy specimen of a stenotic lesion in the ascending colon; in this case the patient underwent a laparotomy and a right hemicolectomy was made.

Besides, we have verified that, when clinical presentation in association with biopsy results are suggestive of sarcoidosis, patients are started on corticosteroid therapy, with significant improvement in their symptoms[13,16,21]. Hilzenrat *et al*[13] showed a complete regression of two obstructive colonic lesions after steroid treatment, as seen on barium enema and colonscopy, where no granulomas were found on mucosal biopsy specimens after therapy.

From our literature review, we have confirmed that there are very few radiologic descriptions of colonic sarcoidosis. CT may show segmental or symmetric wall thickening with preserved wall stratification[14]. In our case, CT showed symmetric thickening of the colonic wall, highly suggestive of a tumor; on the other hand, pathologic enlargement of the appendix observed in the present case is rather uncommon in malignancies.

If a pseudotumor is suspected, however, and the biopsy specimens fail to demonstrate a definitive diagnosis of colonic sarcoidosis, surgical treatment with conventional surgical oncologic principles must be proposed[22]. In the other cases, systemic sarcoidosis is usually readily responsive to corticosteroid therapy and clinical resolution may be reached without surgery.

The colon is an unusual site for gastrointestinal sarcoidosis and its symptomatic involvement may represent the onset of this systemic disease. A preoperative diagnosis of sarcoidosis is difficult to make due to the overlap of imaging and radiologic findings with that of malignancies. In patients with a previous diagnosis of sarcoidosis, however, it should be suspected. In all cases, a histologic examination that reveals noncaseating granulomas allows for a definitive diagnosis. If the histology is unclear, patients will need to undergo surgery for a diagnosis.

COMMENTS

Case characteristics

A 57-year-old man in apparent good health with abdominal pain and constipation.

Clinical diagnosis

New-onset constipation with mild abdominal distention and tenderness of the right lower abdominal quadrant; subtle weight loss.

Differential diagnosis

Malignant tumor of the gastrointestinal tract.

Laboratory analysis

White blood cell count, 8.6 × 10^3/mL with 1.4 × 10^3/mL lymphocytes; hemoglobin, 14.5 g/dL; iron serum, 42 μg/dL with normal values for ferritin (188 ng/mL); carcinoembryonic antigen, 3.6 ng/mL; albumin, 54.4% with a peak of alpha-1 globulin (6.2%); alpha-amylase, 114 mg/dL; liver tests and serum electrolytes were normal.

Imaging diagnosis

An abdominal computed tomography revealed symmetric concentric wall thickening associated with heterogeneous hyperdensity of perivisceral fat tissue and satellite adenopathy at the cecum-ascending colon transition, causing a notable reduction in the enteral lumen; enlargement of the appendix was also found.

Pathological diagnosis

A colonoscopy revealed a stenotic obstructive lesion at the cecum-ascending colon transition; a biopsy showed no evidence of a neoplastic lesion, but rather an acute inflammatory infiltration of the submucosa.

Treatment

The patient underwent a right hemicolectomy.

Related reports

Very few cases of colonic sarcoidosis have been reported in the literature and most of them have been found in patients with a previous diagnosis of this systemic disease.

Term explanation

Sarcoidosis is a multisystem disorder of unknown etiology that has the potential to affect almost every tissue in the body. Polymerase chain reaction for DNA specific to *Mycobacterium tuberculosis* and atypical mycobacteria can be used to rule out these bacteria as the cause of the disease.

Experiences and lessons

This case report presents an unusual onset of systemic sarcoidosis with colonic involvement causing gastrointestinal obstruction-related symptoms.

Peer-review

The authors present the case of a 57-year-old man who presented with an obstructing colonic mass. Colonoscopic biopsies were non-diagnostic and he underwent hemicolectomy and was found to have colonic sarcoidosis. This is an unusual presentation of a highly condition, and a worthwhile case report.

REFERENCES

1 **MacArthur KL**, Forouhar F, Wu GY. Intra-abdominal complications of sarcoidosis. *J Formos Med Assoc* 2010; **109**: 484-492 [PMID: 20654787 DOI: 10.1016/S0929-6646(10)60082-4]

2 Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; **160**: 736-755 [PMID: 10430755 DOI: 10.1164/ajrccm.160.2.ats4-99]

3 **Henke CE**, Henke G, Elveback LR, Beard CM, Ballard DJ, Kurland LT. The epidemiology of sarcoidosis in Rochester, Minnesota: a population-based study of incidence and survival. *Am J Epidemiol* 1986; **123**: 840-845 [PMID: 3962966]

4 **Lynch JP**, Kazerooni EA, Gay SE. Pulmonary sarcoidosis. *Clin Chest Med* 1997; **18**: 755-785 [PMID: 9413657 DOI: 10.1016/S0272-5231(05)70417-2]

5 **Baughman RP**, Teirstein AS, Judson MA, Rossman MD, Yeager H, Bresnitz EA, DePalo L, Hunninghake G, Iannuzzi MC, Johns CJ, McLennan G, Moller DR, Newman LS, Rabin DL, Rose C, Rybicki B, Weinberger SE, Terrin ML, Knatterud GL, Cherniak R. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med* 2001; **164**: 1885-1889 [PMID: 11734441 DOI: 10.1164/ajrccm.164.10.2104046]

6 **Kitaichi M**. Pathology of pulmonary sarcoidosis. *Clin Dermatol* 1986; **4**: 108-115 [PMID: 3542165 DOI: 10.1016/0738-081X(86)90039-8]

7 **Beniwal RS**, Cummings OW, Cho WK. Symptomatic gastrointestinal sarcoidosis: case report and review of the literature. *Dig Dis Sci* 2003; **48**: 174-178 [PMID: 12645806 DOI: 10.1023/

A:1021711204498]
8 **Farman J**, Ramirez G, Rybak B, Lebwohl O, Semrad C, Rotterdam H. Gastric sarcoidosis. *Abdom Imaging* 1997; **22**: 248-252 [PMID: 9107643 DOI: 10.1007/s002619900182]
9 **Ushiki A**, Koizumi T, Kubo K, Suzawa K, Arakura N, Suzawa H. Colonic sarcoidosis presenting multiple submucosal tumor-like lesions. *Intern Med* 2009; **48**: 1813-1816 [PMID: 19834273 DOI: 10.2169/internalmedicine.48.2427]
10 **Vahid B**, Spodik M, Braun KN, Ghazi LJ, Esmaili A. Sarcoidosis of gastrointestinal tract: a rare disease. *Dig Dis Sci* 2007; **52**: 3316-3320 [PMID: 17410465 DOI: 10.1007/s10620-006-9448-y]
11 **Stampfl DA**, Grimm IS, Barbot DJ, Rosato FE, Gordon SJ. Sarcoidosis causing duodenal obstruction. Case report and review of gastrointestinal manifestations. *Dig Dis Sci* 1990; **35**: 526-532 [PMID: 2180656 DOI: 10.1007/BF01536930]
12 **Ryan J**, Sleisenger M. Effects of systemic and extraintestinal disease on the gut. In: Sleisenger M, Fordtran J, editors. Gastrointestinal disease. 5th ed. Philadelphia: Saunders, 1993: 223-224
13 **Hilzenrat N**, Spanier A, Lamoureux E, Bloom C, Sherker A. Colonic obstruction secondary to sarcoidosis: nonsurgical diagnosis and management. *Gastroenterology* 1995; **108**: 1556-1559 [PMID: 7729648 DOI: 10.1016/0016-5085(95)90706-8]
14 **Nchimi A**, Francotte N, Rausin L, Khamis J. Case 61: ileocecal sarcoidosis. *Radiology* 2003; **228**: 452-455 [PMID: 12893903 DOI: 10.1148/radiol.2282011952]
15 **Aaronson HG**, Meir JH, Ulin AW. A case of sarcoidosis of the colon. *J Albert Einstein Med Cent* (Phila) 1957; **6**: 14-16 [PMID: 13480769]
16 **Shimoyama Y**, Kusano M, Uchiyama Y, Mori M. Education and Imaging. Gastrointestinal: rectal sarcoidosis due to paralytic ileus resembling adult-onset Hirschsprung disease. *J Gastroenterol Hepatol* 2010; **25**: 1464 [PMID: 20659240 DOI: 10.1111/j.1440-1746.2010.06423.x]
17 **Daldoul S**, Triki W, El Jeri K, Zaouche A. Unusual presentation of a colonic sarcoidosis. *Case Rep Med* 2012; **2012**: 169760 [PMID: 22536260 DOI: 10.1155/2012/169760]
18 **Veitch AM**, Badger I. Sarcoidosis presenting as colonic polyposis: report of a case. *Dis Colon Rectum* 2004; **47**: 937-939 [PMID: 15073665 DOI: 10.1007/s10350-004-0520-4]
19 **Warshauer DM**, Lee JK. Imaging manifestations of abdominal sarcoidosis. *AJR Am J Roentgenol* 2004; **182**: 15-28 [PMID: 14684507 DOI: 10.2214/ajr.182.1.1820015]
20 **Bat T**, Morgan CM, Marx R, Bailey RS. Colon sarcoidosis presenting with abdominal pain. *Endoscopy* 2014; **46** Suppl 1 **UCTN**: E121 [PMID: 24676820 DOI: 10.1055/s-0034-1364890]
21 **Esmadi M**, Ahmad DS, Odum B, Diaz-Arias A, Hammad H. Sarcoidosis: an extremely rare cause of granulomatous enterocolitis. *J Gastrointestin Liver Dis* 2012; **21**: 423-425 [PMID: 23256126]
22 **Maàmouri N**, Guellouz S, Ben Hariz F, Ketari S, Belkahla N, Ouerghi H, Chelly-Enneifer I, Chouaib S, Moncef Zitouna M, Ben Mami N. [Gastrointestinal sarcoidosis]. *Rev Med Interne* 2010; **31**: 262-267 [PMID: 20170990 DOI: 10.1016/j.revmed.2009.12.003]

P- Reviewer: Cologne KG, Freedberg DE, Kvasnovsky CL
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3388

World J Gastroenterol 2015 March 21; 21(11): 3388-3393
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Giant gastrointestinal stromal tumour of rare sarcomatoid epithelioid subtype: Case study and literature review

Gustaw Lech, Wojciech Korcz, Emilia Kowalczyk, Tomasz Guzel, Marcin Radoch, Ireneusz Wojciech Krasnodębski

Gustaw Lech, Tomasz Guzel, Ireneusz Wojciech Krasnodębski, Department of General, Gastroenterological and Oncological Surgery, Medical University of Warsaw, 02091 Warsaw, Poland
Wojciech Korcz, Emilia Kowalczyk, Marcin Radoch, Students' Scientific Group at Department of General, Gastroenterological and Oncological Surgery, Medical University of Warsaw, 02091 Warsaw, Poland
Author contributions: Lech G wrote the manuscript and provided the original pictures; Korcz W wrote the manuscript; Kowalczyk E and Radoch M collected the clinical and radiological data; Guzel T and Krasnodębski IW reviewed the manuscript.

Correspondence to: Gustaw Lech, MD, PhD, Department of General, Gastroenterological and Oncological Surgery, Medical University of Warsaw, Banacha 1a, 02091 Warsaw, Poland. gustaw.lech@wum.edu.pl
Telephone: +48-22-5992482
Fax: +48-22-5992057
Received: July 26, 2014
Peer-review started: July 27, 2014
First decision: September 15, 2014
Revised: October 18, 2014
Accepted: December 16, 2014
Article in press: December 16, 2014
Published online: March 21, 2015

Abstract

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal tumours of the gastrointestinal tract, but they represent less than 3% of all gastrointestinal tract malignancies. This is a detailed case study of a 52-year-old male patient treated for very uncommon histological subtype of gastric GIST with atypical clinical presentation, asymptomatic progress and late diagnosis. The resected tumour, giant in diameters, was confirmed to represent the most rare histopathologic subtype of GISTs - sarcomatoid epithelioid GIST. We report this case and review the literature with a special focus on pathomorphological evaluation, biological aggressiveness and prognostic factors. To our knowledge this is the first report of giant GIST of very uncommon sarcomatoid epithelioid subtype. It is concluded that clinicians should pay attention to the fact that initial diagnosis may be delayed due to mildly asymptomatic and non-specific clinical presentation. Asymptomatic tumours diagnosed at a late stage, which is often the case, can be large on presentation. Prognosis for patients diagnosed with GIST depend on tumour size, mitotic rate, histopathologic subtype and tumour location. That is why early diagnosis and R0 resection, which is usually feasible and safe even in giant gastric sarcomatoid epithelioid subtype of GISTs, are the key factors for further treatment and good prognosis.

Key words: Gastrointestinal stromal tumour; Sarcomatoid epithelioid gastrointestinal stromal tumour; Gastric gastrointestinal stromal tumour; Gastrointestinal tract tumour

Core tip: This is a detailed case study of a 52-year-old male patient treated for very uncommon histological subtype of gastric gastrointestinal stromal tumour (GIST) with atypical clinical presentation, asymptomatic progress and late diagnosis. The resected tumour, giant in diameters, was confirmed to represent the most rare histopathologic subtype of GISTs - sarcomatoid epithelioid GIST. We report this case and review the literature with a special focus on pathomorphological evaluation, biological aggressiveness and prognostic factors. To our knowledge this is the first report of giant GIST of very uncommon sarcomatoid epithelioid subtype.

Lech G, Korcz W, Kowalczyk E, Guzel T, Radoch M, Krasnodębski IW. Giant gastrointestinal stromal tumour of rare sarcomatoid epithelioid subtype: Case study and literature review. *World J Gastroenterol* 2015; 21(11): 3388-3393 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3388.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3388

INTRODUCTION

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal tumours of the gastrointestinal (GI) tract, representing only less than 3% of all primary gastrointestinal malignancies[1-3]. Interstitial cells of Cajal and stem cells of the smooth muscle tissue are known to be the precursors of GISTs[4]. Tumour size, mitotic rate, localization, tumour vascularisation and infiltration are the key prognostic factors in predicting malignancy potential. The available epidemiological studies provided diverse results, with the incidence rates of around 10-20 new cases per million population per year[2,3]. Incidence determined from data collected in 14 countries which joined the EORTC study totals 4-5 new cases per 1 million population per year[4,5]. GIST is predominantly diagnosed at an advanced stage, most patients (around 75%) are over 50 years old at diagnosis[4,5], and the peak onset is among 55-65 years old[2,4,6]. Age median for gastric GIST is 63 years, with a slight male predominance (55%)[1,7]. Stomach is the most common location for GIST, which accounts for 1%-2% of all gastric cancers[8]. Various sources report that as many as 70% of all GISTs are located in the stomach - most commonly in the gastric body (42.3%) and in the prepyloric area (28.5%)[1,2,4]. Prognosis for gastric GIST is typically better than for GISTs developed in other parts of the GI tract. Stromal tumours can be also found in the small intestine (25%-30%), in the colon and rectum (3%-10%)[2,4,5]. Less than 10% of all GISTs are estimated to be retroperitoneal GISTs or, in late-stage metastatic tumours, the location of the primary tumour can be no longer determined[5].

The molecular pathogenesis of stromal tumours involves a mutation which activates tyrosine kinase (KIT) and PDGFRA membrane receptors[1,3,4]. These receptors regulate key cell functions: proliferation, differentiation and anti-apoptotic signalling[3]. In GIST, ligand-independent activation of these receptors is observed, leading to uncontrolled cell proliferation and stimulation of downstream signalling pathways[3]. The metastatic pattern of GISTs is predominantly intra-abdominal, mainly to the liver[4,5]. Lymph nodal invasion is uncommon, therefore lymphadenectomy is not required with GIST resection[1,4].

In this paper, we study the case of a male patient treated for very uncommon histological subtype of gastric GIST with atypical clinical presentation, with a focus on pathomorphological evaluation, biological aggressiveness and prognostic factors. Authors checked the PubMed and MEDLINE databases for last ten years searching for epihelioid sarcomatoid gastric GIST and to our knowledge this is the first report of giant GIST of very uncommon sarcomatoid epithelioid subtype.

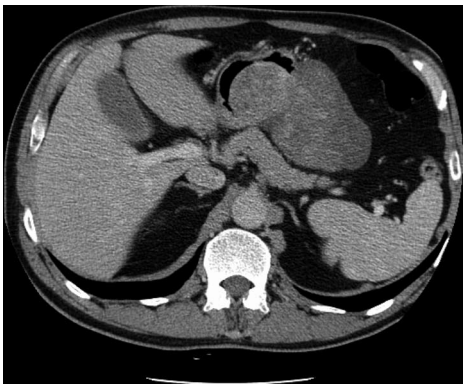


Figure 1 Computed tomography scan revealed a tumour-like mass projecting to the lumen of the stomach, adjacent to the greater curvature area.

CASE REPORT

A 52 years old male patient was transferred to the general and oncological surgery department of the Medical University Teaching Hospital from a district hospital with suspicion of gastric GIST, for continuation of therapy. The patient was previously admitted for the first ever episode of fainting and loss of consciousness. A single tarry stool was reported one day before hospitalisation, and a history of bloody stools for 2 wk before presentation. During this period, the patient did not complain of nausea, vomiting or defecation disorders. Haemoglobin 8.8 g/dL, red blood cell (RBC) 2.8 million/dL, Hct 26% at admittance. No morphological disorders or sources of bleeding into the GI tract were revealed in gastroscopy and colonoscopy. Computed tomography (CT) scan showed a tumour-like mass projecting to the lumen of the stomach, adjacent to the greater curvature area, penetrating downwards and to the left (Figure 1). No interconnection between the intestinal loops and the tumour was demonstrated. Another gastroscopy was performed based on CT results, which revealed an external displacement of otherwise unchanged gastric mucosa over the area of 10 cm × 15 cm, within the angular incisure area. A biopsy sample was collected for histopathological analysis, which revealed signs of chronic inflammation, however, no GIST-type pattern was confirmed. The patient was administered 4 packed red blood cells units and 2 fresh frozen plasma (FFP) units, which improved his CBC parameters.

On the day of admittance to the university department of surgery, the patient did not experience any pain, but reported recurring heartburn and general

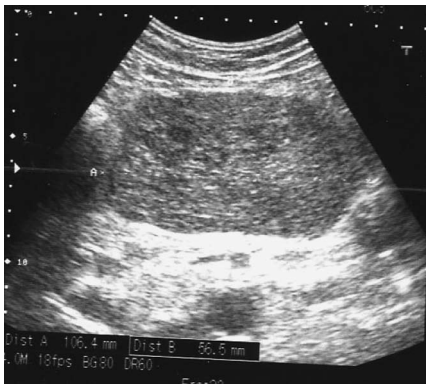


Figure 2 Abdominal ultrasound disclosed hypoechoic, heterogeneous mass filling the epigastrium.

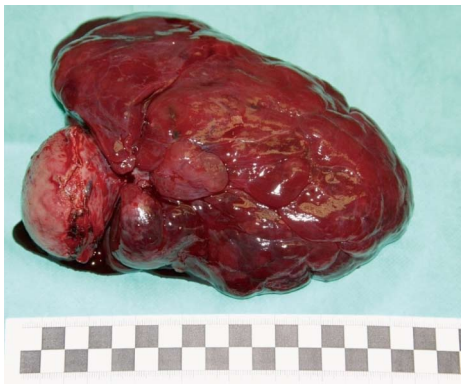


Figure 3 Removed gastric gastrointestinal stromal tumour. The capsule was not damaged.

malaise accompanied by weight loss by around 5 kg within the preceding 2 wk. He did not complain of any other gastrointestinal discomforts. The patient had a history of appendectomy and was also treated for arterial hypertension. In a physical examination, the abdomen was soft and slightly tender in the epigastric region, where a pathological mass was detected, without any signs of peritoneal irritation. During hospitalisation patient underwent an abdominal ultrasound which revealed hypoechoic, heterogeneous tumour of 106 mm × 56 mm × 144 mm in size in the epigastric region, attached to the posterior gastric wall (Figure 2). The tumour itself demonstrated small hypoechoic and anechoic spaces of up to 21 mm. The patient was qualified for surgical treatment with suspicion of gastric GIST. A large cherry-coloured soft-structured mass of 15-18 cm diameter was revealed during surgery within the peritoneal cavity, and more specifically in lesser sac of the peritoneal cavity, attached to the posterior gastric wall of the gastric body. The patient had a wedge resection encompassing the entire tumour mass. The capsule of the tumour was intact (Figure 3). No perioperative complications were observed. The patient tolerated oral dietary intake. He was discharged home on day 7 post surgery in a good general condition. No health concerns were reported during 4 wk follow-up.

In the final histopathologic analysis of a tumour sample, including a 5 cm × 4.5 cm section of gastric wall, the macroscopic appearance of the gastric mucosa was normal. In cross-section, the tumour was shown to penetrate under the gastric mucosa, without infiltrations, and projected beyond the external gastric wall, up to the size of 120 mm × 80 mm × 160 mm. The tumour was yellowish in cross-section, soft, and nested, with areas of haemorrhage. The surgical margin was preserved. Sarcomatoid epithelioid GIST was confirmed in microscopic examination. Immunohistochemical staining revealed the following: Ki67 < 5 mitoses per 50 HPFs, KIT (+/-), CKAE1 (+) AE3 (-), vimentin (+), CD34 (-), S100 (-), CD30 (-), caldesmon (-), actin (-), MCT (-), fat s(-)(Figure 4).

DISCUSSION

Gastrointestinal stromal tumours, although rare, increasingly receive the attention of clinicians. GISTs can develop along the GI tract, which may to some extent explain the broad spectrum of clinical symptoms, which include abdominal pain, gastrointestinal bleeding, anaemia, palpable abdominal mass, dyspepsia, nausea, vomiting and obstruction, constipations, diarrhoeas, swallowing difficulties, and episodes of weight loss[2,3,8]. However, some authors claim that "vague abdominal discomfort" is the most common symptom of stromal tumours, which can affect as many as 70% of all patients[2]. GIST can lead to peritonitis caused by perforation in the abdominal wall, typically accompanied by bleeding into the GI tract lumen[1-4]. Asymptomatic tumours most commonly develop in the stomach and duodenum.

GISTs, especially asymptomatic ones, pose a significant diagnostics challenge and are currently detected mainly with diagnostic imaging techniques and endoscopy. Abdominal contrast-enhanced CT or magnetic resonance (MR) are the recommended diagnostic imaging techniques in determining tumour stage and therapy planning[2,4,5]. With abdominal CT and MR, stromal tumours can be detected in 72% and 91% of patients, respectively[2]. CT images can be useful in evaluating the malignancy potential. Irregular shape, over 10 cm in size, calcification areas, cystic degeneration within the mass, and central necrosis are the main criteria for malignant behaviour[2]. However, some authors question the prognostic value of calcification and colliquative necrosis[1]. On CT images, GISTs are solid well-circumscribed tumours, and patchy enhancement by contrast medium[3,4]. Endoscopic ultrasound (EUS) is another valuable diagnostics tool in which hypoechoic lesions can be precisely marked out across individual layers of the gastrointestinal wall. EUS can be also useful in assessing the depth of invasion. The size of over 4 cm, irregular surface and heterogeneous echogenicity may point to more malignant tumours[3,4].

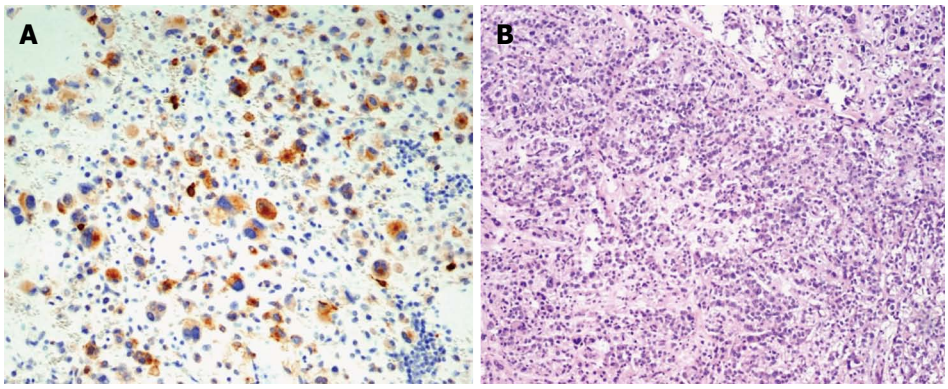


Figure 4 Pathology images. A: Weak immunoreactivity to KIT (CD 117; original magnification × 200); B: Gastric gastrointestinal stromal tumour (HE staining; original magnification × 100).

Endoscopy combined with biopsy and contrast-enhanced radiography is of limited diagnostic value, as it detects only around 33% of cases based on initial diagnosis[2]. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) can be considered the gold diagnostic standard (84% specificity) as it directly visualizes the tumour and provides sufficient cytological material for molecular diagnostics[3].

Gastric GIST comes in three different histological subtypes: spindle cell type (50%), epithelioid type (20%-40%) and mixed type (10%)[1,9,10]. Epithelioid GISTs are also reported to be significantly more common in the stomach[11]. There are now four different subtypes of epithelioid gastric stromal tumours distinguished: sclerosing, hypercellular, heterogeneous and sarcomatoid[1]. The last subtype is the least common subtype of epithelioid GISTs - it is present in only 2.5% of patients with gastric stromal tumours[1]. The sarcomatoid subtype can be found in both, epithelioid and spindle cell tumours[1]. The sarcomatoid pattern typically translates into statistically worse prognosis, especially in GISTs of the spindle cell type[1]. According to some sources, epithelioid stromal tumours involve larger tumour mass and shorter survival period as compared to other histological types (29.2 mo *vs* 96.1 mo for spindle cell tumours, and 61 mo for mixed type tumours)[12]. Pathomorphological evaluation is made up of traditional histopathology accompanied by tests for a number of tumour markers, which are of key importance in diagnosing stromal tumours. Genes responsible for increasing the density of transmembrane tyrosine kinase receptor KIT are universally overexpressed in GIST. KIT receptor can be detected by immunohistochemistry (CD117). CD117 overexpression is the most sensitive and highly specific marker of stromal tumours detected in nearly 90%-95% of cases[1,3,4,9,10]. CD117-negative gastric GISTs predominantly represent the epithelioid subtype, and only 18% of spindle cell tumours show no signs of CD117 expression[1]. Other markers such as nestin (90%) and CD34 (70%) are also representative but less specific[9]. CD34 is almost universally positive in GISTs located in oesophagus or rectum (95%-100%)[9,10]. CD34 is expressed in 80%-88%[1,10,11] of gastric GISTs, whereas α-smooth muscle actin (α-SMA) is present in around 20%-40% of patients in whom CD34 expression is absent[4,7]. The majority of CD34-negative gastric stromal tumours are conclusively identified as epithelioid-type tumours and have been shown to be more aggressive[1,11]. α-SMA (+) is present in only around 30% of patients, but is significantly more common in GISTs located in the stomach or small intestine[9]. In α-SMA-positive patients, the progression-free survival is significantly longer as compared to α-SMA-negative patients (37.7 mo *vs* 15.9 mo), but this correlation was demonstrated on a small population of patients and needs to be confirmed in other studies[12]. S-100 should be also mentioned in the context of pathomorphological evaluation, as it is present in only around 5% of patients and is especially common in epithelioid-type tumours, the majority of which are more malignant[1,11]. A positive relationship with the desmin antibody is rare and can be found in only 1%-3% of gastric stromal tumours, but is more common in epithelioid-type GISTs[9-11].

Biological aggressiveness of primary, resectable GISTs can be determined mainly by relying on tumour size, mitotic rate, and tumour location[4]. The National Institutes of Health (NIH) system for determining GIST malignancy potential is commonly used in daily practice and has been referred to in numerous sources. It involves two major criteria: tumour size and mitotic count[7,8]. The relationship between size and mitotic count *vs* malignancy potential was analysed by Miettinen *et al*[1]. It was based on over 1000 cases of stromal tumours. Based on this analysis, it was determined that the mortality attributed to tumours with less than 5 mitoses per 50 HPFs equals 0% for tumour with the size of less than 2 cm, 2% for tumours of 2-10 cm in size, and 11% for tumours with the size of over 10 cm. The mortality rate significantly increases in tumours showing more than 5 mitotic figures per 50 HPFs: 16% for tumours of 2-5 cm in diameter, 49% for tumours of 5-10 cm in size, and 86% for "large" tumours with the

size of over 10 cm[1]. Mortality associated with primary tumour or metastasis in patients with gastric GIST > 5 cm in size and more than 5 mitotic figures per 50 HPFs amounts to 49%-86%. Epithelioid-type tumours of more than 6 cm in size and other tumour types of more than 7 cm in size can be indicative of higher malignancy and metastatic potential. More favourable prognosis for metastatic tumours can be only expected in patients with large tumours (> 10 cm) and low mitotic count (less than 5 mitotic figures/50 HPFs) - metastases are present in only 12% of cases, and the progression free survival is around 5-15 years[1]. Mean 5-year survival in patients who underwent radical surgical procedure, irrespective of the key major pathomorphological factors, is estimated at 28%-65%, or even 70%[4,7]. The largest gastric GIST reported so far was 37x24x13 cm in size and weighted 8.5 kg[13]. Size median for gastric GISTs is 6 cm. Tumours of 2-5 cm (38.2%) and 5-10 cm (29.7%) in size prevail[1,7]. Large tumours (> 10 cm) on presentation are diagnosed in only 20% of patients[1,7]. Asymptomatic disease progression is the reason of late-stage diagnosis, with metastases present in around 21% of patients on presentation[4,8]. The most malignant gastric stromal tumours are located at the fundus, entrance, and at the gastro-oesophageal junction[1]. Mortality is closely correlated with the mitotic rate and tumour size[1]. Median survival following resection is 12.4 years for all GISTs, and 14.1 years for gastric GISTs, with no signs of recurrence[1,7]. The majority of GIST recur within the first 5 years post resection[7]. Local recurrences limited to gastric walls are rare. Local recurrences result from incomplete resection of the primary tumour or new GISTs[1].

The studied case can be classified as stage Ⅱ tumour according to the TNM system[14]. It is a high-risk (malignant) tumour according to the NIH system[8]. According to Miettinem, the patient should be classified to low or medium risk of death, which translates into 12%-15% risk of cancer-related death[1].

Surgery is the first choice treatment of primary gastrointestinal stromal tumours of more than 2 cm in diameter[4,6]. It is desirable to obtain 1 cm macroscopic margins of healthy tissues to secure negative microscopic margins (R0 resection) with the tumour capsule left intact[3,6]. It is imperative to avoid rupture of the tumour capsule as it can lead to tumour dissemination. Both sporadic and surgery-related ruptures are reported in only around 6% of patients[7]. According to the National Comprehensive Cancer Network guidelines, extended anatomic resection of the stomach is indicated only rarely[6]. Wedge resection and partial resection of the stomach are sufficient in 40% and 34% of cases, respectively[1]. Complete gastrectomy is necessary in only 3% of patients[1]. Infiltration into the peritoneal cavity and adjacent organs is observed in only 5.4% of patients. Laparoscopic surgery can be performed on GISTs of less than 5cm in size[6]. As compared to traditional surgery, laparoscopic approach in gastric GISTs involves reduced blood loss, fewer perioperative complications, shorter hospitalisation, and earlier resumption of oral dietary intake. However, there were no statistically significant differences between the outcomes of different surgical techniques and the number of recurrences, surgical margins, or the overall survival[15].

The key issue in effective post-surgery therapy is to inform the patient of the possibility of recurrence following many years of disease-free period. Therefore, patients should be subject to regular follow-up surveillance. Abdominopelvic contrast-enhanced CT is the mainstay for this type of follow-up. In medium and high risk patients according to NIH, follow-up surveillance should be performed at 3-4 mo intervals during the first 2 years, every 6 mo for 3 more years, and then every 12 mo (5 or more years post-resection). Patients with GISTs of very low and low aggressiveness can undergo the follow-up examinations every 12 mo.

Although surgical tumour resection is the treatment of choice in GIST, the supportive role of pharmacotherapy with tyrosine kinase inhibitors is increasingly highlighted. Adjuvant therapy remains controversial, despite the approval of imatinib in post-surgery treatment of patients with high-risk of recurrence according to NCCN-AFIP-AJCC classification. This type of treatment is most beneficial in patients classified to the highest risk of recurrence. Neoadjuvant imatinib should be also attempted in patients with borderline resectable GIST. In patients at advanced stage of GIST, inclusion criteria for imatinib are: unresectable tumour, distant metastases (liver, dissemination) and local recurrence. Second generation tyrosine kinase inhibitor - sunitinib can be also considered if there is a further disease progression. Cancer treatment is now becoming more and more personalised. However, despite the huge progress in this area, and the introduction of novel anti-cancer drugs, cancer treatment still remains a huge challenge.

COMMENTS

Case characteristics

A 52 years old male patient admitted for the first ever episode of fainting and loss of consciousness with a history of bloody stools for 2 wk before presentation.

Clinical diagnosis

In a physical examination, the abdomen was soft and slightly tender in the epigastric region, where a pathological mass was detected, without any signs of peritoneal irritation.

Differential diagnosis

Malignant tumors, benign neoplasms.

Laboratory diagnosis

Haemoglobin 8.8 g/dL, RBC 2.8 million/dL, Hct 26%, metabolic panel were within normal limits at admittance.

Imaging diagnosis

Computed tomography scan showed a tumour-like mass projecting to the lumen of the stomach, adjacent to the greater curvature area, whereas gastroscopy revealed an external displacement of otherwise unchanged gastric mucosa.

Pathological diagnosis

In the final histopathologic analysis sarcomatoid epithelioid gastrointestinal stromal tumours (GIST) was confirmed and immunohistochemical staining revealed the following: Ki67 < 5 mitoses per 50 HPFs, tyrosine kinase (KIT) (+/-),

CKAE1 (+) AE3 (-), vimentin (+), CD34 (-), S100 (-), CD30 (-), caldesmon (-), actin (-), MCT (-), fats (-).

Treatment

The patient had a wedge resection encompassing the entire gastric tumour mass.

Related reports

To authors' knowledge this is the first report of giant GIST of very uncommon sarcomatoid epithelioid subtype.

Term explanation

KIT is a receptor tyrosine kinase protein found on the surface of gastrointestinal stromal tumors cells.

Experiences and lessons

This case report not only focuses on uncommon symptoms, pathomorphological evaluation, biological aggressiveness of giant gastric sarcomatoid epithelioid GISTs, but also discuss the key factors for further treatment and good prognosis.

Peer-review

This paper includes the overviews on GISTs with clinically relevant info and concise case of uncommon sarcomatoid epithelioid subtype.

REFERENCES

1 **Miettinen M**, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. *Am J Surg Pathol* 2005; **29**: 52-68 [PMID: 15613856 DOI: 10.1097/01.pas.0000146010.92933.de]

2 **Scarpa M**, Bertin M, Ruffolo C, Polese L, D'Amico DF, Angriman I. A systematic review on the clinical diagnosis of gastrointestinal stromal tumors. *J Surg Oncol* 2008; **98**: 384-392 [PMID: 18668671 DOI: 10.1002/jso.21120]

3 **Rammohan A**, Sathyanesan J, Rajendran K, Pitchaimuthu A, Perumal SK, Srinivasan U, Ramasamy R, Palaniappan R, Govindan M. A gist of gastrointestinal stromal tumors: A review. *World J Gastrointest Oncol* 2013; **5**: 102-112 [PMID: 23847717 DOI: 10.4251/wjgo.v5.i6.102]

4 **Cichoz-Lach H**, Kasztelan-Szczerbińska B, Słomka M. Gastrointestinal stromal tumors: epidemiology, clinical picture, diagnosis, prognosis and treatment. *Pol Arch Med Wewn* 2008; **118**: 216-221 [PMID: 18575421]

5 **Rutkowski P**, Wozniak A, Dębiec-Rychter M, Kąkol M, Dziewirski W, Zdzienicki M, Ptaszynski K, Jurkowska M, Limon J, Siedlecki JA. Clinical utility of the new American Joint Committee on Cancer staging system for gastrointestinal stromal tumors: current overall survival after primary tumor resection. *Cancer* 2011; **117**: 4916-4924 [PMID: 21456019 DOI: 10.1002/cncr.26079]

6 **Kong SH**, Yang HK. Surgical treatment of gastric gastrointestinal stromal tumor. *J Gastric Cancer* 2013; **13**: 3-18 [PMID: 23610714 DOI: 10.5230/jgc.2013.13.1.3]

7 **Joensuu H**, Vehtari A, Riihimäki J, Nishida T, Steigen SE, Brabec P, Plank L, Nilsson B, Cirilli C, Braconi C, Bordoni A, Magnusson MK, Linke Z, Sufliarsky J, Federico M, Jonasson JG, Dei Tos AP, Rutkowski P. Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts. *Lancet Oncol* 2012; **13**: 265-274 [PMID: 22153892 DOI: 10.1016/S1470-2045(11)70299-6]

8 **Joensuu H**. Risk stratification of patients diagnosed with gastrointestinal stromal tumor. *Hum Pathol* 2008; **39**: 1411-1419 [PMID: 18774375 DOI: 10.1016/j.humpath.2008.06.025]

9 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors (GISTs): definition, occurrence, pathology, differential diagnosis and molecular genetics. *Pol J Pathol* 2003; **54**: 3-24 [PMID: 12817876]

10 **Patil DT**, Rubin BP. Gastrointestinal stromal tumor: advances in diagnosis and management. *Arch Pathol Lab Med* 2011; **135**: 1298-1310 [PMID: 21970485 DOI: 10.5858/arpa.2011-0022-RA]

11 **Bülbül Doğusoy G**. Gastrointestinal stromal tumors: A multicenter study of 1160 Turkish cases. *Turk J Gastroenterol* 2012; **23**: 203-211 [PMID: 22798108]

12 **Demir L**, Ekinci N, Erten C, Kucukzeybek Y, Alacacioglu A, Somali I, Can A, Dirican A, Bayoglu V, Akyol M, Cakalagaoglu F, Tarhan MO. Does immunohistochemistry provide additional prognostic data in gastrointestinal stromal tumors? *Asian Pac J Cancer Prev* 2013; **14**: 4751-4758 [PMID: 24083738 DOI: 10.7314/APJCP.2013.14.8.4751]

13 **Cappellani A**, Piccolo G, Cardì F, Cavallaro A, Lo Menzo E, Cavallaro V, Zanghì A, Di Vita M, Berretta M. Giant gastrointestinal stromal tumor (GIST) of the stomach cause of high bowel obstruction: surgical management. *World J Surg Oncol* 2013; **11**: 172 [PMID: 23914945 DOI: 10.1186/1477-7819-11-172]

14 **Edge SB**, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, (Eds.). AJCC Cancer Staging Handbook. 7th ed. New York: Springer, 2010

15 **Koh YX**, Chok AY, Zheng HL, Tan CS, Chow PK, Wong WK, Goh BK. A systematic review and meta-analysis comparing laparoscopic versus open gastric resections for gastrointestinal stromal tumors of the stomach. *Ann Surg Oncol* 2013; **20**: 3549-3560 [PMID: 23793362 DOI: 10.1245/s10434-013-3051-1]

P- Reviewer: Mortensen C, Wong GLH
S- Editor: Yu J **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3394

World J Gastroenterol 2015 March 21; 21(11): 3394-3401
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Bronchial bleeding caused by recurrent pneumonia after radical esophagectomy for esophageal cancer

Toshihiro Kitajima, Kota Momose, Seigi Lee, Shusuke Haruta, Masaki Ueno, Hisashi Shinohara, Sakashi Fujimori, Takeshi Fujii, Ryoji Takei, Tadasu Kohno, Harushi Udagawa

Toshihiro Kitajima, Kota Momose, Seigi Lee, Shusuke Haruta, Masaki Ueno, Hisashi Shinohara, Harushi Udagawa, Department of Gastroenterological Surgery, Toranomon Hospital, Tokyo 105-8470, Japan
Sakashi Fujimori, Tadasu Kohno, Department of Thoracic Surgery, Toranomon Hospital, Tokyo 105-8470, Japan
Takeshi Fujii, Department of Pathology, Toranomon Hospital, Tokyo 105-8470, Japan
Ryoji Takei, Department of Radiology, Toranomon Hospital, Tokyo 105-8470, Japan
Author contributions: Kitajima T and Udagawa H designed the report; Momose K, Lee S, Haruta S, Shinohara H, Ueno M, Fujimori S and Kohno T were attending doctors for the patient and performed the operation; Takei R performed bronchial arterial embolization procedure; Fujii T performed the pathological examination; Kitajima T wrote this paper.

Correspondence to: Toshihiro Kitajima, MD, Department of Gastroenterological Surgery, Toranomon Hospital, 2-2-2 Toranomon, Minatoku, Tokyo 105-8470, Japan. t.kitajima0407@gmail.com
Telephone: +81-3-35881111
Fax: +81-3-35827068
Received: August 5, 2014
Peer-review started: August 6, 2014
First decision: August 27, 2014
Revised: September 8, 2014
Accepted: October 15, 2014
Article in press: October 15, 2014
Published online: March 21, 2015

Abstract

We herein report a case of bronchial bleeding after radical esophagectomy that was treated with lobectomy. A 65-year-old male who underwent subtotal esophagectomy with three-field lymph node dissection for esophageal carcinoma was referred to our hospital because of sudden hemoptysis. After the esophagectomy, bilateral vocal cord paralysis was observed, and the patient suffered from repeated episodes of aspiration pneumonia. Bronchoscopy revealed hemosputum in the right middle lobe bronchus, and contrast-enhanced computed tomography showed tortuous arteries arising from the right inferior phrenic artery and left subclavian artery toward the right middle lobe bronchus. Although bronchial arterial embolization was performed twice to control the recurrent hemoptysis, the procedures were unsuccessful. Right middle lobectomy was therefore performed *via* video-assisted thoracic surgery. Engorged bronchial arterys with medial hypertrophy and overgrowth of the small branches were noted near the bronchus in the resected specimen. The patient recovered uneventfully and was discharged on postoperative day 14.

Key words: Hemoptysis; Esophagectomy; Recurrent laryngeal nerve injury; Ectopic/collateral bronchial artery; Lobectomy; Aspiration pneumonia; Bronchial arterial embolization

Core tip: To the best of our knowledge, this is the first report of bronchial bleeding suspected to arise from ectopic/collateral bronchial arteries after radical esophagectomy with three-field lymphadenectomy for esophageal carcinoma. Such clinical course after esophagectomy for esophageal carcinoma is quite meaningful, especially in cases of postoperative recurrent aspiration pneumonia due to bilateral recurrent laryngeal nerve injury.

Kitajima T, Momose K, Lee S, Haruta S, Ueno M, Shinohara H, Fujimori S, Fujii T, Takei R, Kohno T, Udagawa H. Bronchial bleeding caused by recurrent pneumonia after radical esophagectomy for esophageal cancer. *World J Gastroenterol* 2015; 21(11): 3394-3401 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3394.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3394

INTRODUCTION

Hemoptysis is an alarming symptom and a potentially life-threatening complication that can occur in a variety of infectious, inflammatory, and cancerous diseases of the chest. In general, there are several causes of hemoptysis, including bronchogenic carcinoma, chronic inflammatory lung disease due to bronchiectasis and various infections, although the majority of cases are due to tuberculosis[1,2].

According to previous reports, surgeons should consider the potential for locoregional recurrence and aorto-bronchial fistula[3,4] or gastro-tracheobronchial fistula formation[5,6] in cases of hemoptysis after esophagectomy for esophageal carcinoma. To the best of our knowledge, this is the first report of bronchial bleeding suspected to have arisen from ectopic/collateral bronchial arteries after radical esophagectomy with three-field lymphadenectomy for esophageal carcinoma. This clinical course after esophagectomy for esophageal carcinoma is quite meaningful, especially in cases of postoperative recurrent aspiration pneumonia due to bilateral recurrent laryngeal nerve injury.

CASE REPORT

A 65-year-old Japanese male who underwent esophagectomy was admitted to our hospital with sudden hemoptysis. He had smoked 20 cigarettes a day since his twenties and consumed alcohol only on social occasions without regular habitual drinking. On preoperative examination, upper gastrointestinal endoscopy demonstrated a pedunculated lesion located on the posterior wall of the upper thoracic esophagus 21-23 cm distal from the incisors. A biopsy from the pedunculated lesion revealed a well-differentiated tubular adenocarcinoma. The patient had undergone radical esophagectomy with three-field lymphadenectomy 19 mo before the current admission. Surgical reconstruction was performed *via* the posterior mediastinal route using a gastric conduit, followed by esophagogastrostomy and tracheostomy placement, as bilateral recurrent laryngeal nerve injury was noted. The right bronchial artery (BA), which is usually identified and preserved, was suspected to be absent because it was not identified despite a meticulous investigation. Histopathologically, the pedunculated tumor consisted of mixed adenoendocrine carcinoma. None of the 79 widely dissected lymph nodes exhibited metastasis, and no lymphatic invasion was detected. The patient was diagnosed with stage Ⅰ A (pT1bN0M0) disease according to the 7th edition of the UICC/AJCC TNM classification. Postoperatively, bilateral vocal cord paralysis was observed, and the patient suffered from repeated episodes of aspiration pneumonia for six months after the surgery (Figure 1A, B). Because the main cause of the patient's repeated aspiration was postprandial reflux of gastric contents, resection of the pyloric ring and diversion of the gastric conduit in a Roux-en Y fashion was performed to prevent further aspiration of the regurgitant. Thus, it took approximately eight months for the patient to safely resume oral intake. He was discharged from our hospital nine months after surgery. Although the bilateral vocal cord paralysis did not improve, he was able to maintain proper dietary habits through dysphagia rehabilitation and adopting a soft diet. Although no further episodes of aspiration pneumonia or evidence of tumor recurrence were noted during follow-up, consolidation in the right middle lobe, which was observed at discharge, persisted by computed tomography (CT) that was performed immediately before this episode (Figure 1C). A total of 19 mo after esophagectomy, the patient began to cough up small amounts of blood and was admitted to a regional hospital. Upper gastrointestinal endoscopy did not reveal any bleeding from the gastric conduit. Meanwhile, bronchoscopy revealed hemosputum in the entrance of the right middle lobe bronchus; bleeding from the peripheral bronchus was suspected to be the primary cause of this complication. Although the patient exhibited a stable clinical course under conservative therapy, sudden hemoptysis was noted one week after onset, and he was transferred to our hospital. Laboratory tests performed at the time of admission indicated anemia and inflammation. The hemoglobin level was 8.8 g/dL, and the C-reactive protein level was elevated at 16.1 mg/dL. However, the levels of serum Aspergillus antigens, β-D glucan, and tumor markers, such as carcinoembryonic antigen, squamous cell carcinoma antigen, and carbohydrate antigen 19-9 were all within normal limits. The patient demonstrated a negative blood culture, although *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were detected in a sputum culture. Additionally, he was negative for tuberculosis. While bronchoscopy revealed no active hemorrhaging from the respiratory tract, a small level of hemosputum was observed in the entrance of the right middle lobe bronchus (Figure 2A). CT and chest radiography revealed a massive infiltrative shadow in the right middle lobe with atelectasis in the right lower lobe (Figure 2B, C). In addition, bronchoscopy and CT revealed no protruding lesions, suggesting bronchial invasion of the tumor and/or gastro-tracheobronchial/aorto-bronchial fistula formation in the bronchus. With respect to the vessel anatomy, contrast-enhanced

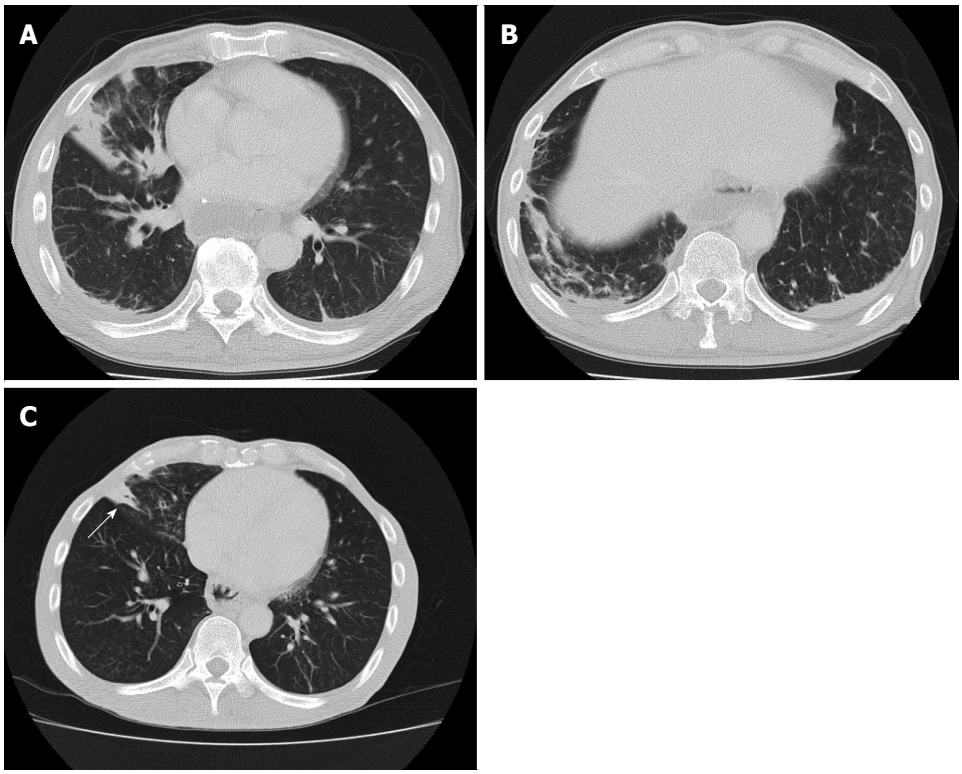


Figure 1 Unenhanced computed tomography after esophagectomy. A: Computed tomography (CT) revealed consolidation in the right middle lobe; B: CT simultaneously demonstrated consolidation in the right lower lobe (A and B); C: Although there was no evidence of newly developed pneumonia, consolidation remained in the right middle lobe 14 mo after esophagectomy (arrow).

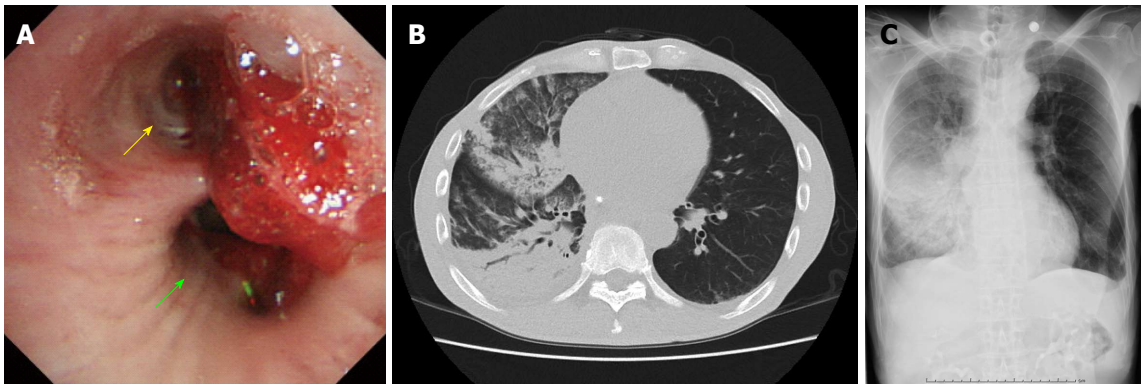


Figure 2 Imaging findings before bronchial arterial embolization. A: Bronchoscopy revealed hemosputum in the entrance of the right middle lobe bronchus (yellow arrow) extending down to the lower lobe bronchus (green arrow); B, C: Computed tomography and chest radiography revealed a massive infiltrative shadow in the right middle lobe with atelectasis in the right lower lobe.

CT showed a tortuous artery ascending from the right inferior phrenic artery directly arising from the aorta near the celiac trunk toward the right middle bronchus (Figure 3A); in addition, we observed several tortuous arteries with hypervascularity arising from the left subclavian artery, which descended obliquely along the right main bronchus (Figure 3B). Although we considered these engorged and tortuous vessels to be ectopic/collateral BAs that caused the patient's hemoptysis, identifying the responsible vessel was difficult because of his hemostasis. Considering the difficulty in performing embolization of the ectopic BAs arising from the left subclavian artery, we first attempted to embolize the right collateral BA arising from the inferior phrenic artery. On the next day from admission, a bronchial arterial embolization (BAE) procedure was performed. No aorto-bronchial fistulas were detected on aortography. The right collateral BA was successfully embolized with gelatin sponge particles (Figure 3C). However, the day after the BAE procedure, the level of recurrent hemoptysis was estimated to be approximately 200-300 mL, and bronchoscopy revealed bleeding from the same site as that observed at disease onset. On the third day after admission, a second BAE procedure was emergently performed. Although selective embolization of the

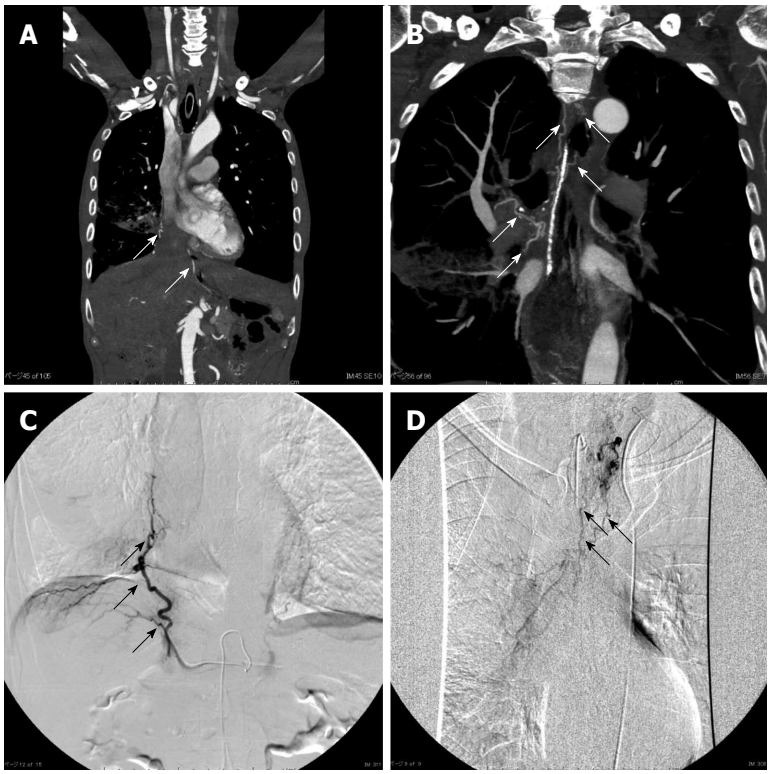


Figure 3 Computed tomography arteriography. A: Computed tomography (CT) arteriography revealed a tortuous artery ascending from the right inferior phrenic artery that arose directly from the aorta (arrows); B: CT arteriography demonstrated several tortuous arteries arising from the left subclavian artery that descended obliquely along the right main bronchus (arrows); C: A collateral right bronchial artery (BA) arising from the inferior phrenic artery. Right inferior phrenic arteriography showed an abnormal tortuous artery (arrows); D: Ectopic BAs arising from the left subclavian artery. Left subclavian arteriography showed abnormal tortuous arteries arising from a root of the left subclavian artery (arrows).

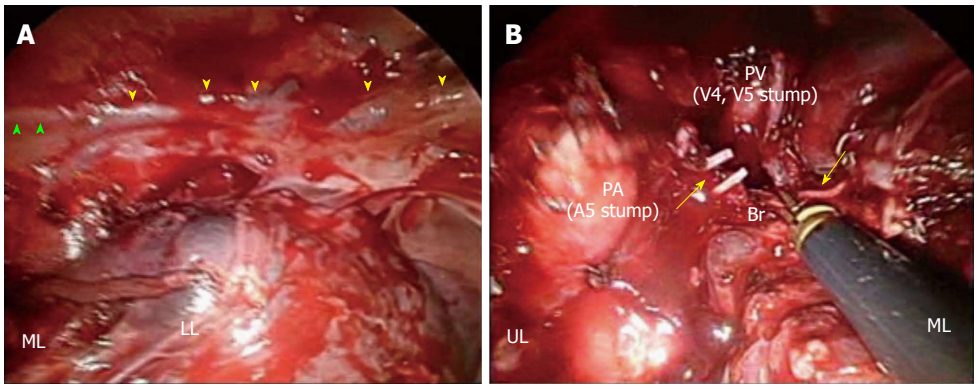


Figure 4 Intraoperative findings. A: The presence of a collateral bronchial artery (BA) arising from the right inferior phrenic artery running along the phrenic nerve (green arrowheads) was confirmed; the tissue was black in color due to the first bronchial arterial embolization procedure (yellow arrowheads). B: Engorged right BAs streaming into the right middle lobe were dissected at the level of bifurcation of the middle lobe bronchus (arrows). UL: Upper lobe; ML: Middle lobe; LL: Lower lobe; Br: Middle lobe bronchus; PA: Pulmonary artery; PV: Pulmonary vein.

ectopic BAs arising from the left subclavian artery was also performed (Figure 3D), CT conducted after the second BAE procedure revealed that the arterial blood supply to the right middle lobe bronchus was decreased but not completely blocked. Because we posited that performing additional embolization of the ectopic vessels would be difficult due to the tortuosity and fragility of the vessels, we planned to perform surgical resection after the patient's hemodynamic parameters stabilized. On the ninth day after admission, right middle lobectomy was performed *via* video-assisted thoracic surgery (VATS). With respect to the intraoperative findings, the presence of a BA arising from the right inferior phrenic artery was confirmed; the vessel was black in color as a result of the first BAE procedure (Figure 4A). Although a few engorged right BAs streaming into the right middle lobe were dissected at the level of bifurcation of the middle lobe bronchus (Figure 4B), it was difficult to detect the root of the dissected arteries. In addition, whitish pleural

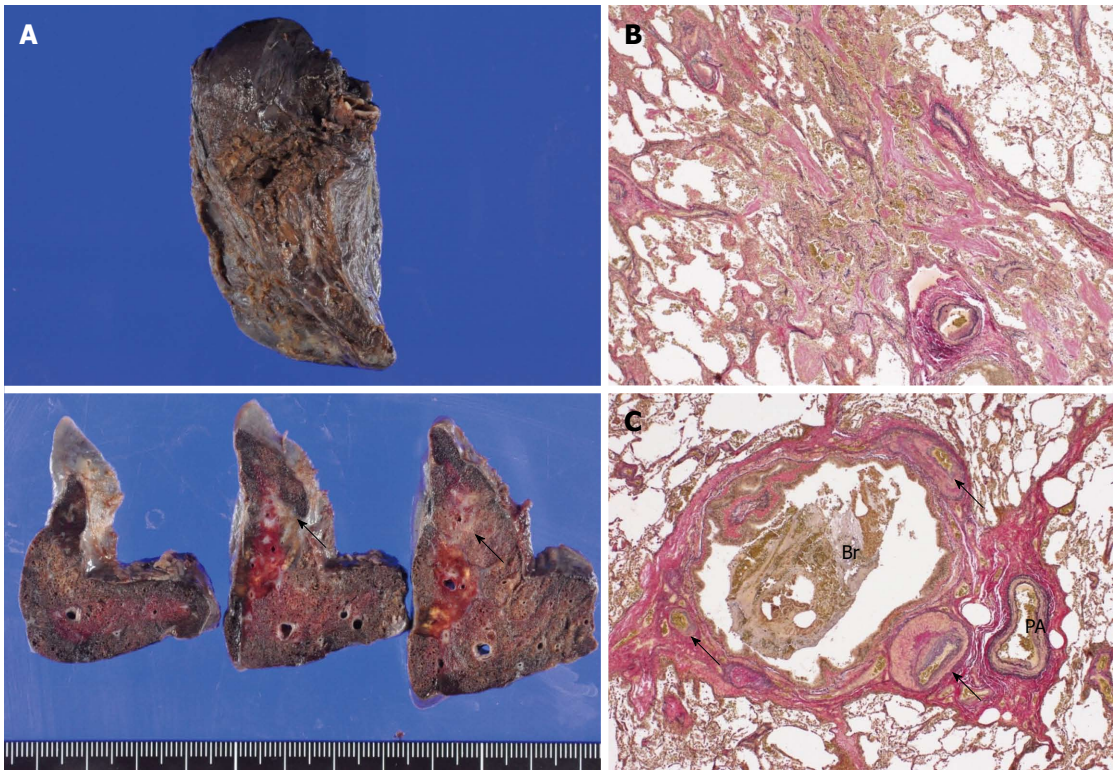


Figure 5 Gross and histopathological findings of the resected specimen. A: An ill-demarcated whitish area with a brownish tint was noted on the cut surface of the resected lung (arrows). Macroscopically, the bleeding origin was not detected; B: Organizing pneumonia with the accumulation of hemosiderin-laden macrophages was observed [Elastica van Gieson stain (EVG), magnification × 24]; C: The bronchus was enlarged, and engorged bronchial artery with medial hypertrophy and overgrowth of small branches were identified near the bronchus (arrows) (EVG, magnification × 24). Br: Bronchus; PA: Pulmonary artery.

thickening of the right middle and lower lobes was significant. Grossly, an ill-demarcated whitish area with a brownish tint was observed on the cut surface of the resected lung (Figure 5A). Histopathologically, organizing pneumonia with the accumulation of hemosiderin-laden macrophages was detected (Figure 5B). The bronchus was enlarged, and engorged BAs with medial hypertrophy and overgrowth of small branches were also noted near the bronchus, although the bleeding origin was not identified (Figure 5C). The patient recovered uneventfully, with the absence of recurrent hemoptysis, and was discharged from our hospital on postoperative day 14. At the time of this writing, he has been doing well for four months, with no evidence of recurrence or hemoptysis.

DISCUSSION

With respect to the major clinical conditions and/or complications inducing hemoptysis after esophagectomy for esophageal carcinoma, surgeons should consider the possibility of locoregional recurrence and aorto-bronchial[3,4] or gastro-tracheobronchial[5,6] fistula formation. We performed a search of PubMed using the following key words: "hemoptysis/bronchial bleeding/respiratory tract hemorrhage after esophagectomy". Fistula formation after esophagectomy is an uncommon and invariably life-threatening complication if left untreated. Only a few cases of aorto-bronchial fistulas after esophagectomy have been reported worldwide in the published literature to date; among the two cases identified in PubMed, gastric tube reconstruction was performed *via* the retrosternal or posterior mediastinal route, and both fistulas were detected between the left main bronchus and descending aorta[3,4]. In contrast, several cases of gastro-tracheobronchial fistulas after esophagectomy have been reported, and tumor recurrence, erosion injury, and radiation necrosis are considered common causes of the late development of these fistulas[5,6]. In the present case, these three conditions were excluded because no protruding/occupied lesions or either fistula type were observed on CT, BAE, or bronchoscopy. In addition, the stage of disease (IA, pT1bN0M0), the presence of bleeding from the peripheral bronchus suspicious on bronchoscopy, and the lack of ulceration necrosis or erosion in the gastric conduit on follow-up gastrointestinal endoscopy supported the exclusion of these clinical conditions.

Based on the intraoperative and pathological findings observed in this case, we judged that the causative vessels of the patient's hemoptysis were the tortuous and engorged arteries arising from the left subclavian artery along the right main bronchus. Retrospectively, orthotopic bilateral BAs originating from the descending aorta at the level of the sixth thoracic vertebra (T6) were detected on contrast-enhanced CT prior to esophagectomy, whereas the ectopic/collateral arteries arising from the right phrenic

artery and left subclavian artery were not. However, it was difficult to precisely evaluate the presence of the ectopic/collateral arteries preoperatively because multiphase contrast-enhanced CT is not routinely performed. According to previous studies, ectopic BAs are defined as arteries originating outside the level of T5-T6 of the thoracic aorta[1,7,8]. Ectopic BAs, which extend along the course of the major bronchus, can be distinguished from collateral arteries arising from the non-bronchial systemic arteries that enter the lung parenchyma *via* the pulmonary ligament or adherent pleura and exhibit a course that is not parallel to the bronchus[1,7]. Additionally, some studies have reported that chronic inflammation of the lungs is associated with engorgement of the BAs[9,10] in which the collateral blood supply from non-bronchial systemic arteries is vascularized and recruited under conditions of chronic inflammation and may arise from branches of the subclavian, axillary, or internal mammary arteries, as well as infradiaphragmatic branches from the inferior phrenic and left gastric arteries[1,11]. In the present case, it was more appropriate to consider the engorged BAs arising from the left subclavian artery to be inherent, having developed after esophagectomy rather than being completely vascularized, due to the dissection of the orthotopic right BAs during radical lymphadenectomy and the effects of postoperative recurrent aspiration pneumonia. These BAs may have been classified as ectopic BAs because they extended along the course of the right main bronchus[1,7]. Alternatively, the tortuous right BA arising from the right inferior phrenic artery may have been classified as a collateral artery arising from non-bronchial systemic arteries because CT detected this artery extending along the pleural surface[1,7]. The frequency of ectopic BAs arising from the subclavian and inferior phrenic arteries is reported to be 10%-13% of all ectopic BAs detected on CT angiograms[12,13].

The mechanism underlying the persistent pneumonia in the right middle lobe observed in this case is thought to be similar to that underlying middle lobe syndrome. This syndrome is characterized by the recurrent or chronic collapse of the right middle lobe and can be classified into obstructive and non-obstructive types[14-16]. Although the etiology of the non-obstructive type has not been fully elucidated, inefficient collateral ventilation, infection, and inflammation are considered to play key roles. Pathophysiologically, the long length and narrow diameter of the middle lobe bronchus can result in poor conditions for drainage[17]. Additionally, collateral ventilation is often prevented by the presence of deep fissures of the middle lobe with few parenchymal connections[18]. In the present case, these anatomical characteristics may also have contributed to the persistence of pneumonia of the right middle lobe, whereas consolidation of the right lower lobe was resolved (Figure 1C).

Furthermore, this case could be diagnosed as secondary racemose hemangioma of BA as these cases have been reported in Japan. Such cases are diagnosed by the existence of remarkably tortuous, enhancing, and meandering BAs[19]. Hyperplastic changes that develop after inflammation, such as bronchiectasis, bronchitis, and pneumonia, are classified as secondary racemose hemangioma[20]. However, in this case, the definitive diagnosis was difficult to obtain due to the lack of clarity of the diagnostic criteria. Although this case could be clinically classified as secondary racemose hemangioma of BA, the hemangioma was also considered to have developed from postoperative recurrent pneumonia, as previously discussed.

BAE has recently become a well-established, effective treatment for controlling recurrent or massive hemoptysis in patients with various pulmonary diseases[21]. Hayakawa *et al*[22] reported a high rate of hemoptysis control and high cumulative survival rate among patients with hemoptysis treated with BAE. However, Chun *et al*[1] reported that recurrent hemoptysis is not uncommon, occurring in 10%-55.3% of cases, although immediate control of hemoptysis is achieved in 73%-99% of treated patients. In addition, several authors have demonstrated that achieving embolization of ectopic BAs is more difficult than that of non-ectopic BAs, and that the complexity of performing selective and definite catheterization explains the increased risk of major complications observed in this patient population[7,23]. In the current case, BAE failed to control the patient's hemoptysis due to incomplete embolization of several ectopic BAs, and surgical resection was eventually required. Although pulmonary resection for hemoptysis is known to carry an increased risk of death and morbidity due to pre-existing comorbidities and a poor respiratory reserve, particularly in the acute phase[19,24], we propose the use of early lobectomy soon after the patient's hemodynamic parameters stabilize following failed BAE as a treatment option, even in cases of recurrent hemoptysis after esophagectomy. Furthermore, the use of lobectomy *via* VATS contributes to a shortened postoperative hospital stay due to the reduced invasiveness of the procedure in patients with poor respiratory function.

We recognize that our discussion includes various limitations. First, there was no evidence suggesting the precise mechanism of the ectopic/collateral BA development in this case. Second, we did not prove, in the resected specimen, that the hemoptysis was induced by rupture of one of the ectopic/collateral BAs, although we surmise that the ectopic BAs originating from the left subclavian artery were responsible based on the patient's clinical course. However, we consider the patient's bronchial bleeding and postoperative recurrent pneumonia to be associated because engorged BAs with medial hypertrophy and overgrowth of small branches were easily identified by histology as being localized near the bronchus and because postoperative recurrent pneumonia was persistently detected in the middle lobe.

In conclusion, to the best of our knowledge, this is the first case report of bronchial bleeding suspected to have derived from the ectopic/collateral bronchial arteries after radical esophagectomy with three-field lymphadenectomy for esophageal carcinoma. We propose the use of early lobectomy soon after the patient's hemodynamic parameters stabilize following failed BAE as a treatment option, even in cases of recurrent hemoptysis after esophagectomy. We strongly believe that the present patient's clinical course after esophagectomy for esophageal carcinoma is significant, particularly with respect to postoperative recurrent pneumonia due to bilateral recurrent laryngeal nerve injury.

COMMENTS

Case characteristics

The patient presented with sudden and recurrent hemoptysis 19 mo after esophagectomy.

Clinical diagnosis

The authors reported a rare case of bronchial bleeding suspected to have arisen from the ectopic/collateral bronchial arteries after radical esophagectomy for esophageal carcinoma with bilateral recurrent laryngeal nerve injury.

Differential diagnosis

Locoregional recurrence and aorto-bronchial or gastro-tracheobronchial fistula formation should be considered as potential causes of bronchial bleeding after esophagectomy for esophageal carcinoma.

Laboratory diagnosis

The hemoglobin level was 8.8 g/dL, and the C-reactive protein level was elevated to 16.1 mg/dL, whereas tumor markers, such as carcinoembryonic antigen, squamous cell carcinoma antigen, and carbohydrate antigen 19-9, were all within normal limits.

Imaging diagnosis

Bronchoscopy revealed a small level of hemosputum in the entrance of the right middle lobe bronchus, and contrast-enhanced computed tomography showed a tortuous artery ascending from the right inferior phrenic artery as well as several tortuous arteries arising from the left subclavian artery, which descended along the right main bronchus.

Pathological diagnosis

Organizing pneumonia with the accumulation of hemosiderin-laden macrophages was detected, and engorged bronchial artery (BA) with medial hypertrophy and overgrowth of small branches were noted near the bronchus.

Treatment

Right middle lobectomy was performed *via* video-assisted thoracic surgery.

Related reports

Chun *et al* demonstrated that chronic inflammation causes recruitment of a collateral blood supply from nonbronchial arteries. However, there are no other reports of bronchial bleeding suspected to arise from the ectopic/collateral bronchial arteries after esophagectomy with bilateral recurrent laryngeal nerve injury.

Term explanation

Middle lobe syndrome is characterized by chronic collapse of the middle lobe, which is more common in females; chronic productive cough and recurrent infections were the most common symptoms.

Experiences and lessons

When encountering a patient with hemoptysis after esophagectomy, the possibility of bronchial bleeding from ectopic/collateral BAs must be considered, especially in cases of postoperative recurrent pneumonia due to bilateral recurrent laryngeal nerve injury.

Peer-review

This case report described the significant clinical course after esophagectomy for esophageal carcinoma. However, a limitation of the report is that there was no evidence indicating the precise mechanism for ectopic/collateral BA developments.

REFERENCES

1 **Chun JY**, Morgan R, Belli AM. Radiological management of hemoptysis: a comprehensive review of diagnostic imaging and bronchial arterial embolization. *Cardiovasc Intervent Radiol* 2010; **33**: 240-250 [PMID: 20058006 DOI: 10.1007/s00270-009-9788-z]

2 **Jean-Baptiste E**. Clinical assessment and management of massive hemoptysis. *Crit Care Med* 2000; **28**: 1642-1647 [PMID: 10834728]

3 **Li HP**, Hsieh CC, Chiang HH, Wang TH, Lee JY, Huang MF, Chou SH. Aortobronchial fistula after esophagectomy for esophageal cancer -- a very rare complication. *Kaohsiung J Med Sci* 2011; **27**: 247-250 [PMID: 21601172 DOI: 10.1016/j.kjms.2010.09.005]

4 **Egan C**, Szontagh-Kishazi P, Flavin R. Aortic fistula after neoadjuvant chemoradiotherapy and esophagectomy for esophageal carcinoma: an unusual cause of sudden death. *Am J Forensic Med Pathol* 2012; **33**: 270-272 [PMID: 22854882 DOI: 10.1097/PAF.0b013e318252e5e7]

5 **Yasuda T**, Sugimura K, Yamasaki M, Miyata H, Motoori M, Yano M, Shiozaki H, Mori M, Doki Y. Ten cases of gastro-tracheobronchial fistula: a serious complication after esophagectomy and reconstruction using posterior mediastinal gastric tube. *Dis Esophagus* 2012; **25**: 687-693 [PMID: 22292530 DOI: 10.1111/j.1442-2050.2011.01309.x]

6 **Sahebazamani M**, Rubio E, Boyd M. Airway gastric fistula after esophagectomy for esophageal cancer. *Ann Thorac Surg* 2012; **93**: 988-990 [PMID: 22364996 DOI: 10.1016/j.athoracsur.2011.06.052]

7 **Sancho C**, Escalante E, Domínguez J, Vidal J, Lopez E, Valldeperas J, Montañá XJ. Embolization of bronchial arteries of anomalous origin. *Cardiovasc Intervent Radiol* 1998; **21**: 300-304 [PMID: 9688797]

8 **Cauldwell EW**, Siekert RG. The bronchial arteries; an anatomic study of 150 human cadavers. *Surg Gynecol Obstet* 1948; **86**: 395-412 [PMID: 18905113]

9 **Ferris EJ**. Pulmonary hemorrhage. Vascular evaluation and interventional therapy. *Chest* 1981; **80**: 710-714 [PMID: 7307594]

10 **Marshall TJ**, Jackson JE. Vascular intervention in the thorax: bronchial artery embolization for haemoptysis. *Eur Radiol* 1997; **7**: 1221-1227 [PMID: 9377505]

11 **McDonald DM**. Angiogenesis and remodeling of airway vasculature in chronic inflammation. *Am J Respir Crit Care Med* 2001; **164**: S39-S45 [PMID: 11734465]

12 **Hartmann IJ**, Remy-Jardin M, Menchini L, Teisseire A, Khalil C, Remy J. Ectopic origin of bronchial arteries: assessment with multidetector helical CT angiography. *Eur Radiol* 2007; **17**: 1943-1953 [PMID: 17285281]

13 **Ponnuswamy I**, Sankaravadivelu ST, Maduraimuthu P, Natarajan K, Sathyanathan BP, Sadras S. 64-detector row CT evaluation of bronchial and non-bronchial systemic arteries in life-threatening haemoptysis. *Br J Radiol* 2012; **85**: e666-e672 [PMID: 22595498 DOI: 10.1259/bjr/24730002]

14 **Einarsson JT**, Einarsson JG, Isaksson H, Gudbjartsson T, Gudmundsson G. Middle lobe syndrome: a nationwide study on clinicopathological features and surgical treatment. *Clin Respir J* 2009; **3**: 77-81 [PMID: 20298381 DOI: 10.1111/j.1752-699X.2008.00109.x]

15 **Gudbjartsson T**, Gudmundsson G. Middle lobe syndrome: a review of clinicopathological features, diagnosis and treatment. *Respiration* 2012; **84**: 80-86 [PMID: 22377566 DOI: 10.1159/000336238]

16 **Shaikhrezai K**, Khorsandi M, Zamvar V. Middle lobe syndrome associated with major haemoptysis. *J Cardiothorac Surg* 2013; **8**: 84 [PMID: 23587098 DOI: 10.1186/1749-8090-8-84]

17 **Bertelsen S**, Struve-Christensen E, Aasted A, Sparup J. Isolated middle lobe atelectasis: aetiology, pathogenesis, and treatment of the so-called middle lobe syndrome. *Thorax* 1980; **35**: 449-452 [PMID: 7434301]

18 **Ayed AK**. Resection of the right middle lobe and lingula in

children for middle lobe/lingula syndrome. *Chest* 2004; **125**: 38-42 [PMID: 14718418]

19 **Kimura M**, Kuwabara Y, Ishiguro H, Takeyama H. Esophageal cancer with racemose hemangioma of the bronchial arteries. *Gen Thorac Cardiovasc Surg* 2012; **60**: 149-152 [PMID: 22419183 DOI: 10.1007/s11748-011-0804-2]

20 **Iwasaki M**, Kobayashi H, Nomoto T, Arai T, Kondoh T. Primary racemose hemangioma of the bronchial artery. *Intern Med* 2001; **40**: 650-653 [PMID: 11506310]

21 **Fernando HC**, Stein M, Benfield JR, Link DP. Role of bronchial artery embolization in the management of hemoptysis. *Arch Surg* 1998; **133**: 862-866 [PMID: 9711960]

22 **Hayakawa K**, Tanaka F, Torizuka T, Mitsumori M, Okuno Y, Matsui A, Satoh Y, Fujiwara K, Misaki T. Bronchial artery embolization for hemoptysis: immediate and long-term results. *Cardiovasc Intervent Radiol* 1992; **15**: 154-158; discussion 158-159 [PMID: 1628281]

23 **Rabkin JE**, Astafjev VI, Gothman LN, Grigorjev YG. Transcatheter embolization in the management of pulmonary hemorrhage. *Radiology* 1987; **163**: 361-365 [PMID: 3562815]

24 **Andréjak C**, Parrot A, Bazelly B, Ancel PY, Djibré M, Khalil A, Grunenwald D, Fartoukh M. Surgical lung resection for severe hemoptysis. *Ann Thorac Surg* 2009; **88**: 1556-1565 [PMID: 19853112 DOI: 10.1016/j.athoracsur.2009.06.011]

P- Reviewer: Chiarioni G, Marangoni G, Pal S **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3402

World J Gastroenterol 2015 March 21; 21(11): 3402-3408
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Endoscopic ultrasound-guided drainage of postoperative intra-abdominal abscesses

Koichiro Mandai, Koji Uno, Kenjiro Yasuda

Koichiro Mandai, Koji Uno, Kenjiro Yasuda, Department of Gastroenterology, Kyoto Second Red Cross Hospital, Kamigyo-ku, Kyoto 602-8026, Japan
Author contributions: Mandai K collected the data and wrote the paper; Uno K and Yasuda K revised the paper.
Ethics approval: The study was reviewed and approved by the Kyoto Second Red Cross Hospital Institutional Review Board.
Informed consent: All study participants provided informed consent prior to study enrollment.
Conflict-of-interest: There are no conflicts of interest to declare.

Correspondence to: Koichiro Mandai, MD, Department of Gastroenterology, Kyoto Second Red Cross Hospital, 355-5 Haruobi-cho, Kamigyo-ku, Kyoto 602-8026, Japan. mandai@gmail.com
Telephone: +81-75-2315171
Fax: +81-75-2563451
Received: October 4, 2014
Peer-review started: October 6, 2014
First decision: October 29, 2014
Revised: November 12, 2014
Accepted: January 8, 2015
Article in press: January 8, 2015
Published online: March 21, 2015

Abstract

Although endoscopic ultrasound (EUS)-guided drainage has become the standard procedure for pancreatic pseudocysts in recent years and is generally regarded as a safe and effective method, there have been few reports of EUS-guided drainage of postoperative intra-abdominal abscesses. Here we report our experience with 4 cases of postoperative intra-abdominal abscesses for which EUS-guided drainage was performed between May 2011 and May 2014. Distal pancreatectomy had been performed in 3 cases, whereas low anterior resection for rectal cancer was performed in the remaining case. All patients underwent transgastric naso-cystic drainage, which resulted in clinical improvement without complications, even when performed within 4 wk after surgery. On average, the naso-cystic drain was removed 10 d after placement, with no abscess recurrence. Based on these findings, we believe that EUS-guided drainage of postoperative intra-abdominal abscesses is a safe and effective method, although further large-scale investigations are required to confirm our findings.

Key words: Postoperative intra-abdominal abscess; Endoscopic ultrasonography; Endoscopic ultrasound-guided drainage; Transgastric drainage; Naso-cystic drainage

Core tip: There have been few reports of endoscopic ultrasound (EUS)-guided drainage of postoperative intra-abdominal abscesses, although EUS-guided drainage has become the standard procedure for pancreatic pseudocysts in recent years. Here we report our experience with 4 cases. Transgastric naso-cystic drainage was performed for all patients and resulted in clinical improvement without complications, even when performed within 4 wk after surgery. On average, the naso-cystic drain was removed 10 d after placement, with no abscess recurrence. We believe that EUS-guided drainage of postoperative intra-abdominal abscesses is a safe and effective method, although further large-scale investigations are required to confirm our findings.

Mandai K, Uno K, Yasuda K. Endoscopic ultrasound-guided

drainage of postoperative intra-abdominal abscesses. *World J Gastroenterol* 2015; 21(11): 3402-3408 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3402.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3402

INTRODUCTION

Traditionally, postoperative intra-abdominal abscesses have been managed by percutaneous or surgical drainage. Image-guided percutaneous drainage is reported to be a minimally invasive and effective method[1].

Although endoscopic ultrasound (EUS)-guided drainage for pancreatic pseudocysts is regarded as a safe and effective standard method[2-7], there have been few reports of EUS-guided drainage of postoperative intra-abdominal abscesses[8,9].

Herein, we examined 4 cases of EUS-guided drainage of postoperative intra-abdominal abscesses performed at our institution to evaluate the safety and efficacy of this procedure.

CASE REPORT

Between May 2011 and May 2014, we experienced 4 cases of postoperative intra-abdominal abscesses for which EUS-guided drainage was performed. The indication of EUS-guided drainage was limited to cases in which adhesion of the abscess and digestive wall was highly suspected on computed tomography (CT).

All procedures were performed using a convex-type echoendoscope (GE-UC2000P or GF-UCT260; Olympus Medical Systems, Tokyo, Japan). A 19-gauge needle (EchoTip; Cook Medical, Winston-Salem, NC, United States or Expect; Boston Scientific Japan, Tokyo, Japan, or SonoTip; Medi-Globe, Rosenheim, Germany) was used to puncture the abscess. The fluid content was aspirated, and a sample of the aspirate was sent for Gram staining and culture. Subsequently, a small dose of contrast agent was injected, and a 0.025-inch guidewire (VisiGlide; Olympus Medical Systems) was introduced through the needle and coiled into the abscess under fluoroscopic guidance. In cases of only naso-cystic drainage, the fistula was dilated using a 7-Fr dilation catheter (Soehendra Biliary Dilation Catheter; Cook Medical), and a 6-Fr pigtail nasal biliary catheter (nasal biliary drainage set; Cook Medical) was subsequently placed. In cases of both naso-cystic and internal drainage, the fistula was dilated using a 7-Fr dilation catheter (Soehendra Biliary Dilation Catheter; Cook Medical) and a balloon catheter (Maxforce TTS biliary balloon dilator, 6 mm; Boston Scientific Japan). Next, two 0.025-inch guidewires (VisiGlide; Olympus Medical Systems) were placed in the abscess using a double lumen catheter (Uneven Double Lumen Catheter; PIOLAX, Kanagawa, Japan), and a 6-Fr pigtail nasal biliary catheter (nasal biliary drainage set; Cook Medical) and 7-Fr double pigtail biliary stent (Zimmon Biliary Stent; Cook Medical) were subsequently placed. The naso-cystic drainage catheter was irrigated once daily with 10-20 mL of sterile saline in all patients. Characteristics of the 4 cases are summarized in Table 1.

Case 1

A 60-year-old woman underwent laparoscopic low anterior resection for rectal cancer. She developed a high fever after surgery, and CT revealed fluid collection around the anastomotic intestine and in the subdiaphragmatic area. We diagnosed this fluid collection as an abscess, and surgical drainage was consequently performed on postoperative day 17. Although the drain was removed and the patient was discharged on postoperative day 44, she was readmitted for high fever on postoperative day 64. CT revealed an encapsulated fluid collection (maximum axis, 61 mm) in the left subdiaphragmatic area close to the fornix of the stomach (Figure 1A). We again diagnosed this fluid collection as an abscess; and, as antibiotic administration did not improve the patient's condition, EUS-guided transgastric drainage was performed, and a 6-Fr pigtail nasal biliary catheter was placed on postoperative day 69 (Figure 1B, C). Gram staining and culture of a sample of the aspirate confirmed extended-spectrum beta lactamase (ESBL)-producing *Escherichia coli*, *Enterococcus*, and anaerobic bacteria. Antibiotics were administered until postoperative day 74, and CT on postoperative day 75 showed that the abscess had decreased in size (Figure 1D). The nasal biliary catheter was removed on postoperative day 80. The patient was discharged on postoperative day 99. At the latest follow-up (3 years after discharge), no abscess recurrence was noted.

Case 2

A 46-year-old woman underwent distal pancreatectomy for a mucinous cystic neoplasm of the pancreas. She developed high fever on postoperative day 24, and CT revealed irregular fluid collection (maximum axis, 54 mm) around the resection stump of the pancreas close to the gastric wall (Figure 2A). The fluid collection was diagnosed as an abscess; and, accordingly, EUS-guided transgastric drainage was performed and a 6-Fr pigtail nasal biliary catheter was placed on postoperative day 26 (Figure 2B-D). Gram staining and culture of a sample of the aspirate confirmed methicillin-resistant *Staphylococcus aureus* (MRSA). CT on postoperative day 37 showed that the abscess had decreased in size (Figure 2E), and the nasal biliary catheter was removed on the same day. Antibiotics were administered until postoperative day 38. The patient was discharged on postoperative day 40. At the latest follow-up (2 years after discharge), no abscess

Table 1 Patient characteristics

Case	Age (yr)/ sex	Primary disease	Surgical procedure	Abscess size (mm)	Primary treatment for abscess	EUS-D route	Time to EUS-D (d)	EUS-D modality	Drainage length (d)	Complications/ abscess recurrence
1	60/F	Rectal cancer	Low anterior rectal resection	61 × 55	Surgical drainage	TG	69 p.o.	6-Fr NB	11	None
2	46/F	MCN	Distal pancreatectomy	54 × 33	None	TG	26 p.o.	6-Fr NB	11	None
3	74/M	PDAC	Distal pancreatectomy	62 × 35	None	TG	10 p.o.	7-Fr stent	20	None
				66 × 42	None	TG	21 p.o.	6-Fr NB	8	None
4	69/M	IPMN	Distal pancreatectomy	70 × 45	Percutaneous drainage	TG	18 p.o.	7-Fr stent, 6-Fr NB	7-Fr stent: no removal 6-Fr NB: 10 per-cutaneous: 24	None

EUS-D: Endoscopic ultrasound-guided drainage; TG: Transgastric; p.o.: Postoperative day; MCN: Mucinous cystic neoplasm; NB: Nasal biliary catheter; PDAC: Pancreatic ductal adenocarcinoma; IPMN: Intraductal papillary mucinous neoplasm.

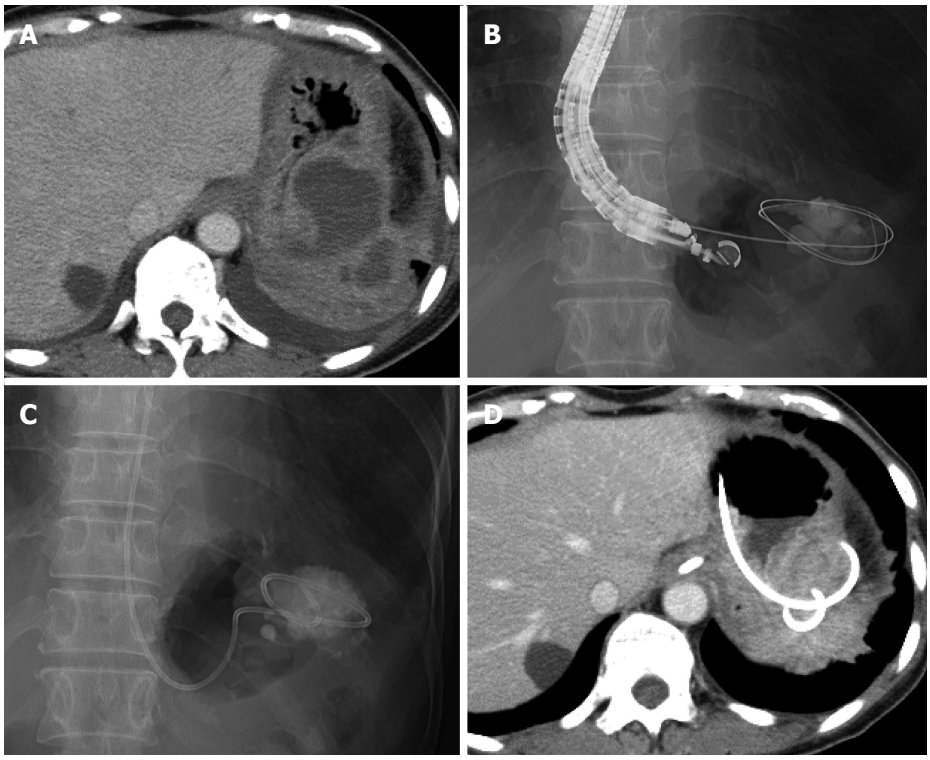


Figure 1 Case 1. A: Computed tomography (CT) reveals an encapsulated fluid collection in the left subdiaphragmatic area close to the fornix of the stomach; B and C: Fluoroscopy image showing the guidewire inserted into the fluid collection under endoscopic ultrasound (EUS)-guidance and placement of a 6-Fr nasal biliary catheter; D: Six days after EUS-guided drainage, CT reveals that the drain was placed and that the fluid collection decreased.

recurrence was observed.

Case 3

A 74-year-old man underwent distal pancreatectomy for pancreatic ductal adenocarcinoma. He developed high fever on postoperative day 9, and CT revealed fluid collection (maximum axis, 62 mm) around the resection stump of the pancreas close to the gastric wall (Figure 3A). We diagnosed this fluid collection as an abscess, and EUS-guided transgastric drainage was performed and a 7-Fr double pigtail stent was placed on postoperative day 10 (Figure 3B). Gram staining and culture of a sample of the aspirate confirmed methicillin-susceptible *Staphylococcus aureus* (MSSA), and antibiotics were administered until postoperative day 15. However, the patient developed high fever after drainage, and CT on postoperative day 17 revealed another fluid collection (maximum axis, 66 mm) at the left side of the previously drained abscess (Figure 3C). EUS-guided transgastric drainage was performed again, and a 6-Fr pigtail nasal biliary catheter was placed on postoperative day 21 (Figure 3D, E). Gram staining and culture of a sample of the aspirate again

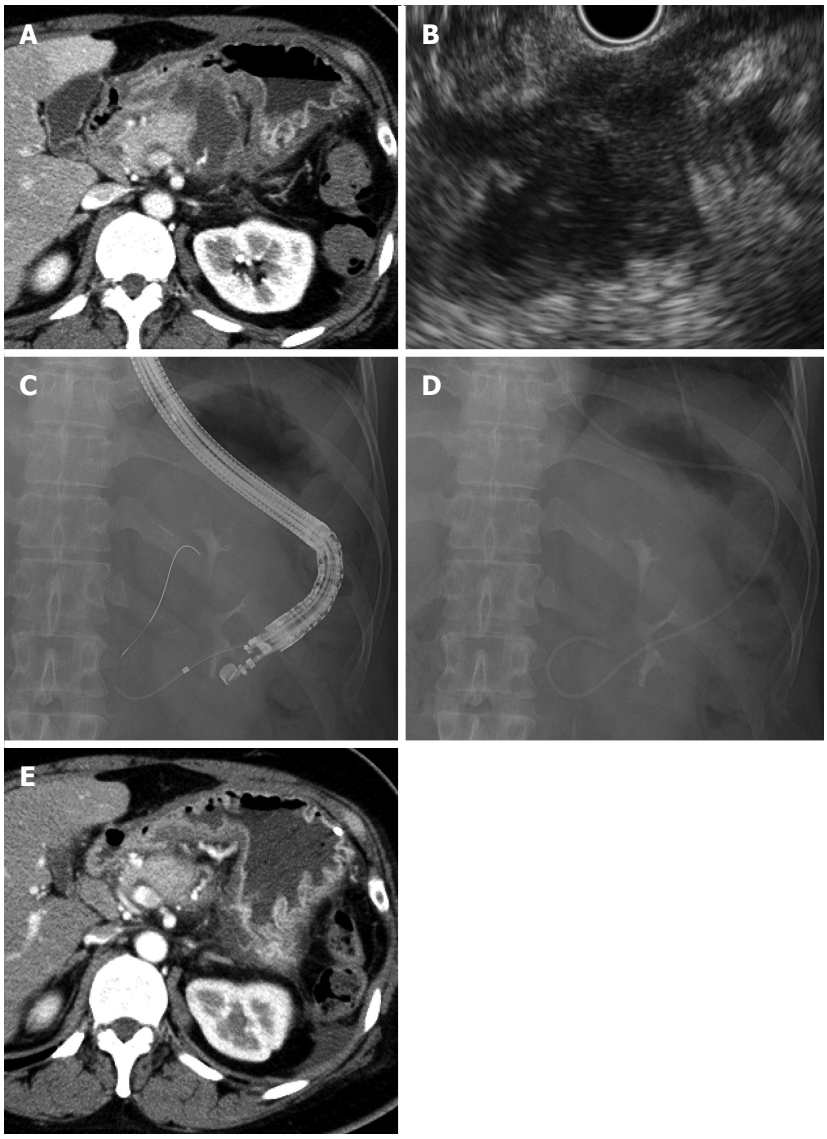


Figure 2 Case 2. A: Computed tomography (CT) reveals irregular fluid collection around the resection stump of the pancreas at the dorsal side of the stomach; B: Endoscopic ultrasound (EUS) reveals an irregular and cloudy fluid collection close to the gastric wall; C and D: Fluoroscopy image showing the dilation catheter inserted over the guidewire and placement of a 6-Fr nasal biliary catheter; E: Eleven days after EUS-guided drainage, CT reveals that the fluid collection decreased.

confirmed MSSA, and antibiotics were administered until postoperative day 24. CT on postoperative day 28 showed that the abscess had decreased in size (Figure 3F), and the nasal biliary catheter and the 7-Fr double pigtail stent were removed on postoperative days 29 and 30, respectively. The patient was discharged on postoperative day 32. At the latest follow-up (1 year after discharge), no abscess recurrence was observed.

Case 4

A 69-year-old man underwent distal pancreatectomy for intraductal papillary mucinous neoplasm of the pancreas. He developed high fever on postoperative day 9, and CT revealed a fluid collection (maximum axis, 70 mm) at the dorsal side of the fornix close to the gastric wall (Figure 4A). We diagnosed this fluid collection as an abscess, and conventional ultrasound-guided percutaneous drainage was performed on the same day. However, the high fever persisted after drainage, and EUS-guided transgastric drainage was consequently performed together with placement of a 7-Fr double pigtail stent and a 6-Fr pigtail nasal biliary catheter on postoperative day 18 (Figure 4B-D). Gram staining and culture of a sample of the aspirate revealed *Enterococcus*, and antibiotics were administered until postoperative day 21. CT on postoperative day 28 showed that the abscess had decreased in size (Figure 4E), and the nasal biliary catheter and the percutaneous drain were removed on postoperative days 28 and 33, respectively. The 7-Fr double pigtail stent was not removed, and the patient was discharged on postoperative day 36. At the latest follow-up (6 mo after discharge), no abscess recurrence was noted.

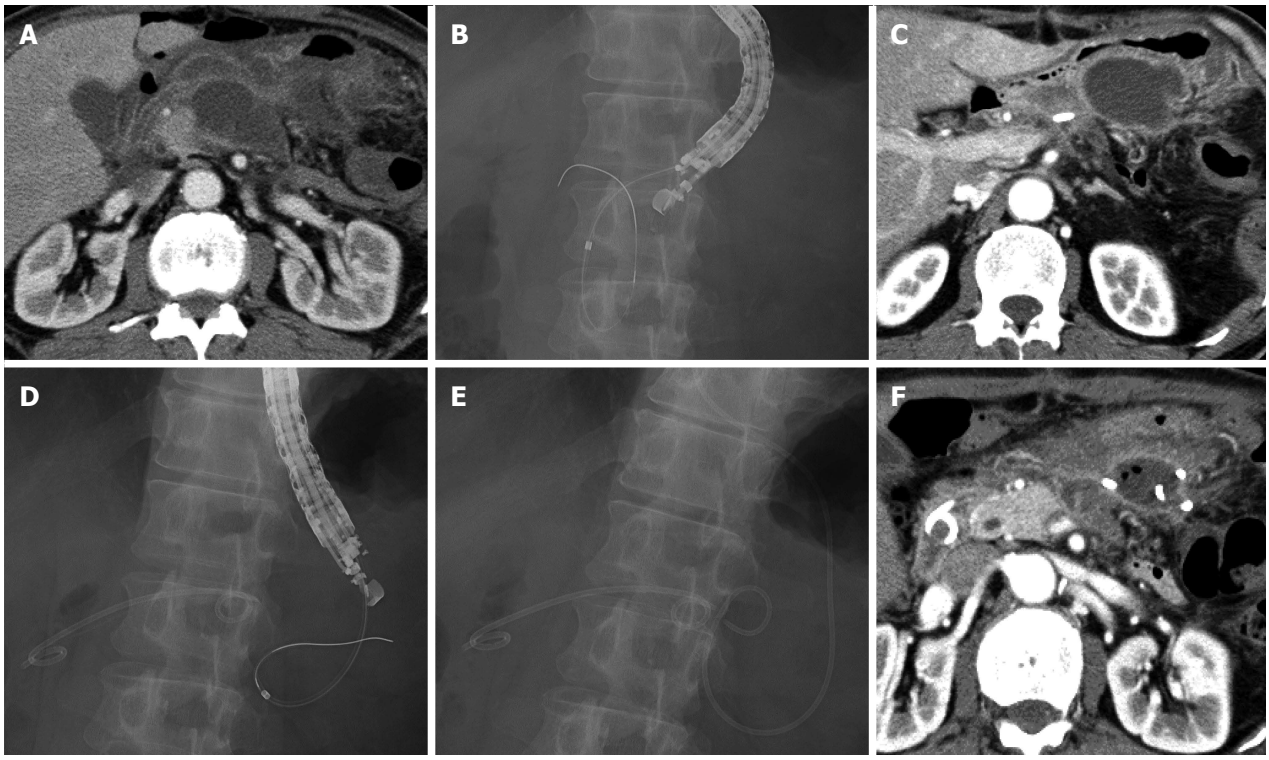


Figure 3 Case 3. A: Computed tomography (CT) reveals fluid collection around the resection stump of the pancreas; B: Fluoroscopy image showing the dilation catheter inserted over the guidewire; C: Seven days after endoscopic ultrasound (EUS)-guided internal drainage, CT reveals another fluid collection on the left side of the previously drained abscess; D and E: Fluoroscopy image showing the placement of the 7-Fr double pigtail stent and insertion of the dilation catheter into the new abscess. Moreover, a 6-Fr nasal biliary catheter was placed; F: Seven days after EUS-guided naso-cystic drainage, CT reveals that the fluid collection decreased.

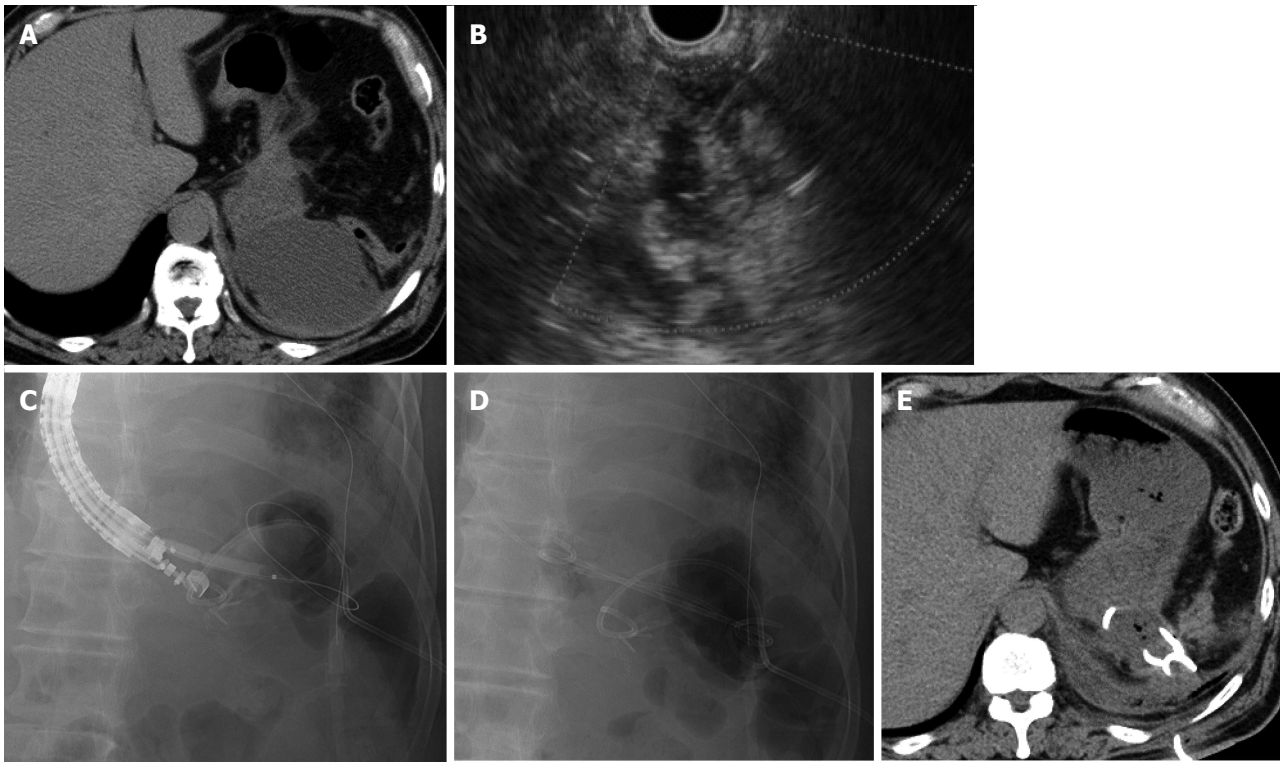


Figure 4 Case 4. A: Computed tomography (CT) reveals fluid collection at the dorsal side of the fornix close to the gastric wall; B: Endoscopic ultrasound (EUS) showing the fluid collection, which was punctured by a 19-gauge needle; C and D: Fluoroscopy image showing placement of the percutaneous drain and insertion of the 6-mm balloon catheter over the guidewire. Both a 7-Fr double pigtail stent and a 6-Fr nasal biliary catheter are placed; E: Ten days after EUS-guided drainage, CT reveals that the fluid collection decreased.

DISCUSSION

Image-guided percutaneous drainage has been performed for postoperative intra-abdominal abscesses as a minimally invasive method. Azeem *et al*[10] reported that endoscopic drainage with or without EUS was as effective as percutaneous drainage in patients with pancreatic fluid collection after distal pancreatectomy, and that primary endoscopic drainage may be associated with a shorter hospital stay. Although their report suggested the importance of endoscopic drainage of pancreatic fluid collection after pancreatic resection, to our knowledge, there have been no reports comparing the outcomes of EUS-guided drainage and image-guided percutaneous drainage for the treatment of postoperative intra-abdominal abscesses.

In this study, EUS-guided transgastric drainage for postoperative intra-abdominal abscesses was performed in all 4 patients and resulted in clinical improvement in all cases. Furthermore, naso-cystic drainage was performed within 4 wk of surgery in 3 of the 4 patients, with no complications in any patient, suggesting that early EUS-guided drainage after surgery is safe. Similarly, Tilara *et al*[8] also reported that early drainage (within 30 d after surgery) of postoperative pancreatic fluid collections was not associated with an increased risk of complications.

In all 4 of our cases, naso-cystic drainage was performed. The naso-cystic drain was removed on day 11 after placement in cases 1 and 2, day 8 in Case 3, and day 10 in Case 4 (mean, day 10 after naso-cystic drainage), and there was no recurrence of the abscess after naso-cystic drain removal in any case. Taken together, these outcomes suggest the efficacy of naso-cystic drainage, but further investigations are required to confirm these results and to determine whether naso-cystic drainage alone is effective, as internal drainage and percutaneous drainage were also performed before naso-cystic drainage in Cases 3 and 4, respectively. Furthermore, the necessity of internal drainage stent removal and the appropriate time of stent removal should be investigated in cases of both naso-cystic and internal drainage. Several recent studies of EUS-guided drainage of pancreatic pseudocysts recommend both internal and naso-cystic drain placement by first using the double guidewire technique, followed by naso-cystic drain removal after improvement of infection[3,6]; however, there is no consensus regarding the optimal drainage method and time for drain removal.

Thus, based on our present cases, we believe that EUS-guided drainage of postoperative intra-abdominal abscesses is a safe and effective method; however, further large-scale investigations are required to confirm our findings.

COMMENTS

Case characteristics

The 4 patients (2 male, 2 female) developed high fever after abdominal surgery (pancreatectomy or low anterior resection for rectal cancer).

Clinical diagnosis

In addition to high fever, all patients had general fatigue.

Differential diagnosis

Postoperative intra-abdominal fluid collection was performed to diagnose infection.

Laboratory diagnosis

Gram staining and culture of samples of collected fluid confirmed extended-spectrum beta lactamase-producing *Escherichia coli*, *Enterococcus*, and anaerobic bacteria in the first patient, methicillin-resistant *Staphylococcus aureus* in the second patient, methicillin-susceptible *Staphylococcus aureus* in the third patient, and *Enterococcus* in the fourth patient.

Imaging diagnosis

Computed tomography revealed fluid collection close to the gastric wall in all 4 patients.

Pathological diagnosis

Pathological examination of the fluid collection was not performed in any of the patients.

Treatment

All 4 patients underwent endoscopic ultrasound (EUS)-guided transgastric naso-cystic drainage, and in 2 of the 4 patients, internal drainage was also performed.

Related reports

Earlier reports state that EUS-guided drainage of postoperative peripancreatic fluid collections is safe and effective, and we believe that this case report adds our findings that early EUS-guided transgastric drainage within 4 wk of surgery is also safe and effective.

Term explanation

Naso-cystic drainage describes a method of external drainage via the nose.

Experiences and lessons

This case report presents data showing that early EUS-guided drainage within 4 wk of surgery for postoperative intra-abdominal abscesses is safe and effective.

Peer-review

This is a well-written case report of a novel application of therapeutic EUS. Although 3 of 4 cases death with post-pancreatic surgery fluid collections, the 4th case is the first to report drainage of an abscess by EUS after colorectal surgery.

REFERENCES

1 **Kassi F**, Dohan A, Soyer P, Vicaut E, Boudiaf M, Valleur P, Pocard M. Predictive factors for failure of percutaneous drainage of postoperative abscess after abdominal surgery. *Am J Surg* 2014; **207**: 915-921 [PMID: 24280147 DOI: 10.1016/j.amjsurg.2013.07.041]

2 **Lopes CV**, Pesenti C, Bories E, Caillol F, Giovannini M. Endoscopic-ultrasound-guided endoscopic transmural drainage of pancreatic pseudocysts and abscesses. *Scand J Gastroenterol* 2007; **42**: 524-529 [PMID: 17454865 DOI: 10.1080/00365520601065093]

3 **Itoi T**, Itokawa F, Tsuchiya T, Kawai T, Moriyasu F. EUS-guided pancreatic pseudocyst drainage: simultaneous placement of stents and nasocystic catheter using double-guidewire technique. *Dig Endosc* 2009; **21** Suppl 1: S53-S56 [PMID: 19691736 DOI: 10.1111/j.1443-1661.2009.00851.x]

4 **Seewald S**, Ang TL, Richter H, Teng KY, Zhong Y, Groth S, Omar S, Soehendra N. Long-term results after endoscopic drainage and necrosectomy of symptomatic pancreatic fluid collections. *Dig Endosc* 2012; **24**: 36-41 [PMID: 22211410 DOI: 10.1111/j.1443-1661.2011.01162.x]

5 **Sadik R**, Kalaitzakis E, Thune A, Hansen J, Jönson C. EUS-guided drainage is more successful in pancreatic pseudocysts compared

with abscesses. *World J Gastroenterol* 2011; **17**: 499-505 [PMID: 21274380 DOI: 10.3748/wjg.v17.i4.499]

6 **Kato S**, Katanuma A, Maguchi H, Takahashi K, Osanai M, Yane K, Kim T, Kaneko M, Takaki R, Matsumoto K, Matsumori T, Gon K, Tomonari A. Efficacy, Safety, and Long-Term Follow-Up Results of EUS-Guided Transmural Drainage for Pancreatic Pseudocyst. *Diagn Ther Endosc* 2013; **2013**: 924291 [PMID: 23554548 DOI: 10.1155/2013/924291]

7 **Lin H**, Zhan XB, Sun SY, Yang XJ, Jin ZD, Zou DW, Li ZS. Stent selection for endoscopic ultrasound-guided drainage of pancreatic fluid collections: a multicenter study in china. *Gastroenterol Res Pract* 2014; **2014**: 193562 [PMID: 25018767 DOI: 10.1155/2014/193562]

8 **Tilara A**, Gerdes H, Allen P, Jarnagin W, Kingham P, Fong Y, DeMatteo R, D'Angelica M, Schattner M. Endoscopic ultrasound-guided transmural drainage of postoperative pancreatic collections. *J Am Coll Surg* 2014; **218**: 33-40 [PMID: 24099888 DOI: 10.1016/j.jamcollsurg.2013.09.001]

9 **Varadarajulu S**, Wilcox CM, Christein JD. EUS-guided therapy for management of peripancreatic fluid collections after distal pancreatectomy in 20 consecutive patients. *Gastrointest Endosc* 2011; **74**: 418-423 [PMID: 21679939 DOI: 10.1016/j.gie.2011.03.1242]

10 **Azeem N**, Baron TH, Topazian MD, Zhong N, Fleming CJ, Kendrick ML. Outcomes of endoscopic and percutaneous drainage of pancreatic fluid collections arising after pancreatic tail resection. *J Am Coll Surg* 2012; **215**: 177-185 [PMID: 22634120 DOI: 10.1016/j.jamcollsurg.2012.03.015]

P- Reviewer: Chisthi MM, Teshima CW, Yoshida H **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3409

World J Gastroenterol 2015 March 21; 21(11): 3409-3413
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Pediatric gastric cancer presenting with massive ascites

Chien-Heng Lin, Wei-Ching Lin, I-Hsiu Lai, Shu-Fen Wu , Kang-Hsi Wu, An-Chyi Chen

Chien-Heng Lin, I-Hsiu Lai, Shu-Fen Wu, Kang-Hsi Wu, An-Chyi Chen, Department of Pediatrics, Children's Hospital, China Medical University Hospital, Taichung 404, Taiwan
Wei-Ching Lin, Shu-Fen Wu, An-Chyi Chen, College of Medicine, School of Medicine, China Medical University, Taichung 404, Taiwan
Kang-Hsi Wu, College of Chinese Medicine, School of Chinese Medicine, China Medical University, Taichung 404, Taiwan
Wei-Ching Lin, Department of Radiology, China Medical University Hospital, Taichung 404, Taiwan
Author contributions: Lin CH is the main author of this article; Chen AC performed the majority of experiments; Lin WC provided vital reagents and analytical tools and also the description of the imaging; Wu SF and Wu KH were attending doctors and performed clinical treatment and were also involved in editing the manuscript; Lai IH provided the collection of all the human materials; all authors have reviewed the manuscript and approved the final manuscript.
Supported by grants from the Research laboratory of pediatrics, Children's Hospital of China Medical University; and China Medical University Hospital, No. DMR-101-042.

Correspondence to: An-Chyi Chen, MD, Department of Pediatrics, Children's Hospital, China Medical University Hospital, No. 2 Yuh-Der Road, Taichung 404, Taiwan. d8427@mail.cmuh.org.tw
Telephone: +886-4-22052121
Fax: +886-4-22032798
Received: September 9, 2014
Peer-review started: September 9, 2014
First decision: October 14, 2014
Revised: October 23, 2014
Accepted: November 11, 2014
Article in press: November 11, 2014
Published online: March 21, 2015

Abstract

Gastric adenocarcinoma is quite rare in children and as a result very little experience has been reported on with regards to clinical presentation, treatment and outcome. We describe the case of a 16-year-old boy presenting with abdominal fullness and poor appetite for 7 d. Sonography showed massive ascites and computed tomography imaging revealed the presence of gastric mucosa thickness with omentum caking. The diagnosis of gastric adenocarcinoma was biopsy-proven endoscopically. Despite gastric adenocarcinoma being quite rare in the pediatric patient population, we should not overlook the possibility of gastric adenocarcinoma when a child presents with distended abdomen and massive ascites.

Key words: Gastric adenocarcinoma; Ascites; Children

Core tip: Gastric adenocarcinoma is rare in pediatric patient. It should be suspected in a child with gastric ulcers and massive ascites. Upper gastrointestinal endoscopy and endoscopic biopsies are crucial in children with vague gastrointestinal symptoms and massive ascites in whom CT fails to demonstrate the primary site of the malignancy.

Lin CH, Lin WC, Lai IH, Wu SF, Wu KH, Chen AC. Pediatric gastric cancer presenting with massive ascites. *World J Gastroenterol* 2015; 21(11): 3409-3413 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3409.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3409

INTRODUCTION

Primary gastric adenocarcinoma is a rare cancer

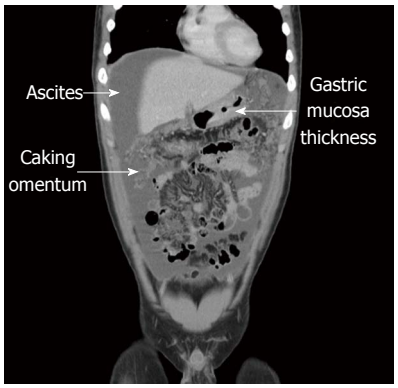


Figure 1 Massive ascites, gastric mucosa thickness, and caking omentum are shown by arrows on the coronal view of abdominal computed tomography.

in children, and occurs in 0.05% of all childhood cancers[1]. The initial clinical presentations were mostly nonspecific abdominal symptoms, such as dyspepsia, epigastric pain, nausea/vomiting, weight loss, and gastrointestinal bleeding. The etiology of gastric adenocarcinoma in adults could be related to lifestyle factors or infectious factors[2]. However, the role of these factors in children is unknown. The prognosis for the gastric adenocarcinoma in children is very poor, with a median survival time of 5 mo and average survival time of 7.5 mo[3], and relatively little information exists regarding the 5-year disease-free survival rate for children[3-5]. Because of its rarity, the diagnosis and treatment in a pediatric population with gastric adenocarcinoma remains challenging. Herein we describe a 16-year-old boy with gastric adenocarcinoma who presented with abdominal fullness with massive ascites.

CASE REPORT

A 16-year-old boy presented with abdominal fullness and poor appetite for one week. It took approximately 2 wk from initial symptom occurrence to patient hospitalization. A body weight loss of 8 kilograms was noted initially. The diet habits of the patient were standard without any obvious personal favorites regarding food. The patient also had the social habit of smoking with a consumption of 1 pack of cigarettes every 3 d for about 1 year. His family history showed gastric cancer in his grandfather, who died at the age of 51 years. The physical examination show distended abdominal wall with dull on percussion, and epigastric local tenderness. Neck lymph nodes were enlarged on level V. No resistance and no enlargement of liver or spleen were noted.

Laboratory investigations revealed normal results except for mild leukocytosis (11.19 × 1000/μL, normal range: 3.99-10.39 × 1000/μL) with predominant neutrophil (80.4%, normal range: 40%-74%) and decreased lactate dehydrogenase (LDH) level (90 U/L, normal range: 98-192 U/L). The level of tumor marker was significantly increased in CA125 (109.1 U/mL, normal range: < 35 U/mL) but was normal in CEA (0.51 ng/mL, normal range: < 5 ng/mL) and CA199 (6.8 U/mL, normal range: < 35 U/mL).

Abdominal sonography confirmed massive ascites. Abdominal computed tomography (CT) scan revealed massive ascites, gastric mucosa thickness, and caking omentum (Figure 1). The gastroscopy revealed a large bizarre gastric ulcer (A2) (4 cm × 4 cm in size) which appeared as a snake skin with multiple nodular appearance over peri-antrum area (Figure 2). Four biopsy specimens were obtained on the margin of the ulcers during histological examination, and poorly differentiated adenocarcinoma with the presence of signet ring cells was observed (Figure 3). *Helicobacter pylori* (*H. pylori*) were also detected in the patient's biopsy tissue specimens (Figure 4).

Study of the ascites showed RBC count: 4000/μL, and WBC count: 10707/μL with neutrophil 89% and biochemistry revealed glucose: 97 mg/dL, LDH: 288 U/L, total protein: 4.2 g/dL, albumin: 2.9 g/dL (normal range: 3.8-5.3 g/dL). The cytology of ascites from abdominal tapping reported malignant cells. The genetic work-up for our patient was all shown to be normal.

The ascites study revealed many tumor nests, which were all negative for CD45, CD3, CD19, CD20, CD33, myeloperoxidase and TdT in the immunohistochemical study. These results indicated that lymphoma was not likely.

The infectious factor of Helicobacter bacilli was evident in pathology from gastric biopsy. However, the EBV DNA PCR study was negative (< 600 copies). Hepatitis was excluded as both HBsAg and HCV antibody were negative.

No local radiotherapy or surgery was planned because of extensive metastatic disease (the TNM staging was T4N × M1, Ⅳ); the patient was placed on steroid as dexamethasone phosphate and a chemotherapy regimen of oxaliplatin and capecitabine. The patient died 10 mo after diagnosis.

DISCUSSION

Gastric adenocarcinoma is primarily a disease that impacts older individuals, and is generally rare in individuals under the age of 30 years, and even rarer in the children[6-8]. The presentation and biologic behavior of primary gastric adenocarcinoma in children are similar to those seen in adults. However, the etiology of pediatric gastric cancer is more unclear and may be associated with gene mutations[9], the incidence is very rare, the management is not well-established, and is associated with very poor outcome. In a review of the related literature, Sasaki *et al*[3] previously reported 22 cases of primary gastric adenocarcinoma under the age of 21 years, and Lu *et al*[8] recently reported 16 cases

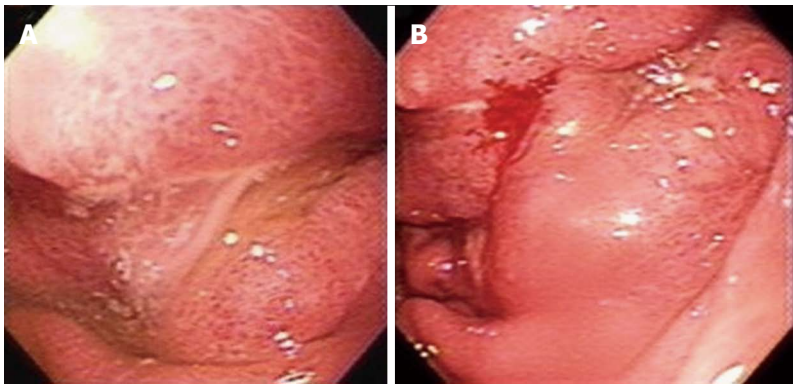


Figure 2 Endoscopy of our patient revealed blizzard morphology and nodular appearance on gastric mucosa (A), and with antral ulcers (B). Biopsy was performed on the margin of the ulcers.

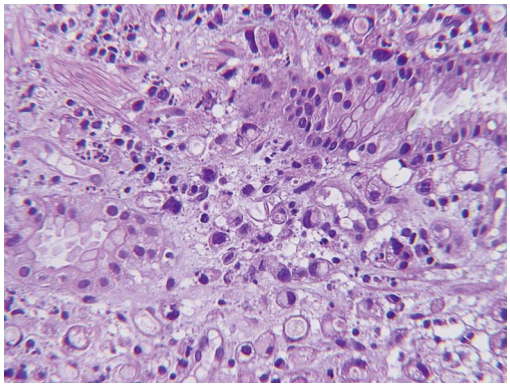


Figure 3 Histologic analysis of the specimen by the endoscopic gastric mucosal biopsy shows round-oval neoplastic cells arranged in abortive glandular pattern or individually over the lamina propria and signet-ring tumor cells are identified. Hematoxylin and eosin staining, magnification × 400.

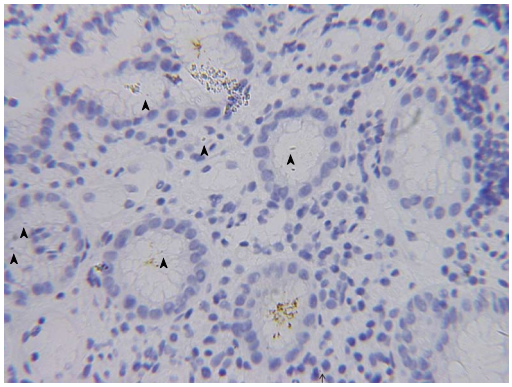


Figure 4 *Helicobacter bacilli* can be identified on Warthin-Starry stains (× 400), as shown by arrowheads.

with gastric cancer under the age of 18 years[8]. Of these 39 cases, only 2 patients survived over 2 years (102 mo and 30 mo, respectively), which demonstrated that primary gastric adenocarcinoma during childhood is strongly associated with a very poor clinical outcome as compared to adults. This is possibly because of its rarity and nonspecific presentation results in a failure to consider potential malignancy and thus contributes to a delay in diagnosis, and as a result, few cases of appropriate clinical treatment.

The most common presentations in pediatric gastric adenocarcinoma are abdominal pain and vomiting, which may mimic other disease of acute abdomen[10,11]. Others symptoms include, hematemesis, melena and weight loss. In general, pediatric physician would not routinely do more invasive examination technique on pediatric patient with nonspecific presentation. Therefore, early gastric cancer is rare on children, and almost pediatric gastric adenocarcinoma was diagnosed as terminal stage, which quite different from adult. Our patient complained of abdominal fullness for one week, and massive ascites were found by sonography. The major causes of ascites in pediatric patients are related to diseases of the liver and kidney. However, ascites can also result from heart disease, malignancy, peritonitis, eosinophilic gastroenteritis and pancreatitis. The upper gastrointestinal endoscopy was performed on our patient because of gastric mucosa thickness with caking omentum seen by CT, and a pathologic result of gastric adenocarcinoma was proven by biopsy. Although ascites due to peritoneal tumor seeding and metastasis are well-known symptoms in adult patients with gastric cancer, they are not frequently encountered in children. According Lu *et al*[8] in a review of 16 cases of pediatric gastric adenocarcinoma, only one patient presented with abdominal fullness. In this case, the importance of gastroscopy for prompt diagnosis in pediatric patients with ascites is emphasized[2,12,13].

The most important risk factor of pediatric adenocarcinoma is *H. pylori* infection, which can cause chronic active inflammation in the gastric mucosa**;** and furthermore, gastric atrophy can develop predominantly in the antrum[1,4,14]. Pediatric patient got *H. pylori* infection at a very early age has been related to a much higher gastric cancer risk, especially in the setting of a positive family history of gastric cancer[15,16]. Other risk factors such high intake of salt, smoked food, nitrates and carbohydrates, alcohol consumption, smoking, blood groups, and cancer family history are associated with gastric adenocarcinoma in adults, but its role in children is unknown[4]. Taken together, several risk factors for tumor development were present in our patient, including

his grandfather dying of gastric cancer, having a history of smoking for one year, and *H. pylori* infection proven by biopsy. Despite the patient having *H. pylori* infection, we can suspect the gastric adencarcinoma may be related to an organism infection.

Because gastric adenocarcinoma rarely affects children, the management of this disease in children is not well-established, and must be based on the principles used in adults for the time being. Radical gastrectomy with extended lymph node dissection is the only curative management in patients with localized gastric adenocarcinoma; however, recurrence within 2 years is still quite common[1,4,16,17]. Preoperative chemoradiation or post-operative adjuvant chemoradiation is commonly practiced and has been shown to improve survival[18]. In the patients with unresectable tumors, such as our patient who had extensive metastatic disease, palliative chemotherapy, such as 5-fluorouracil, leucovorin, adriamycin, cisplatin, etoposide, and epirubicin-containing protocols can be effective when attempting to control the symptoms, and may provide some limited improvement in terms of survival rates.

In conclusion, although gastric adenocarcinoma is rare in children, it should be suspected in a child with gastric ulcers and massive ascites. Upper gastrointestinal endoscopy and endoscopic biopsies are crucial in children with vague gastrointestinal symptoms and massive ascites in whom CT fails to demonstrate the primary site of the malignancy.

COMMENTS

Case characteristics

The main symptom is abdominal fullness and poor appetite for 7 d.

Clinical diagnosis

The physical sign of the patient was massive ascites; upon physical examination show distended abdominal wall with dull on percussion, and epigastric local tenderness. Neck lymph nodes were enlarged on level Ⅴ.

Differential diagnosis

The differential diagnosis of massive ascites included heart disease, malignancy, peritonitis, eosinophilic gastroenteritis and pancreatitis.

Laboratory diagnosis

Laboratory investigations revealed normal results except for mild leukocytosis (11.19 × 1000/μL, normal range: 3.99-10.39 × 1000/μL) with predominant neutrophil (80.4%, normal range: 40%-74%) and decreased lactate dehydrogenase (LDH) level (90 U/L, normal range: 98-192 U/L). The level of tumor marker was significantly increased in CA125 (109.1 U/mL, normal range: < 35 U/mL) but was normal in CEA (0.51 ng/mL, normal range: < 5 ng/mL) and CA199 (6.8 U/mL, normal range: < 35 U/mL).

Imaging diagnosis

Abdominal computed tomography (CT) scan revealed massive ascites, gastric mucosa thickness, and caking omentum. The gastroscopy revealed a large bizarre gastric ulcer (A2) (4 cm × 4 cm in size) which appeared as a snake skin with multiple nodular appearance over peri-antrum area.

Pathological diagnosis

Histological examination revealed poorly differentiated adenocarcinoma with the presence of signet ring cells was observed. *Helicobacter pylori* were also detected in the patient's biopsy tissue specimens.

Treatment

No local radiotherapy or surgery was planned because of extensive metastatic disease; the patient was placed on steroid as dexamethasone phosphate and a chemotherapy regimen of oxaliplatin and capecitabine.

Related reports

Very few cases of pediatric gastric adenocarcinoma have been reported in the literature. The clinical and pathological characteristics of pediatric gastric adenocarcinoma remain unclear and the treatment is controversial.

Term explanation

Gastric adenocarcinoma, is a rare disease in children that primarily affects adults.

Experiences and lessons

This case report presents the clinical characteristics of gastric adenocarcinoma and also discusses the diagnosis of gastric adenocarcinoma. The authors recommend that upper gastrointestinal endoscopy and endoscopic biopsies are crucial in children with vague gastrointestinal symptoms and massive ascites in whom CT fails to demonstrate the primary site of the malignancy.

Peer-review

The authors have described a case of gastric adenocarcinoma presenting with initial presentation of massive ascites. The article highlights the rare clinical characteristics of this tumor and provides insights into the diagnostic implications.

REFERENCES

1 **Harting MT**, Blakely ML, Herzog CE, Lally KP, Ajani JA, Andrassy RJ. Treatment issues in pediatric gastric adenocarcinoma. *J Pediatr Surg* 2004; **39**: e8-e10 [PMID: 15300556 DOI: 10.1016/j.jpedsurg.2004.04.043]

2 **Milne AN**, Carneiro F, O'Morain C, Offerhaus GJ. Nature meets nurture: molecular genetics of gastric cancer. *Hum Genet* 2009; **126**: 615-628 [PMID: 19657673 DOI: 10.1007/s00439-009-0722-x]

3 **Sasaki H**, Sasano H, Ohi R, Imaizumi M, Shineha R, Nakamura M, Shibuya D, Hayashi Y. Adenocarcinoma at the esophageal gastric junction arising in an 11-year-old girl. *Pathol Int* 1999; **49**: 1109-1113 [PMID: 10632934 DOI: 10.1046/j.1440-1827.1999.00993.x]

4 **Subbiah V**, Varadhachary G, Herzog CE, Huh WW. Gastric adenocarcinoma in children and adolescents. *Pediatr Blood Cancer* 2011; **57**: 524-527 [PMID: 21744476 DOI: 10.1002/pbc.23051]

5 **Schwartz MG**, Sgaglione NA. Gastric carcinoma in the young: overview of the literature. *Mt Sinai J Med* 1984; **51**: 720-723 [PMID: 6335567]

6 **Strobel CT**, Smith LE, Euler AR. Primary gastric adenocarcinoma in the pediatric population. *J Pediatr* 1978; **92**: 850-851 [PMID: 641643 DOI: 10.1016/s0022-3476(78)80177-2]

7 **Goldthorn JF**, Canizaro PC. Gastrointestinal malignancies in infancy, childhood, and adolescence. *Surg Clin North Am* 1986; **66**: 845-861 [PMID: 3738705 DOI: 10.1016/s0022-3468(87)80307-x]

8 **Lu J**, Huang CM, Zheng CH, Li P, Xie JW, Wang JB, Lin JX. [Gastric carcinoma in a 12-year-old girl: a case report and literature review]. *Zhonghua Weichang Waike Zazhi* 2012; **15**: 967-970 [PMID: 22990936]

9 **Chang VY**, Federman N, Martinez-Agosto J, Tatishchev SF, Nelson SF. Whole exome sequencing of pediatric gastric adenocarcinoma reveals an atypical presentation of Li-Fraumeni syndrome. *Pediatr Blood Cancer* 2013; **60**: 570-574 [PMID: 23015295 DOI: 10.1002/pbc.24316]

10 **Wu HP**, Yang WC, Wu KH, Chen CY, Fu YC. Diagnosing appendicitis at different time points in children with right lower quadrant pain: comparison between Pediatric Appendicitis Score and the Alvarado score. *World J Surg* 2012; **36**: 216-221 [PMID: 22009520 DOI: 10.1007/s00268-011-1310-5]

11 **Lee YT**, Ng EK, Hung LC, Chung SC, Ching JY, Chan WY, Chu WC, Sung JJ. Accuracy of endoscopic ultrasonography in diagnosing ascites and predicting peritoneal metastases in gastric cancer patients. *Gut* 2005; **54**: 1541-1545 [PMID: 15955787 DOI: 10.1136/gut.2004.055772]

12 **Aydoğan A**, Corapçioğlu F, Elemen EL, Tugay M, Gürbüz Y, Oncel S. A case report: gastric adenocarcinoma in childhood. *Turk J Pediatr* 2009; **51**: 489-492 [PMID: 20112606]

13 **Peek RM**, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2002; **2**: 28-37 [PMID:

11902583 DOI: 10.1038/nrc703]
14 **Kato S**, Kikuchi S, Nakajima S. When does gastric atrophy develop in Japanese children? *Helicobacter* 2008; **13**: 278-281 [PMID: 18665937 DOI: 10.1111/j.1523-5378.2008.00611.x]
15 **Brenner H**, Arndt V, Stürmer T, Stegmaier C, Ziegler H, Dhom G. Individual and joint contribution of family history and Helicobacter pylori infection to the risk of gastric carcinoma. *Cancer* 2000; **88**: 274-279 [PMID: 10640957 DOI: 10.1002/(SICI)1097-0142(20000115)]
16 **Blaser MJ**, Chyou PH, Nomura A. Age at establishment of Helicobacter pylori infection and gastric carcinoma, gastric ulcer, and duodenal ulcer risk. *Cancer Res* 1995; **55**: 562-565 [PMID: 7834625]
17 **Slotta JE**, Heine S, Kauffels A, Krenn T, Grünhage F, Wagner M, Graf N, Schilling MK, Schuld J. Gastrectomy with isoperistaltic jejunal parallel pouch in a 15-year-old adolescent boy with gastric adenocarcinoma and autosomal recessive agammaglobulinemia. *J Pediatr Surg* 2011; **46**: e21-e24 [PMID: 21929971 DOI: 10.1016/j.jpedsurg.2011.06.005]
18 **Varadhachary G**, Ajani JA. Preoperative and adjuvant therapies for upper gastrointestinal cancers. *Expert Rev Anticancer Ther* 2005; **5**: 719-725 [PMID: 16111471 DOI: 10.1586/14737140.5.4.719]

P- Reviewer: Codoner-Franch P, Qi F **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3414

World J Gastroenterol 2015 March 21; 21(11): 3414-3419
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Hepatocellular carcinoma with concomitant hepatic angiomyolipoma and cavernous hemangioma in one patient

Xiao-Wen Ge, Hai-Ying Zeng, Akesu Su-Jie, Min Du, Yuan Ji, Yun-Shan Tan, Ying-Yong Hou, Jian-Fang Xu

Xiao-Wen Ge, Hai-Ying Zeng, Akesu Su-Jie, Min Du, Yuan Ji, Yun-Shan Tan, Ying-Yong Hou, Jian-Fang Xu, Department of Pathology, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Author contributions: Ge XW and Xu JF made the pathological diagnosis of this case; Zeng HY, Su-Jie A and Du M contributed analytic tools; and Ge XW, Ji Y, Tan YS, Hou YY and Xu JF wrote the paper.

Correspondence to: Jian-Fang Xu, MD, Department of Pathology, Zhongshan Hospital, Fudan University, No. 180 Fenglin Road, Shanghai 200032, China. jianfangxu01@hotmail.com
Telephone: +86-21-64041990
Fax: +86-21-64038472

Received: August 8, 2014
Peer-review started: August 8, 2014
First decision: September 27, 2014
Revised: October 27, 2014
Accepted: December 1, 2014
Article in press: December 1, 2014
Published online: March 21, 2015

Abstract

The risk of developing hepatocellular carcinoma (HCC) is strongly associated with hepatitis B virus infection. Hepatic angiomyolipoma (AML), a rare benign tumor, is composed of a heterogeneous mixture of adipose cells, smooth muscle cells and blood vessels. Here, we report the case of a 44-year-old man who developed HCC with a concomitant hepatic AML and a cavernous hemangioma, in the absence of cirrhosis. To our knowledge, based on an extensive literature search using the www.pubmed.gov website, this is the first report of an HCC case with both concomitant AML and cavernous hemangioma at the same position in the liver. The presence of the hepatitis B surface antigen was detected, but the liver function was normal. Clinical and pathological data were collected before and during the treatment. Hepatic AML was diagnosed based on the typical histological characteristics and immunohistochemical staining, which revealed, a positive staining with a melanocytic cell-specific monoclonal antibody. There was no evidence of tuberous sclerosis complex in this patient. Although the HCC was poor- to moderately-differentiated, the characteristics of the AML and the cavernous hemangioma in this patient did not match any criteria for malignancy. Hepatectomy followed by transarterial chemoembolization treatment were effective therapeutic methods for the adjacent lesions in this patient. This case is an interesting coincidence.

Key words: Angiomyolipoma; Hepatocellular carcinoma; Cavernous hemangioma; Hepatitis B virus infection; Concomitant

Core tip: Hepatic angiomyolipoma (AML) is a benign tumor which is composed of a heterogeneous mixture of adipose cells, smooth muscle cells and blood vessels. Here, we report the case of a 44-year-old man who developed Hepatocellular carcinoma (HCC) with concomitant hepatic AML and cavernous hemangioma, in the absence of cirrhosis. To our knowledge, based on an extensive literature search using the PubMed, this is the first report of an HCC case with both concomitant AML and cavernous hemangioma in the liver. The characteristics of the AML and cavernous hemangioma in this patient did not match any criteria for malignancy.

Ge XW, Zeng HY, Su-Jie A, Du M, Ji Y, Tan YS, Hou YY, Xu JF. Hepatocellular carcinoma with concomitant hepatic angiomyolipoma and cavernous hemangioma in one patient.

World J Gastroenterol 2015; 21(11): 3414-3419 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3414.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3414

INTRODUCTION

Hepatocellular carcinoma (HCC), the most common malignancy of the liver[1], ranks as the 5th most common cancer and the 3rd most common cause of cancer-related mortality worldwide. HCC may occur as a consequence of every pathologic condition inducing chronic liver damage. Indeed, cirrhosis is largely recognized to be a nodal step in hepatocarcinogenesis. The risk of developing HCC is strongly increased when hepatitis B virus (HBV) is involved[2].

Angiomyolipoma (AML), a rare benign tumor, is composed of a heterogeneous mixture of adipose cells, smooth muscle cells and blood vessels. AML occurs most commonly in the kidneys. The liver represents the second most frequent site of AML development[3,4]. Spontaneous hemorrhage or malignant transformation can occur during the progression of AML[5-7]. Because the radiological features of AML depend on the relative proportion of adipose cells in the tumor, AML is not easy to distinguish from HCC[8,9], especially when the tumor is small. The relationship between HBV infection and the occurrence of hepatic AML is still unclear.

We recently encountered a patient with hepatitis B surface antigen (HBsAg) expression who also presented with simultaneous HCC, hepatic AML and cavernous hemangioma in liver. To our knowledge, no case of HCC with concomitant hepatic AML and cavernous hemangioma has been reported to date in the scientific literature. Here, we present the histological features of the tumors from this patient and discuss the diagnosis and its practical significance.

CASE REPORT

In December 2012, a 44-year-old Chinese man was admitted to the hospital for intrahepatic nodules. The nodules were found incidentally on an abdominal computerized tomography (CT) scan performed as part of a general health examination. Laboratory analyses showed normal liver function, and the test for HBsAg expression was positive (+288.6 COI). The viral load was 1.16×10^3 IU/mL, and the alanine aminotransferase (ALT) level was 45 IU/L (normal value is 9-50 IU/L). The serum concentration of alpha-fetoprotein (AFP) was 4.1 μg/mL (normal value is < 20 μg/mL). The results of the routine urine test and the renal function were normal.

Abdominal ultrasonography (US) revealed a well-defined hypoechoic mass, measuring 8.8 cm × 7.8 cm, located in the right hepatic lobe (Figure 1A). Color Doppler sonography showed a filiform vascular distribution pattern (Figure 1B). Also in the right hepatic lobe, enhanced CT showed a hepatic mass measuring 9.6 cm × 8.0 cm and presenting an early-phase hypoattenuation and late-phase hypoattenuation. The morphological aspects of the liver tumor mass observed on the CT scan and on the US were typical of HCC. However, there is no pathognomonic clinical sign for tuberous sclerosis. After informed consent was obtained, the patient requested surgical treatment. Resection of partial right hepatic lobe containing the tumors was performed.

Upon macroscopic examination, the tumor was displayed an elastic consistency and presented a diameter of 9.0 cm. The cut surface of the tumor was grayish-white and grayish-green. The tumor was encapsulated and clearly demarcated from the normal hepatic parenchyma. Scarring and broad fibrous septa were observed (Figure 2A). There was no sign of fibrosis in the liver tissue surrounding the tumor. Underneath and adjacent to the tumor capsule, we identified a small yellowish soft area measuring 1.0 cm in diameter. There was a white nodule in the middle of the yellowish area with a diameter of 0.3 cm (Figure 2B).

The 9.0 cm tumor was histopathologically diagnosed as HCC, with a poor- to moderate-differentiation level (Figure 3A). A vascular tumor embolus was identified outside of the HCC mass (Figure 3B). The yellowish area was mainly composed of smooth muscle cells, adipose cells and blood vessels. Therefore, the three defining characteristics of AML were present. Nuclear pleomorphism was absent, and mitotic figures were rarely observed. The white nodule measuring 0.3 cm and located in the middle of the AML tumor was also an HCC nodule. This small nodule, which presented similar components as the 9.0 cm HCC tumor, may represent a disseminated nodule originating from the bigger tumor mass (Figure 3C, D). Adjacent to the edge of the 9.0 cm HCC tumor, a small typical cavernous hemangioma lesion was found (Figure 3E). *Via* immunohistochemical stainings, the AML area was found negative for Desmin and CD34 expression, but positive for HMB45 (melanocytic cell-specific monoclonal antibody) and A103 (Melan-A) expression (Figure 4A-D).

One month after the hepatectomy, the patient requested a transarterial chemoembolization (TACE) treatment. Although the tumors were completely removed by local resection of right hepatic lobe, there were vascular tumor embolus identified outside of the HCC mass, which was a potential recurrence factor. A dose of 750 mg of FuDR was used in the TACE treatment in order to prevent recurrence of the HCC. A dosage of 100 mg QD of Lamivudine was administered as antiviral Therapy. The post-TACE course was uneventful, and so far, there has been no evidence of postoperative recurrence or metastasis.

DISCUSSION

Cases describing HCC with a concomitant hepatic

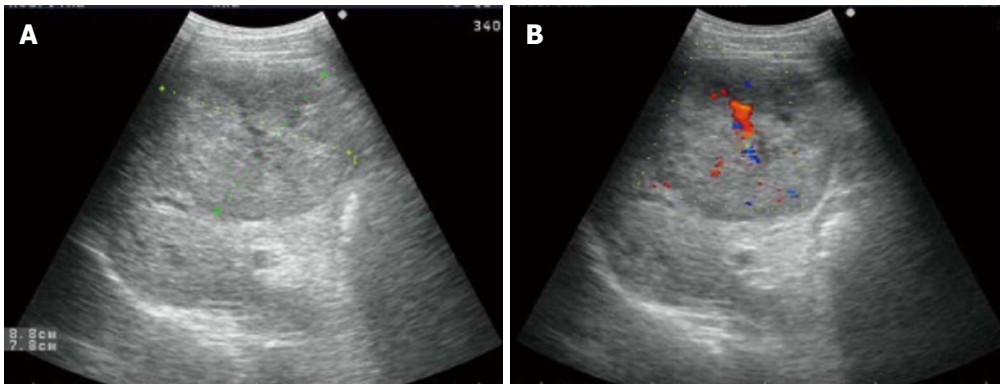


Figure 1 Tumor was hypoechoic on ultrasonography. A: Measuring 8.8 cm × 7.8 cm well-defined hypoechoic mass; B: Color Doppler sonography showing a filiform vascular distribution pattern.

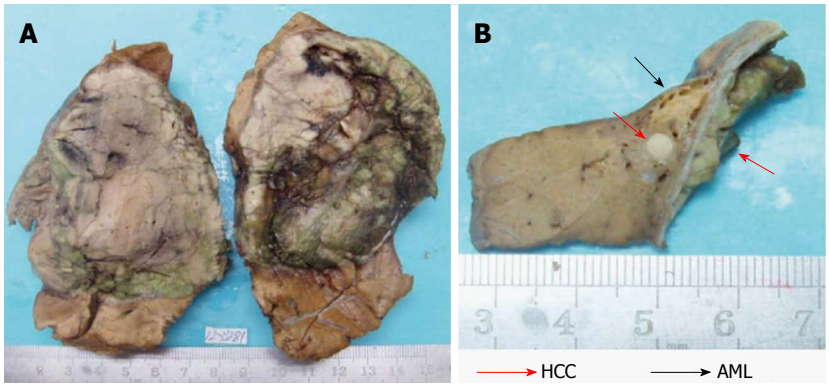


Figure 2 Macroscopical features of the tumors in this case. A: The 10% neutral buffered formalin-fixed tumor occupied a large area of the right lobe. The encapsulated tumor was grayish-white and grayish-green. Scarring and broad fibrous septa was observed; B: Underneath the tumor capsule, an adjacent small yellowish soft area measuring 1.0 cm in diameter was observed, along with a small white nodule in it.

AML, or describing hepatic AML with a concomitant cavernous hemangioma are very rare[10,11]. The occurrence of HCC concurrently with both hepatic AML and cavernous hemangioma in the same patient is even less common. To our knowledge, based on a thorough literature search in PubMed (www.pubmed.gov), this is the first report of an HCC case with a concomitant AML and cavernous hemangioma located at the same position in the liver.

AML is considered as being included in the perivascular epithelioid cell tumor (PEComa) family, which was described by Bonetti *et al*[12] in 1992. PEComas are defined as mesenchymal tumors composed of histologically and immunohistochemically distinctive perivascular epithelioid cells. These tumors are consistently immunoreactive to HMB45 and A103 monoclonal antibodies, which are used as markers for melanoma. In diagnostic radiology, the imaging features of AML vary due to the differences in the proportion of adipose cells, smooth muscle cells and blood vessels. Therefore, sometimes it is difficult to differentiate hepatic AML from HCC in radiological diagnosis[13,14]. In our case, the marginal 1.0 cm AML was too small to be differentiated from the 9.0 cm HCC mass using both the US and CT scan methods. MRI is considered to be the best modality to determine the components of AML[15]. Hyper- or hypointensity on the T1-weighted image and hyperintensity on the T2-weighted image are observed depending on the component of tumor tissue[16,17]. Contrast-enhanced US (CEUS) was reported as an effective diagnostic tool for AML[17,18]. Li *et al*[18] reported that CEUS showed an inhomogeneous hyperenhancing pattern in the arterial phase and prolonged enhancement during the portal and Kupffer phases of AML. However, MRI and CEUS were not performed on this patient. Occasionally, the AML is part of a systemic disease called tuberous sclerosis complex (TSC). TSC is an autosomal dominant disorder involving multiple organs[19,20].In the setting of TSC, hepatic AML is often accompanied by bilateral renal AML. However, in this case, we found no evidence of TSC.

Cavernous hemangioma is the most common benign primary liver tumor. In most cases, the size of the cavernous hemangioma is stable[21]. Most hepatic cavernous hemangiomas can be diagnosed accurately from the characteristic imaging features identified on CT or MRI. However, in instances such as ours, where the cavernous hemangioma is located underneath the hepatic capsule, adjacent to the HCC[22], the hemangioma is occasionally too small to distinguish from the adjacent HCC.

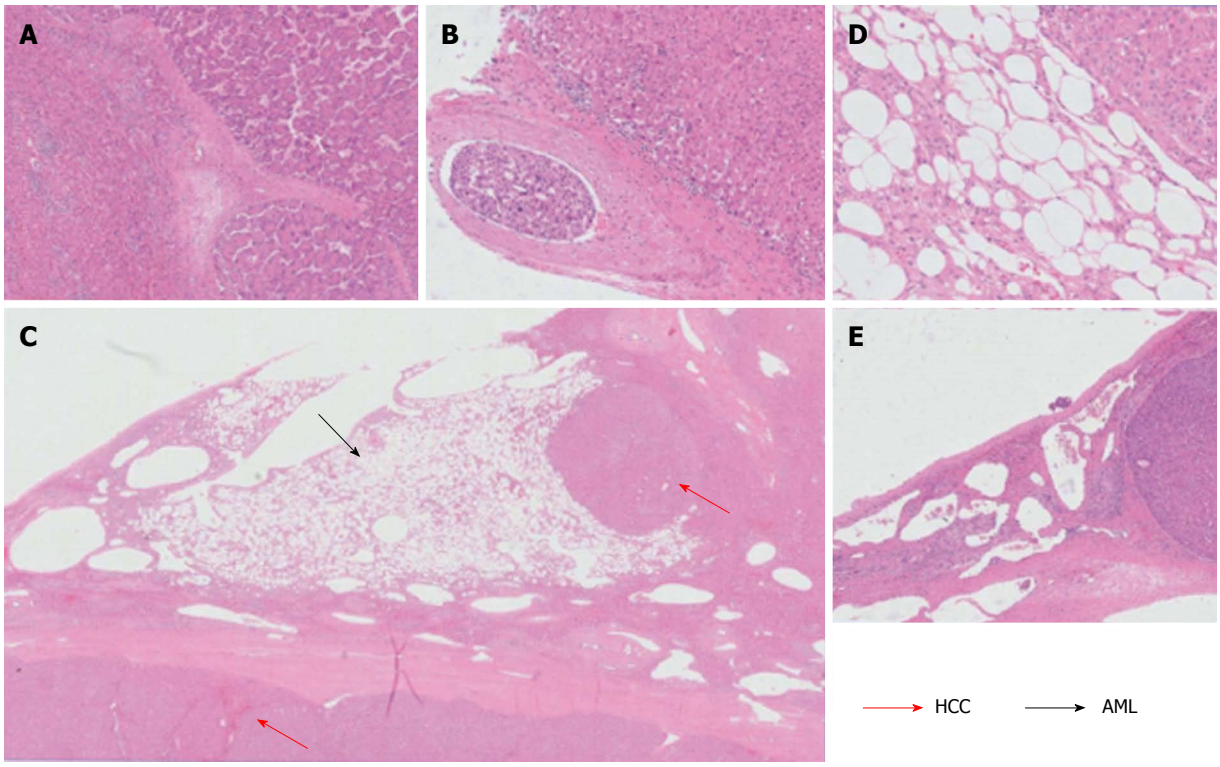


Figure 3 Histological features of the tumors in this case. A: The HCC, showing a poor- to moderately-differentiation level, was demarcated from the surrounding liver tissue with a relatively clear boundary (HE staining, magnification × 50); B: A tumor emboli of HCC was found in a blood vessel located outside of the HCC mass (HE staining, magnification × 200); C and D: The AML component was composed of smooth muscle cells, adipose cells and blood vessels. The 0.3 cm white nodule in the middle of the AML was an HCC nodule with the same composition as the 9.0 cm HCC mass (C: HE staining, magnification × 10; D: HE staining, magnification × 200); E: The cavernous hemangioma areas were composed of large thin-walled vascular spaces, lined by a monolayer flat endothelial cells (HE staining, magnification × 50). HCC: Hepatocellular carcinoma; AML: Angiomyolipoma; HE: Hematoxylin and eosin.

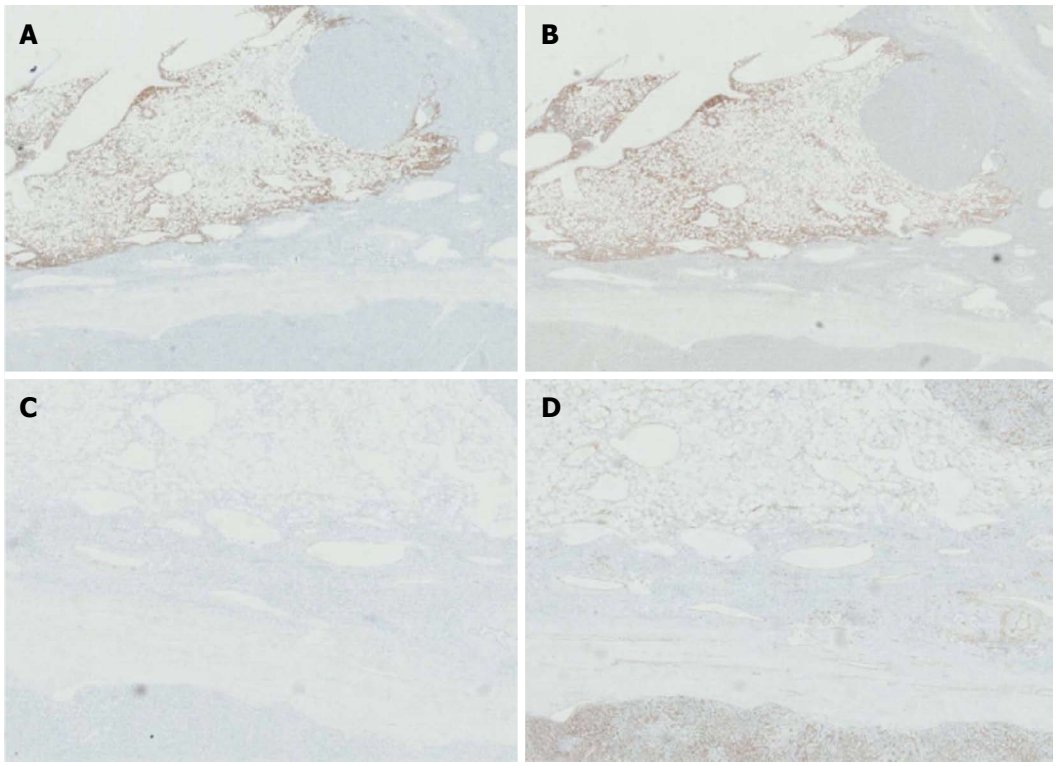


Figure 4 Immunohistochemical stainings. A and B: The AML cells were positive for HMB45 (magnification × 10) and for A103 staining (magnification × 10); C and D: The AML cells were negative for Desmin (magnification × 20) and for CD34 staining (magnification × 20). AML: Angiomyolipoma.

The origin of hepatic AML remains unclear, although recent studies have shown that AML originates from perivascular epithelioid cells. These cells can multi-directionally differentiate into vascular smooth muscle and epithelial cells, and are characterized by the expression of differentiation-associated markers of melanoma cell[6]. However, the origin of cavernous hemangioma is totally different. Hepatic AML most often occurs in females, although this patient was a male with HBV infection. Until now, there is no direct evidence revealing the relation between HBV and AML. Likewise, the correlation between HBV and cavernous hemangioma is also unclear. In HCC, in addition to the long-lasting hepatitis produced by a chronic infection, the HBV may exert a direct oncogenic role through several different mechanisms, including viral DNA integration into the host genome and production of proteins with pro-oncogenic properties[1,2]. HBV DNA integration occurs randomly in the context of the human genome and may involve multiple sites located on different chromosomes[2,23,24]. In cases similar to this one, the HBV may promote liver cell transformation and cancer progression even independently of cirrhosis development[1,2]. In the present case, it is difficult to tell if the origin of the AML and the cavernous hemangioma was induced by HBV DNA integration. Nevertheless, the characteristics of AML and the cavernous hemangioma observed in this case did not match any of the criteria for malignancy, such as necrosis, infiltrative growth patterns, large size, and high mitotic index.

Most cases of hepatic AML and cavernous hemangioma have a good prognosis regardless of the management strategy[25], but hepatocellular carcinoma should be removed surgically. We report the extremely rare case of an HCC tumor accompanied with a concomitant hepatic AML and a hepatic cavernous hemangioma occurring in adjacent positions. Nevertheless, in this case, which may be a pure coincidence, the patient benefited from surgical resection.

COMMENTS

Case characteristics

A 44-year-old man who developed hepatocellular carcinoma (HCC) with concomitant hepatic angiomyolipoma (AML) and cavernous hemangioma.

Clinical diagnosis

Intrahepatic nodules were found incidentally on an abdominal computerized tomography (CT) scan performed as part of a general health examination.

Differential diagnosis

Hepatocellular carcinoma, angiomyolipoma, cavernous hemangioma.

Laboratory diagnosis

HBsAg +288.6 COI; HBV load 1.16 × 10^3 IU/mL; AFP 4.1 μg/mL. Liver function test were within normal limits.

Imaging diagnosis

Abdominal ultrasonography revealed a well-defined hypoechoic mass measuring 8.8 cm × 7.8 cm located in the right hepatic lobe and enhanced CT showed a hepatic mass measuring 9.6 cm × 8.0 cm, presenting an early-phase hyperattenuation and late-phase hypoattenuation.

Pathological diagnosis

The 9.0 cm tumor was histopathologically diagnosed as HCC and adjacent AML and cavernous hemangioma were found located at the same position in the liver. A small HCC nodule located in the middle of the AML tumor. The AML area was found negative for Desmin and CD34 expression but positive for HMB45 and A103 expression.

Treatment

Resection of the right hepatic lobe containing the tumors was performed and one month after the hepatectomy, a transarterial chemoembolization (TACE) treatment was performed.

Related reports

Very few cases of HCC with concomitant hepatic AML and a few cases of HCC with concomitant cavernous hemangioma have been reported in the literature. The origin of HCC with concomitant hepatic AML remains unclear.

Term explanation

AML is considered as being included in the perivascular epithelioid cell tumor family, which is composed of a heterogeneous mixture of adipose cells, smooth muscle cells and blood vessels.

Experiences and lessons

This case report presents the clinical characteristics of HCC with a concomitant hepatic AML and a cavernous hemangioma in the absence of cirrhosis. Hepatectomy followed by TACE treatment were effective therapeutic methods for the adjacent lesions in this patient.

Peer-review

Well written case report given information about a rare tumour combination that could be only a mere coincidence, but it could be worth it to be published to draw the attention for future research to disclose the cause of this association in case more cases are described.

REFERENCES

1 **Bréchot C**. Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. *Gastroenterology* 2004; **127**: S56-S61 [PMID: 15508104]

2 **Pollicino T**, Saitta C, Raimondo G. Hepatocellular carcinoma: the point of view of the hepatitis B virus. *Carcinogenesis* 2011; **32**: 1122-1132 [PMID: 21665892 DOI: 10.1093/carcin/bgr108]

3 **Petrolla AA**, Xin W. Hepatic angiomyolipoma. *Arch Pathol Lab Med* 2008; **132**: 1679-1682 [PMID: 18834230]

4 **Vagefi PA**, Eilers H, Hiniker A, Freise CE. Liver transplantation for giant hepatic angiomyolipoma. *Liver Transpl* 2011; **17**: 985-986 [PMID: 21462294 DOI: 10.1002/lt.22310]

5 **Nonomura A**, Mizukami Y, Kadoya M. Angiomyolipoma of the liver: a collective review. *J Gastroenterol* 1994; **29**: 95-105 [PMID: 8199705]

6 **Yang CY**, Ho MC, Jeng YM, Hu RH, Wu YM, Lee PH. Management of hepatic angiomyolipoma. *J Gastrointest Surg* 2007; **11**: 452-457 [PMID: 17436129 DOI: 10.1007/s11605-006-0037-3]

7 **Dalle I**, Sciot R, de Vos R, Aerts R, van Damme B, Desmet V, Roskams T. Malignant angiomyolipoma of the liver: a hitherto unreported variant. *Histopathology* 2000; **36**: 443-450 [PMID: 10792486]

8 **Agaimy A**, Vassos N, Croner RS, Strobel D, Lell M. Hepatic angiomyolipoma: a series of six cases with emphasis on pathological-radiological correlations and unusual variants diagnosed by core needle biopsy. *Int J Clin Exp Pathol* 2012; **5**: 512-521 [PMID: 22949933]

9 **Shintaku M**. Hepatic angiomyolipoma with 'oncocyte-like' features. *Histopathology* 1998; **33**: 581-583 [PMID: 9870158]

10 **Yang B**, Chen WH, Shi PZ, Xiang JJ, Xu RJ, Liu JH. Coincidence of hepatocelluar carcinoma and hepatic angiomyolipomas in tuberous sclerosis complex: a case report. *World J Gastroenterol* 2008; **14**: 812-814 [PMID: 18205279]

11 **Tani A**, Yoshida H, Mamada Y, Taniai N, Mineta S, Yoshioka M, Kawano Y, Ueda J, Naito Z, Uchida E. Hepatic angiomyolipoma with a giant hemangioma. *J Nippon Med Sch* 2011; **78**: 317-321 [PMID: 22041879]

12 **Bonetti F**, Pea M, Martignoni G, Zamboni G. PEC and sugar. *Am J Surg Pathol* 1992; **16**: 307-308 [PMID: 1599021]

13 **Takahara M**, Miyake Y, Matsumoto K, Kawai D, Kaji E, Toyokawa T, Nakatsu M, Ando M, Hirohata M. A case of hepatic

angiomyolipoma difficult to distinguish from hepatocellular carcinoma. *World J Gastroenterol* 2009; **15**: 2930-2932 [PMID: 19533821]
14 **Prasad SR**, Wang H, Rosas H, Menias CO, Narra VR, Middleton WD, Heiken JP. Fat-containing lesions of the liver: radiologic-pathologic correlation. *Radiographics* 2005; **25**: 321-331 [PMID: 15798052]
15 **Kamimura K**, Nomoto M, Aoyagi Y. Hepatic angiomyolipoma: diagnostic findings and management. *Int J Hepatol* 2012; **2012**: 410781 [PMID: 23320180 DOI: 10.1155/2012/410781]
16 **Ding GH**, Liu Y, Wu MC, Yang GS, Yang JM, Cong WM. Diagnosis and treatment of hepatic angiomyolipoma. *J Surg Oncol* 2011; **103**: 807-812 [PMID: 21283992 DOI: 10.1002/jso.21814]
17 **Krebs S**, Esposito I, Lersch C, Gaa J, Schmid RM, Ebert O. Preoperative radiological characterization of hepatic angiomyolipoma using magnetic resonance imaging and contrast-enhanced ultrasonography: a case report. *J Med Case Rep* 2011; **5**: 481 [PMID: 21943146 DOI: 10.1186/1752-1947-5-481]
18 **Li R**, Zhang X, Hua X, Cai P, Zhong H, Guo Y, Ding S, Yan X. Real-time contrast-enhanced ultrasonography of resected and immunohistochemically proven hepatic angiomyolipomas. *Abdom Imaging* 2010; **35**: 676-682 [PMID: 20020286 DOI: 10.1007/s00261-009-9592-x]
19 **Roach ES**, Gomez MR, Northrup H. Tuberous sclerosis complex consensus conference: revised clinical diagnostic criteria. *J Child Neurol* 1998; **13**: 624-628 [PMID: 9881533]
20 **Maria BL**, Deidrick KM, Roach ES, Gutmann DH. Tuberous sclerosis complex: pathogenesis, diagnosis, strategies, therapies, and future research directions. *J Child Neurol* 2004; **19**: 632-642 [PMID: 15563008]
21 **Yamagata M**, Kanematsu T, Matsumata T, Utsunomiya T, Ikeda Y, Sugimachi K. Management of haemangioma of the liver: comparison of results between surgery and observation. *Br J Surg* 1991; **78**: 1223-1225 [PMID: 1958991]
22 **Karatzas T**, Smirnis A, Dimitroulis D, Patsouras D, Evaggelou K, Kykalos S, Kouraklis G. Giant pedunculated hepatocellular carcinoma with hemangioma mimicking intestinal obstruction. *BMC Gastroenterol* 2011; **11**: 99 [PMID: 21939543 DOI: 10.1186/1471-230X-11-99]
23 **Pollicino T**, Vegetti A, Saitta C, Ferrara F, Corradini E, Raffa G, Pietrangelo A, Raimondo G. Hepatitis B virus DNA integration in tumour tissue of a non-cirrhotic HFE-haemochromatosis patient with hepatocellular carcinoma. *J Hepatol* 2013; **58**: 190-193 [PMID: 22989571 DOI: 10.1016/j.jhep.2012.09.005]
24 **Bonilla Guerrero R**, Roberts LR. The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. *J Hepatol* 2005; **42**: 760-777 [PMID: 15826727 DOI: 10.1016/j.jhep.2005.02.005]
25 **Yeh CN**, Chen MF, Hung CF, Chen TC, Chao TC. Angiomyolipoma of the liver. *J Surg Oncol* 2001; **77**: 195-200 [PMID: 11455557 DOI: 10.1002/jso.1094]

P- Reviewer: Christodoulidis G, Hashimoto N, Larrubia JR
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3420

World J Gastroenterol 2015 March 21; 21(11): 3420-3424
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Partial splenectomy using a laparoscopic bipolar radiofrequency device: A case report

Wei-Dong Wang, Jie Lin, Zhi-Qiang Wu, Qing-Bo Liu, Jing Ma, Xiao-Wu Chen

Wei-Dong Wang, Jie Lin, Zhi-Qiang Wu, Qing-Bo Liu, Jing Ma, Xiao-Wu Chen, Second Department of General Surgery, the First People's Hospital of Shunde, Shunde 528300, Guangdong Province, China

Author contributions: Wang WD, Lin J, Wu ZQ, Liu QB and Chen XW participated in the study design and performance; Ma J collected the clinical data.

Supported by grants from First People's Hospital of Shunde, Guangdong Province, China.

Ethics approval: The study was reviewed and approved by the Institutional Review Board of The First People's Hospital of Shunde.

Informed consent: The study participant provided informed written consent prior to study enrollment.

Conflict-of-interest: No potential conflicts of interest relevant to this article were reported.

Correspondence to: Xiao-Wu Chen, MD, PhD, Second Department of General Surgery, the First People's Hospital of Shun De, No. 1 Penglai Road, Shunde 528300, Guangdong Province, China. sdchenxiaowu1968@163.com
Telephone: +86-757-22318705
Fax: +86-757 -22223899

Received: September 25, 2014
Peer-review started: September 28, 2014
First decision: October 29, 2014
Revised: November 25, 2014
Accepted: December 16, 2014
Article in press: December 16, 2014
Published online: March 21, 2015

Abstract

We report a 51-year-old female patient with a solitary lymphangioma located in the upper splenic pole which was managed successfully with laparoscopic partial splenectomy. Surgery lasted 170 min and did not require blood transfusions. The patient recovered well post-operatively and was asymptomatic at the 3-mo follow-up. She had a normal platelet count and no recurrence on ultrasonography or computed tomography. Laparoscopic partial splenectomy is a safe, minimally invasive technique for the treatment of solitary splenic lymphangiomas in the splenic pole. We performed the procedure using the Habib™ 4X device. This laparoscopic bipolar radiofrequency device ensured a "bloodless" splenic parenchymal resection.

Key words: Laparoscopic partial splenectomy; Splenic lymphangioma; Laparoscopic bipolar radiofrequency device; Habib™ 4X

Core tip: We report the first case of a solitary splenic lymphangioma managed successfully by laparoscopic partial splenectomy using Habib™ 4X, a laparoscopic bipolar radiofrequency device, which allowed for a "bloodless" splenic parenchymal resection.

Wang WD, Lin J, Wu ZQ, Liu QB, Ma J, Chen XW. Partial splenectomy using a laparoscopic bipolar radiofrequency device: A case report. *World J Gastroenterol* 2015; 21(11): 3420-3424 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3420.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3420

INTRODUCTION

Primary splenic tumors are relatively uncommon, but are occasionally found during routine physical examinations or elective abdominal imaging studies, with no symptoms[1]. Splenic lymphangiomas are

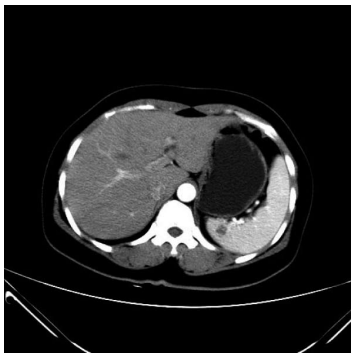


Figure 1 Enhancing computed tomography confirmed a hypo-dense ellipse occupying the upper pole of the spleen.

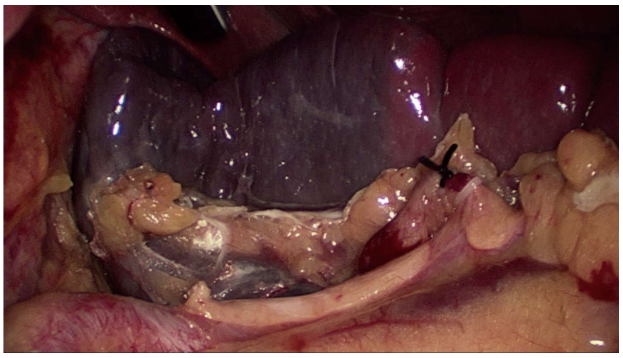


Figure 2 After ligating the upper branches of the splenic artery, an ischemic demarcation line on the splenic surface is pronounced.

rare, benign, primary splenic tumors resulting from congenital malformations of the lymphatic system, which are mainly found in children, but occasionally in adults[2]. Laparoscopic total splenectomy was traditionally recommended for the treatment of splenic neoplasms. However, with the better understanding of the importance of the splenic immune functions in both children and adults, changes have taken place regarding the surgical management and approach to splenic lesions. Laparoscopic partial splenectomy is minimally invasive and has become an elective procedure for preserving the splenic immune functions[3]. But the procedure is technically difficult. We herein report a rare case of a solitary splenic lymphangioma located at the upper pole of the spleen which was successfully managed by laparoscopic partial splenectomy using a Habib™ 4X laparoscopic bipolar radiofrequency device.

CASE REPORT

A 51-year-old female patient presented with a 2-year history of left upper abdominal discomfort. She was admitted to the Department of General Surgery at The First People's Hospital of Shunde in December 12, 2013. The patient had previously undergone a hysterectomy *via* laparotomy because of hysteromyoma. She had no history of trauma, no weight loss, and no family history of cancer. Physical examination yielded no remarkable findings. Laboratory tests were normal. Gastroscopy revealed chronic superficial gastritis and enteroscopy detected no abnormalities. Ultrasonography showed a mildly hyperechoic splenic mass with no blood flow on Doppler images and with a clear boundary. A hemangioma was diagnosed based on the ultrasonographic findings. A computed tomography (CT) scan revealed the size of spleen to be approximately 8.9 cm × 7.9 cm × 3.5 cm and confirmed a hypo-dense ellipse mass, measuring 1.5 cm × 1.0 cm, occupying the upper pole of the spleen (Figure 1). The mass was differentiated from an angioma and a metastatic tumor. Magnetic resonance imaging (MRI) showed a single, well-defined, heterogeneous ellipse with high T2 and low T1 signals in the spleen with no centripetal fill-in of contrast on delayed images. MRI findings were suggestive of a splenic cyst.

Under general anesthesia, the patient underwent a four-trocar laparoscopic upper pole splenectomy in a semi-lateral position. A 10-mm port was placed sub-umbilically for a 30-degree telescope. Two 12-mm trocars were placed at the middle point of the line between the appendix ensiformis and the umbilicus, and parallel to the umbilicus in the left anterior axillary line, respectively. A 5-mm port was placed parallel to the umbilicus in the left mid-clavicular line. The surgeon stood at the right of the patient. The first assistant, who handled the laparoscope, stood to the right of the surgeon.

The procedure began with a thorough search of the abdominal cavity. The left side of the gastrocolic ligament and the splenogastric ligament including the short gastric vessel were divided to expose the splenic vascular pedicle. The branches of the splenic artery and vein that supply the upper half of the spleen were separated and divided after ligation with a hem-o-lok vascular clip, resulting in an ischemic demarcation line (Figure 2). Laparoscopic ultrasound was used to identify the tumor location. A bipolar radiofrequency device, the Habib™ 4X (Generator 1500X, RITA Medical Systems, Inc., California, United States), was inserted into the parenchyma of the spleen along the well-defined demarcation line for coagulating and closing blood vessels (Figure 3). The radiofrequency power was set at 80 W. The coagulated spleen tissue was divided bloodlessly using an Ultracision Harmonic Scalpel (Ethicon Endo-Surgery Inc., Cincinnati, United States) (Figure 4). Thick ducts were clamped with the hem-o-lok vascular clip and cut off for safety. After the splenophrenic ligaments were divided, the whole upper pole of the spleen containing the tumor, was separated completely. After surgery, the specimen was placed in a bag first and then removed from an enlarged sub-umbilical port.

The duration of the operation was 170 min, and the estimated blood loss was minimal (approximately

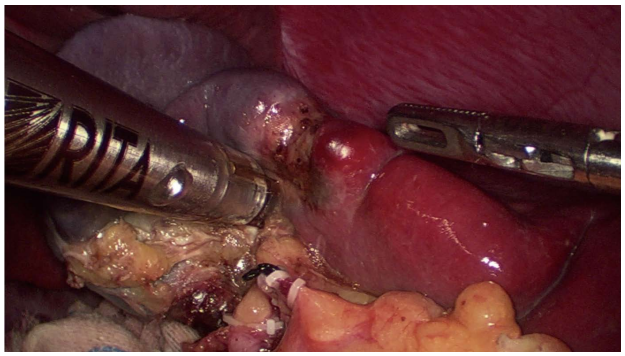


Figure 3 Habib™ 4X was inserted into the splenic parenchyma along the well-defined ischemic demarcation line for coagulating and sealing blood vessels.

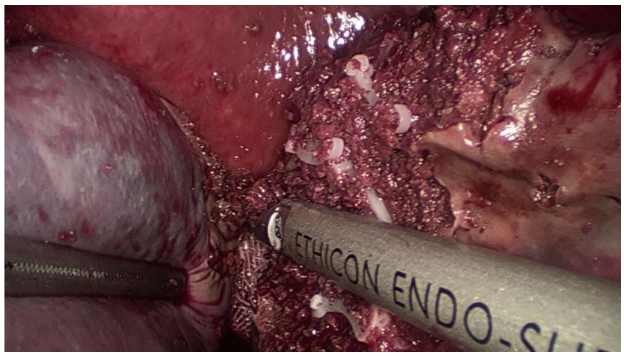


Figure 4 Splenic parenchyma was divided bloodlessly with an Ultracision Harmonic Scalpel.

30 mL). The patient was discharged on day 7 post-operatively with no complications. Histological diagnosis was splenic lymphangioma (Figure 5). At the 3-mo follow-up visit, CT scan showed that the remnant lower pole of the spleen was normal (the size of spleen was approximately 6.1 cm × 4.1 cm × 3.1 cm). The patient was asymptomatic with no recurrence and had a normal platelet count.

DISCUSSION

Splenic lymphangioma presents as a cystic, solid, or honeycomb mass that is either solitary or multifocal. The main clinical symptoms are typically associated with large cysts and include vague abdominal pain and symptoms due to compression of adjacent organs. Cystic lymphangioma is the most common type and is characterized by a honeycomb of large and small thin-walled cysts containing lymph-like clear fluid. Solid lymphangiomas have also been described with sclerotic changes and papillary endothelial proliferation[4]. Solitary splenic lymphangioma is extremely rare and appears as a cystic lesion. Imaging characteristics are hypo- or anechoic lesions detected by ultrasound, low attenuation and non-enhancing masses detected by CT, and high T2 and low T1 signal intensity on MRI[5]. Different imaging characteristics of splenic lymphangiomas, such as a solid-cystic mass, mildly increased echogenicity on ultrasound, and fill-in contrast enhancement on delayed CT images and MRI, are also described in some cases[6-8]. Nevertheless, a solitary splenic lymphangioma is difficult to differentiate from a splenic cyst/angioma or a metastatic tumor. In this report, the imaging characteristics were not compatible with the commonly published form of lymphangioma, making it difficult to diagnose preoperatively.

Currently, laparoscopic splenectomy, which leads to a good short-term outcome and is minimally invasive, is still the most widely accepted standard surgical treatment for splenic disorders. However, with the better understanding of the important role of the spleen in immune defense, changes have taken place

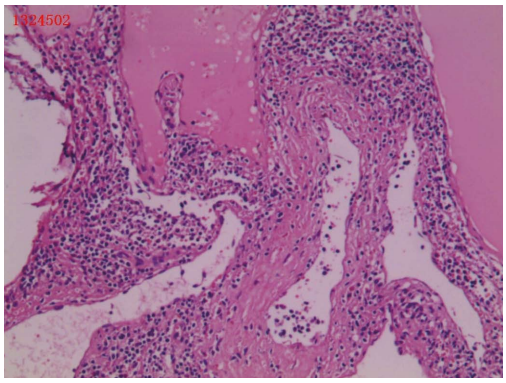


Figure 5 Pathological examination of the splenic lymphangioma. HE staining, original magnification × 40.

in the surgical strategy for splenic surgery. Several studies have indicated that total splenectomy could increase the risks of postoperative infection, secondary atherosclerosis events, pulmonary hypertension, and thrombocytic events[9,10]. Therefore, splenectomy should be avoided if possible. Partial splenectomy can preserve the immune function of the spleen if at least 25% of the normal splenic tissue by weight is preserved[11,12]. Since Uranüs *et al*[13] reported the first case of laparoscopic partial splenectomy in 1995, showing the benefits of a splenic parenchyma-preserving procedure with minimally invasive surgery, it has become an elective procedure for focal splenic disorders[3,14]. Uranues *et al*[14] reported 38 patients who underwent laparoscopic partial splenectomy with a median operating time of 110 min and with no post-operative mortality. However, laparoscopic partial splenectomy still remains a challenging procedure as bleeding from the cut edge of the spleen is difficult to control and can result in death.

In this report, the perioperative bleeding risk was limited, and there were no complications. We found that laparoscopic partial splenectomy is safe for treatment of splenic lymphangiomas located in the pole of the spleen. To succeed in this technique, the surgeon should be skilled at the procedure. The spleen has a segmental blood supply, and tearing the splenic peplos or splenic hilum often results in uncontrolled

bleeding. The surgeon must therefore move the spleen gently when dividing the splenic ligaments. Targeted segmental devascularization of the spleen plays a key role in partial splenectomy. The splenic artery is divided into two or three groups of branches that provide blood supply to the upper and lower poles and an intermediate segment. In some patients, the number of segments ranges from three to seven[15]. The surgeon should isolate and ligate the segmental vessel and make a clear ischemic demarcation line, as shown in our case, to significantly reduce bleeding from the splenic parenchymal section. An advanced laparoscopic instrument ensures a safe transsection of the splenic parenchyma. Currently, important technical advances for a safe laparoscopic partial splenectomy have been made using argon beams, ultrasound scalpels and ligature devices, staplers, or radiofrequency ablative devices[16,17]. In our case, we used a new bipolar radiofrequency device, the Habib™ 4X, which offers an ideal "bloodless" parenchymal resection in the liver[18], resulting in coagulation necrosis of the splenic tissue and sealing of blood vessels prior to transsection. To the best of our knowledge, there are no published data about using this device for a laparoscopic partial splenectomy.

In conclusion, solitary splenic lymphangiomas are extremely rare and can be easily misdiagnosed. Laparoscopic partial splenectomy offers a safe and minimally invasive technique for the treatment of solitary splenic lymphangiomas located in the pole of the spleen. The Habib™ 4X allows for a bloodless splenic parenchyma transsection.

ACKNOWLEDGMENTS

We thank Professor Habib NA, who provided the Habib™ 4X device.

COMMENTS

Case characteristics

A 51-year-old female with a history of hysterectomy *via* laparotomy presented with left upper abdominal discomfort.

Clinical diagnosis

A benign lesion located at the upper pole of the spleen.

Differential diagnosis

Splenic cyst, splenic angioma, and a metastatic tumor.

Laboratory diagnosis

Laboratory tests were normal.

Imaging diagnosis

Ultrasonography revealed a mildly hyperechoic splenic mass with no blood flow and with a clear boundary. Computed tomography confirmed a hypodense ellipse mass (1.5 cm × 1.0 cm) occupying the upper pole of the spleen. Magnetic resonance imaging showed a single, well-defined, heterogeneous ellipse with high T2 and low T1 signals in the spleen with no centripetal fill-in of contrast on delayed images.

Pathological diagnosis

Splenic lymphangioma.

Treatment

The patient was treated with laparoscopic partial splenectomy using Habib™ 4X.

Related reports

Solitary splenic lymphangiomas are extremely rare. There are few reports about laparoscopic partial splenectomy for the treatment of splenic lymphangiomas. There are no published data about using Habib™ 4X for a laparoscopic partial splenectomy.

Term explanation

Habib™ 4X, a bipolar radiofrequency device causing coagulation necrosis of the liver tissue and sealing of blood vessels and bile duct, offers an ideal "bloodless" parenchymal resection in the liver.

Experiences and lessons

This case report represents not only a rare case of a solitary splenic lymphangioma, but also shares the skills of laparoscopic partial splenectomy using Habib™ 4X, which ensured a "bloodless" splenic parenchymal resection.

Peer-review

This article presents the recent progress in laparoscopic partial splenectomy.

REFERENCES

1 **Chen LW**, Chien RN, Yen CL, Chang LC. Splenic tumour: a clinicopathological study. *Int J Clin Pract* 2004; **58**: 924-927 [PMID: 15587770 DOI: 10.1111/j.1742-1241.2004.00009.x]
2 **Kaza RK**, Azar S, Al-Hawary MM, Francis IR. Primary and secondary neoplasms of the spleen. *Cancer Imaging* 2010; **10**: 173-182 [PMID: 20713317 DOI: 10.1102/1470-7330.2010.0026]
3 **Héry G**, Becmeur F, Méfat L, Kalfa D, Lutz P, Lutz L, Guys JM, de Lagausie P. Laparoscopic partial splenectomy: indications and results of a multicenter retrospective study. *Surg Endosc* 2008; **22**: 45-49 [PMID: 17943384 DOI: 10.1007/s00464-007-9509-0]
4 **Giovagnoni A**, Giorgi C, Goteri G. Tumours of the spleen. *Cancer Imaging* 2005; **5**: 73-77 [PMID: 16154823 DOI: 10.1102/1470-7330.2005.0002]
5 **Eghtedari M**, Sicklick J, Kono Y, Peterson MR, Santillan CS. Unusual imaging profile of a solitary splenic lymphangioma. *Acta Radiol Short Rep* 2012; **1**: arsr.2012.120033 [PMID: 23986850 DOI: 10.1258/arsr.2012.120033]
6 **Takayama A**, Nakashima O, Kobayashi K, Kojiro M. Splenic lymphangioma with papillary endothelial proliferation: a case report and review of the literature. *Pathol Int* 2003; **53**: 483-488 [PMID: 12828616 DOI: 10.1046/j.1440-1827.2003.01493.x]
7 **Chang WC**, Liou CH, Kao HW, Hsu CC, Chen CY, Yu CY. Solitary lymphangioma of the spleen: dynamic MR findings with pathological correlation. *Br J Radiol* 2007; **80**: e4-e6 [PMID: 17267469]
8 **Yang F**, Chen WX. Splenic lymphangioma that manifested as a solid-cystic mass: a case report. *World J Gastroenterol* 2013; **19**: 781-783 [PMID: 23429434 DOI: 10.3748/Wjg.v19.i5.781]
9 **Schilling RF**. Spherocytosis, splenectomy, strokes, and heat attacks. *Lancet* 1997; **350**: 1677-1678 [PMID: 9400518 DOI: 10.1016/S0140-6736(05)64276-6]
10 **Lima M**, Reinberg O, Ruggeri G, De Buys Roessingh AS, Gargano T, Soler L, Mogiatti M, Cantone N. 3D virtual rendering before laparoscopic partial splenectomy in children. *J Pediatr Surg* 2013; **48**: 1784-1788 [PMID: 23932623 DOI: 10.1016/j.jpedsurg.2013.06.011]
11 **de Buys Roessingh AS**, de Lagausie P, Rohrlich P, Berrebi D, Aigrain Y. Follow-up of partial splenectomy in children with hereditary spherocytosis. *J Pediatr Surg* 2002; **37**: 1459-1463 [PMID: 12378454 DOI: 10.1053/jpsu.2002.35412]
12 **Jahn S**, Bauer B, Schwab J, Kirchmair F, Neuhaus K, Kiessig ST, Volk HD, Mau H, von Baehr R, Specht U. Immune restoration in children after partial splenectomy. *Immunobiology* 1993; **188**: 370-378 [PMID: 8244444 DOI: 10.1016/S0171-2985(11)80220-2]
13 **Uranüs S**, Pfeifer J, Schauer C, Kronberger L, Rabl H, Ranftl G, Hauser H, Bahadori K. Laparoscopic partial splenic resection. *Surg Laparosc Endosc* 1995; **5**: 133-136 [PMID: 7773460]
14 **Uranues S**, Grossman D, Ludwig L, Bergamaschi R. Laparoscopic partial splenectomy. *Surg Endosc* 2007; **21**: 57-60 [PMID: 17031738 DOI: 10.1007/s00464-006-0124-2]

15 **Redmond HP**, Redmond JM, Rooney BP, Duignan JP, Bouchier-Hayes DJ. Surgical anatomy of the human spleen. *Br J Surg* 1989; **76**: 198-201 [PMID: 2702458 DOI: 10.1002/bjs.1800760230]
16 **Patrzyk M**, Glitsch A, Hoene A, von Bernstorff W, Heidecke CD. Laparoscopic partial splenectomy using a detachable clamp with and without partial splenic embolisation. *Langenbecks Arch Surg* 2011; **396**: 397-402 [PMID: 20683622 DOI: 10.1007/s00423-010-0701-7]
17 **Gumbs AA**, Bouhanna P, Bar-Zakai B, Briennon X, Gayet B. Laparoscopic partial splenectomy using radiofrequency ablation. *J Laparoendosc Adv Surg Tech A* 2008; **18**: 611-613 [PMID: 18721016 DOI: 10.1089/lap.2007.0194]
18 **Ayav A**, Jiao L, Dickinson R, Nicholls J, Milicevic M, Pellicci R, Bachellier P, Habib N. Liver resection with a new multiprobe bipolar radiofrequency device. *Arch Surg* 2008; **143**: 396-401; discussion 401 [PMID: 18427028 DOI: 10.1001/Archsurg.143.4.396]

P- Reviewer: Kamocki ZK, Monclova JL **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3425

World J Gastroenterol 2015 March 21; 21(11): 3425-3428
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Congenital left intrahepatic bile duct draining into gastric wall mimicking biliary reflux gastritis

Jian Guan, Ling Zhang, Jian-Ping Chu, Shao-Chun Lin, Zi-Ping Li

Jian Guan, Jian-Ping Chu, Shao-Chun Lin, Zi-Ping Li, Department of Medical Imaging, the First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, Guangdong Province, China
Ling Zhang, Department of Medical Imaging and Interventional Radiology, Cancer Center, Sun Yat-Sen University, Guangzhou 510080, Guangdong Province, China
Author contributions: Guan J and Chu JP designed the report; Zhang L and Li ZP collected the patient's clinical data; Lin SC performed MRCP scan; Chu JP and Guan J analyzed the data and wrote the paper.

Correspondence to: Jian-Ping Chu, MD, Department of Medical Imaging, the First Affiliated Hospital, Sun Yat-Sen University, 58th the Second Zhongshan Road, Guangzhou 510080, Guangdong Province, China. truechu@hotmail.com
Telephone: +86-20-87755766
Fax: +86-20-87615805
Received: October 6, 2014
Peer-review started: October 7, 2014
First decision: October 14, 2014
Revised: November 4, 2014
Accepted: December 1, 2014
Article in press: December 1, 2014
Published online: March 21, 2015

Abstract

Abnormalities and variations of the biliary ducts are not rare. Most aberrant bile ducts eventually drain into the descending part of duodenum through the papilla of vater. However, drainage of the left hepatic bile duct into the stomach is extremely rare. A 29-year old man was admitted to the hospital with the diagnosis of biliary reflux gastritis. Comprehensive imaging modalities were performed including electronic endoscopy, endoscopic ultrasonography, endoscopic retrograde cholangiopancreatography and magnetic resonance cholangio-pancreatography. Finally, congenital ectopic left intrahepatic bile duct draining into the stomach was found, which caused biliary reflux gastritis. The patient did not receive any surgery. Good recovery was achieved by medical treatment.

Key words: Ectopic left intrahepatic bile duct; Endoscopic ultrasonography; Endoscopic retrograde cholangiopancreatography; Magnetic resonance; Cholangiopancreatography

Core tip: Abnormalities and variations of the biliary ducts are not rare. Most aberrant bile ducts eventually drain into the descending part of duodenum through the papilla of vater. However, drainage of the left hepatic bile duct into the stomach is extremely rare. We report a case with congenital left intrahepatic bile duct draining into gastric wall. The clinical symptoms are similar to bile reflux gastritis and the imaging changes are easily misdiagnosed as gastric tumor. The comprehensive imaging examination is necessary for correct diagnosis.

Guan J, Zhang L, Chu JP, Lin SC, Li ZP. Congenital left intrahepatic bile duct draining into gastric wall mimicking biliary reflux gastritis. *World J Gastroenterol* 2015; 21(11): 3425-3428 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3425.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3425

INTRODUCTION

Knowledge of the hepatic duct variants is necessary when interpreting images and performing surgical or

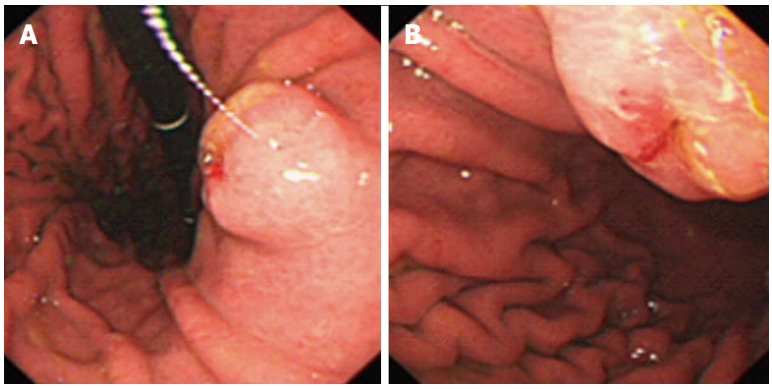


Figure 1 Electronic gastroscopy showed a papillary mass in lesser curvature of the stomach, and some bile on the surface of the mass.

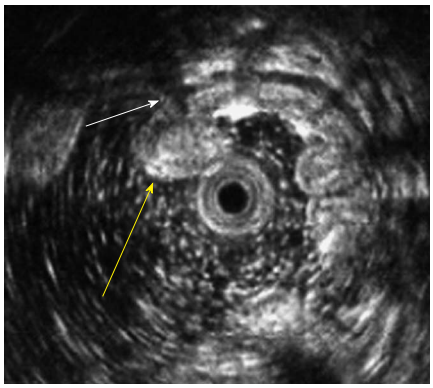


Figure 2 Endoscopic ultrasonography revealed a homogeneously hyperechoic submucosal mass (yellow arrow) with clear margin and a hypoechoic tunnel inside (white arrow).

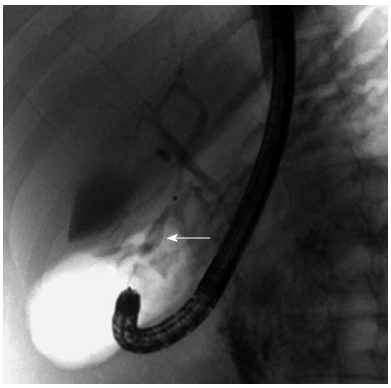


Figure 3 Endoscopic retrograde cholangiopancreatography showed a tubular structure (white arrow) between left hepatic lobe and lesser curvature of the stomach. GB: Gallbladder.

non-surgical therapeutic procedures. Sharma *et al*[1] described that the branching pattern of intrahepatic bile ducts (IHD) was atypical in 47% patients. Guerra *et al*[2] reported that one case of ectopic papilla of vater in the pylorus. Although anatomical variations of IHD present several types of branching patterns[1-4], anomalous opening of IHD located outside the biliary tree was rare and only described in case reports. The purpose of this study is to describe an extremely rare anatomic variation of left intrahepatic duct draining into the lesser curvature of stomach.

CASE REPORT

A 29-year old man was admitted to our hospital and presented with upper abdominal pain and burning sensation for more than a year. These symptoms aggravated especially under the circumstance of waking-up in the early morning and starvation, and then relieved after eating and activity. This patient did not have any history of gastrointestinal disease and surgery. Physical examination revealed soft and plain abdomen without tenderness and rebound pain.

The electronic endoscopy showed congestive swelling in gastric antral mucosa and a submucosal papillary mass in the lesser curvature of the stomach. This mass manifested as smooth surface projecting to gastric lumen with a size of 1.0 cm × 1.0 cm. There was some bile-like liquid draining out from the fissure-shaped opening of the papillary mass (Figure 1). Gastric mucosa biopsy was performed. Endoscopic ultrasonography revealed a homogeneous hyperechoic submucosal mass of around 8 mm in diameter with clear margin. The structure of the affected gastric wall was normal and intact. There was a hypoechoic tunnel crossing the muscularis propria and serous layer of gastric wall (Figure 2). Pathologic result of mucosa biopsy indicated gastric inflammation. Then endoscopic retrograde cholangiopancreatography (ERCP) was performed. Small amount of contrast agent (Ultravist) was injected through the opening of the papillary mass. We observed that contrast agent was traced through the opening into the bile duct-like structures, and then into the left intrahepatic bile duct, and finally into the common bile duct and the duodenum (Figure 3). In order to entirely display the variant bile duct, magnetic resonance cholangiopancreatography (MRCP) was carried out. It showed the variant bile duct arising from the left hepatic duct connected with gastric wall and formed a papillary orifice in the lesser curvature of the gastric body (Figure 4). A hyperintense tubular structure of around 4 mm in diameter was seen

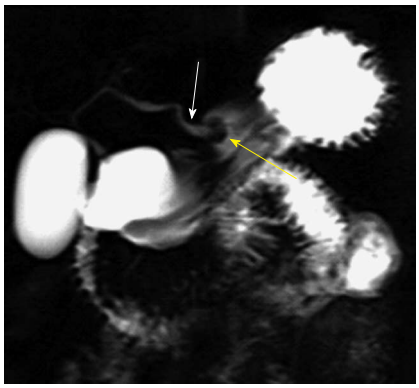


Figure 4 Magnetic resonance cholangiopancreatography revealed the variant bile duct (white arrow) originating from left hepatic duct drained into the lesser curvature of stomach. Note the hyperintense tubular structure (yellow arrow).

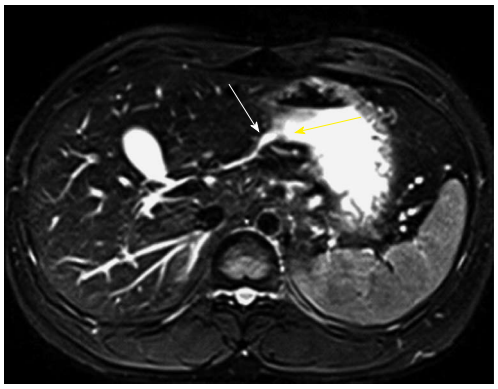


Figure 5 Axial T2WI, a tubular structure (white arrow) with hyperintense signal and its opening (yellow arrow) in stomach can be found as well.

between the left hepatic lobe and stomach on axial T2 weighted imaging (Figure 5). The common bile duct, the right and left hepatic duct, and pancreatic duct were normal without dilatation; and moreover, the gallbladder was also in normal size.

The final diagnosis was congenital ectopic left intrahepatic bile duct draining into stomach, which caused biliary reflux gastritis (Figure 6). The mild biliary reflux gastritis was not indicative for surgery, so the patient was requested to change his dietary habits avoiding greasy food to protect gastric mucosa from biliary stimulation. Prokinetic drugs (Domperidone) and conjugated bile salts drug (Almadrate Sulfate) were prescribed to control the symptoms. The follow-up included periodic monitoring of endoscopy and Helicobacter pylori infection. Until now, the symptoms have been alleviated and no evidence of Helicobacter pylori infection has been got.

DISCUSSION

Abnormalities and variations of the biliary ducts are not rare. There are different drainage types. Most aberrant bile ducts eventually drain into the descending part

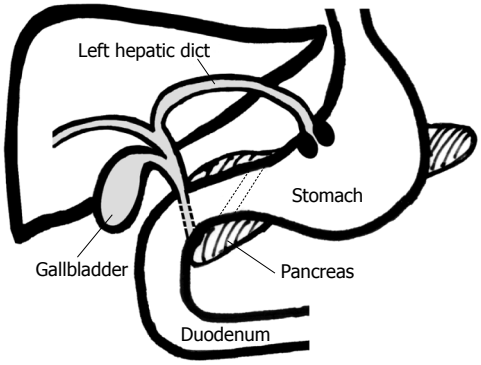


Figure 6 Hand drawing of this case better shows the relationship between variant bile duct and the lesser curvature of the stomach.

of the duodenum through papilla of vater[1,2]. Ectopic papilla of vater located in the duodenal bulb and the pylorus has been described as case reports[4,5]. However, anomalous opening of the left hepatic bile duct into the stomach is very rare.

In our case, anomalous drainage of the left intrahepatic duct into the lesser curvature of the stomach was found. The biliary duct abnormalities may be explained as the overgrowth of left intrahepatic bile duct extending into the stomach wall in embryonic stage. Part of bile was drained into the stomach *via* this anomalous opening, which caused the symptoms similar to bile reflux gastritis. Therefore, for bile gastritis cases, the common causes include uncoordinated movement disorders of stomach and duodenum, insufficiency of pyloric function and delayed gastric emptying, in addition to the causes mentioned above, the anomalous opening of bile duct in stomach should also be considered.

The anomalous opening of bile duct in the stomach formed papillary structure mimicking submucosal tumors, so it was difficult to make accurate diagnosis only by electronic gastroscopy. It should be differentiated from many submucosal masses, such as interstitialoma, lipoma, carcinoid, schwannoma, ectopic pancreas, *etc*. Although endoscopic ultrasound can be some helpful in excluding submucosal tumors by demonstrating its internal echo characteristics and location in the gastric wall, especially the tubular hypoechoic structure inside "submucosal papillary mass", it was still difficult to make a qualitative diagnosis as the lack of tracing the tubular structure outside the gastric wall. MRCP is a noninvasive examination as effective as the ERCP in showing the drainage path and opening morphology of ectopic bile duct. The findings by MRCP were in consistent with those by ERCP, which indicates that MRCP might be the first choice for diagnosis.

In our case, the patient didn't receive any surgery and he was well controlled by medical treatment. In addition, periodic follow-up of endoscopy was normal and no helicobacter pylori infection was found. So we continued follow-up.

The ectopic duct is likely to be misdiagnosed as submucosal tumors due to its special structure. Thus, the patient may receive unnecessary surgical procedures or suffer intraoperative accidental injury to the bile duct, resulting in biliary fistula, bile peritonitis or other medical complications. Therefore, it is essential to fulfill comprehensive examinations before proper intervention.

COMMENTS

Case characteristics

This is a rare case about the ectopic opening of the left bile duct into the stomach. The clinical manifestations are very similar to bile reflux gastritis, which presents as abdominal pain and a burning sensation when fasting.

Clinical diagnosis

According to its bile irritation symptoms, this case is diagnosed as bile reflux gastritis.

Differential diagnosis

Clinically, the main differential diagnosis is bile reflux gastritis. The radiological differential diagnosis includes gastric tumor and ectopic pancreas.

Laboratory diagnosis

Laboratory diagnosis is non-specific.

Imaging diagnosis

Imaging findings revealed that the ectopic opening of left hepatic duct situated in the gastric wall, which allows bile to drain into the stomach directly.

Pathological diagnosis

Histopathological examination confirmed gastritis.

Treatment

Treatment including eating little but often, inhibition of gastric acid and gastric mucosa protection can effectively relieve symptoms.

Related reports

As the limit of our knowledge, no related case was reported.

Term explanation

No specific terms were used in our case report.

Experiences and lessons

Bile reflux gastritis is not necessarily caused by abnormal pyloric sphincter. It may be caused by the ectopic biliary opening on the gastric wall, so the comprehensive imaging examination is necessary.

Peer-review

The ectopic opening of the left bile duct into the stomach is a very special anatomic variation of bile duct. The clinical symptoms are similar to bile reflux gastritis and the imaging changes are easily misdiagnosed as gastric tumor. This patient only received symptomatic treatment and long-term follow-up. We still lack of experience in the treatment of the disease since it is a rare condition.

REFERENCES

1 **Sharma V**, Saraswat VA, Baijal SS, Choudhuri G. Anatomic variations in intrahepatic bile ducts in a north Indian population. *J Gastroenterol Hepatol* 2008; **23**: e58-e62 [PMID: 18700937 DOI: 10.1111/j.1440-1746.2008.05418.x]

2 **Guerra I**, Rábago LR, Bermejo F, Quintanilla E, García-Garzón S. Ectopic papilla of Vater in the pylorus. *World J Gastroenterol* 2009; **15**: 5221-5223 [PMID: 19891024 DOI: 10.3748/wjg.15.5221]

3 **Elmunzer BJ**, Taylor JR. Aberrant right hepatic duct with patent ducts of Luschka. *Gastrointest Endosc* 2011; **74**: 196; discussion 197 [PMID: 21531408 DOI: 10.1016/j.gie.2011.02.025]

4 **Uchiyama D**, Fujimoto K, Fujimoto N, Hayabuchi N. Anatomic variation of the intrahepatic bile duct. *Intern Med* 2008; **47**: 1631 [PMID: 18797125]

5 **Disibeyaz S**, Parlak E, Cicek B, Cengiz C, Kuran SO, Oguz D, Güzel H, Sahin B. Anomalous opening of the common bile duct into the duodenal bulb: endoscopic treatment. *BMC Gastroenterol* 2007; **7**: 26 [PMID: 17610747 DOI: 10.1186/1471-230X-7-26]

P- Reviewer: Intagliata E, Iso Y, Zhong YS
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3429

World J Gastroenterol 2015 March 21; 21(11): 3429-3434
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Rare case of *Helicobacter pylori*-positive multiorgan IgG4-related disease and gastric cancer

Min Li, Qiang Zhou, Kun Yang, David R Brigstock, Lu Zhang, Ming Xiu, Li Sun, Run-Ping Gao

Min Li, Qiang Zhou, Kun Yang, Lu Zhang, Ming Xiu, Li Sun, Run-Ping Gao, Department of Hepatic-Biliary-Pancreatic Medicine, The First Hospital of Jilin University, Changchun 130021, Jilin Province, China
David R Brigstock, The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205, United States
David R Brigstock, Division of Pediatric Surgery, Department of Surgery, The Ohio State University, Columbus, OH 43205, United States
Author contributions: Li M, Zhou Q, Yang K, Zhang L, Xiu M, and Sun L contributed to the data collection; Gao RP designed the study and wrote the manuscript; Brigstock DR coordinated the study and edited the manuscript.
Supported by National Natural Scientific Foundation of China (to Gao RP), No. 81070370 and No. 81270544.

Correspondence to: Run-Ping Gao, Professor, MD, PhD, Department of Hepatic-Biliary-Pancreatic Medicine, The First Hospital of Jilin University, No. 71 Xinmin Avenue, Changchun 130021, Jilin Province, China. gao_runping@yahoo.com
Telephone: +86-431-88785110
Fax: +86-431-65612468
Received: September 15, 2014
Peer-review started: September 19, 2014
First decision: October 29, 2014
Revised: November 17, 2014
Accepted: December 22, 2014
Article in press: December 22, 2014
Published online: March 21, 2015

Abstract

A 61-year-old male from Northeast China presented with a 2-mo history of abdominal distension, pruritus and jaundice. Laboratory testing revealed an elevated serum IgG4 level. A computed tomography scan showed a typical feature of autoimmune pancreatitis (AIP) and cholecystocholangitis. Early gastric cancer was incidentally discovered when endoscopic untrasound-guided fine needle aspiration (EUS-FNA) of the pancreas was carried out. The patient underwent radical subtotal gastrectomy for gastric cancer combined with cholecystectomy. *Helicobacter pylori* (*H. pylori*) and IgG4-positive plasmacytes were detected in gastric cancer tissue, pancreatic EUS-FNA sample and resected gallbladder specimen by immunohistochemistry. The patient was diagnosed with *H. pylori*-positive IgG4-related AIP and sclerosing cholecystocholangitis as well as *H. pylori*-positive gastric cancer. He responded well to steroid therapy and remains healthy with no signs of recurrence at one year follow-up. We speculate that *H. pylori* might act as a trigger *via* direct or indirect action in the initiation of onset of gastric cancer and multiorgan IgG4-related disease.

Key words: IgG4-related disease; *Helicobacter pylori*; Type 1 autoimmune pancreatitis; Sclerosing cholecystocholangitis; Gastric cancer

Core tip: We report a rare case of a 61-year-old male patient who suffered from *Helicobacter pylori* (*H. pylori*)-positive IgG4-related autoimmune pancreatitis and sclerosing cholecystocholangitis as well as *H. pylori*-positive gastric cancer. The patient responded well to corticosteroid therapy after he underwent radical subtotal gastrectomy for gastric cancer combined with cholecystectomy. This report supports the role of *H. pylori* in the initiation of onset of gastric cancer and multiorgan IgG4-related disease.

Li M, Zhou Q, Yang K, Brigstock DR, Zhang L, Xiu M, Sun L, Gao RP. Rare case of *Helicobacter pylori*-positive multiorgan

IgG4-related disease and gastric cancer. *World J Gastroenterol* 2015; 21(11): 3429-3434 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3429.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3429

INTRODUCTION

IgG4-related disease (IgG4-RD) is a recently recognized disorder that is characterized by the enlargement of involved organs, elevated levels of serum IgG4, and abundant infiltration of IgG4-positive plasmacytes in the affected organs[1]. Autoimmune pancreatitis (AIP) has been divided into types 1 and 2. Type 1 AIP is the pancreatic manifestation of IgG4-RD[2]. IgG4-associated cholangitis and chronic sclerosing sialadenitis are other common manifestations of IgG4-RD[3]. Most cases of AIP in Japan and Korea are of the type 1 form, whereas type 2 is quite rare[2]. The prevalence and clinical features of AIP and other forms of IgG4-RD in China have yet to be fully clarified.

Recent investigations from Japan indicate that the standardized incidence ratio for malignances in IgG4-RD patients is higher than that in the general population and that the affected cancerous tissues can be infiltrated by IgG4 positive plasmacytes to various degrees[4,5]. On the contrary, the latest report from the United States indicates that cancer risk before and after diagnosis of AIP is similar to that in control subjects[6]. Malignancies in patients with IgG4-RD have included lung cancer, colon cancer, prostate cancer and lymphoma[4,6-9]. The question of whether synchronous carcinoma and IgG4-RD have a true association or are the result of a nonspecific peri-cancerous IgG4 reaction remains to be clarified.

Infection with *Helicobacter pylori* (*H. pylori*) has been shown to play a major role in gastric carcinogenesis[10,11]. The interplay of infectious agents with other etiological factors such as the genetic susceptibility of the host or the external environment is becoming increasingly recognized as an important component in the occurrence of gastric cancer. Over the last decade, *H. pylori* was thought to contribute to the development of AIP through induction of autoimmunity and apoptosis[12,13]. However, the relationship between *H. pylori* infection and multiorgan IgG4-RD has yet to be clarified.

In this report, we describe a rare case of concurrent *H. pylori*-positive gastric cancer and multiorgan IgG-RD from northeast China.

CASE REPORT

A 61-year-old male from northeast China presented with a two-month history of abdominal distension, pruritus, jaundice and a 25-pound weight loss. The patient denied any history of alcohol, tobacco, or illicit drug use. On physical examination, the patient had yellow staining of the skin and sclera. He had mild epigastric tenderness to deep palpation.

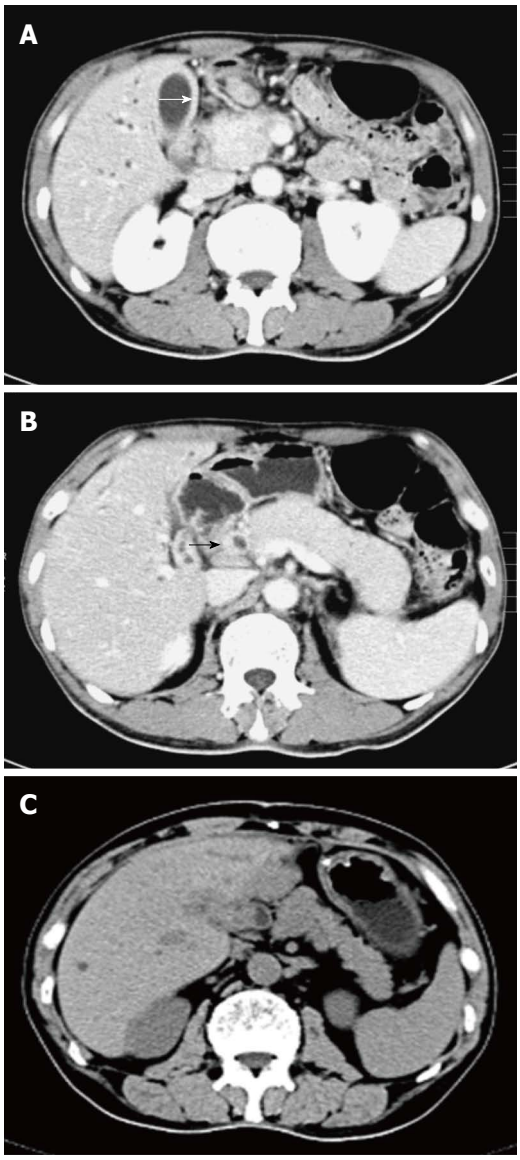


Figure 1 Computed tomography images of autoimmune cholecystocholangitis and pancreatitis. Diffuse gallbladder wall thickening (white arrow) and intrahepatic bile duct dilatation (A), thickening of the common bile duct wall (black arrow) and diffuse swelling pancreas with loss of lobulation (B), and a dramatic recovery in the size of the pancreas after 4 wk of steroid therapy (C).

Routine blood tests showed a high percentage (11%) of eosinophils in the white blood cell count, and the erythrocyte sedimentation rate (ESR) was markedly raised (89 mm/h). Serum biochemical data on admission were as follows: total bilirubin 179 μmol/L, total bile acids 184.1 μmol/L, alkaline phosphatase 487 U/L, alanine aminotransferase (ALT) 319 U/L, aspartate aminotransferase (AST) 128 U/L, amylase 183 U/L, and lipase 127 U/L. Serum immunological testing displayed high levels of IgG4 (17.5 g/L) and IgG (18.1 g/L).

A computed tomography scan of the abdomen revealed diffuse gallbladder wall thickening and intrahepatic bile duct dilatation (Figure 1A), a

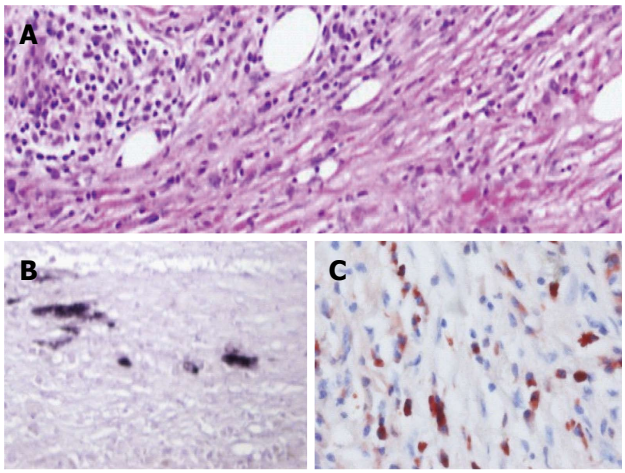


Figure 2 Histological findings of the needle biopsy specimen of the pancreas. HE staining shows numerous lymphoplasmacyte infiltration and storiform fibrosis (A). Immunostaining shows *Helicobacter pylori*-positive cells (B) or IgG4-positive plasma cells (C) in the needle specimen sections of the patient. Original magnification × 400 (A, B and C).

patchy thickening of the distal common bile duct and diffuse enlargement of the pancreas with loss of lobulation, consistent with AIP (Figure 1B). Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNA) of the pancreas revealed dense lymphoplasmacyte infiltration and storiform-type fibrosis (Figure 2A). Immunohistochemical staining showed several *H. pylori*-positive cells (Figure 2B) and numerous IgG4-positive plasma cells (Figure 2C) in the needle specimen sections of the patient, which met the diagnostic criteria for IgG4-related AIP with *H. pylori* infection.

Endoscopic biopsy specimens from the pylorus showed an early moderately differentiated gastric adenocarcinoma limited to the mucosal and submucosal layers with abundant infiltration of lymphoplasmacytes and eosinophil cells in the tumor stroma by HE staining (Figure 3A), as well as the presence of *H. pylori* in the epithelial cells, cancer cells or mesenchymal cells by immunohistochemistry (Figure 3B, C). In contrast, only sparse and patchy IgG4-positive or IgG-positive plasma cells were seen in the tumor stroma by immunohistochemical staining (Figure 3D, E). Neither dense fibrosis nor phlebitis was observed in the gastric specimen of the patient (Figure 3A).

The patient underwent radical subtotal gastrectomy for gastric cancer combined with cholecystectomy and T-tube drainage on the 14th day after admission. HE staining of the resected gallbladder specimen revealed numerous lymphoplasmacyte and eosinophil cell infiltration as well as fibrosis (Figure 4A). Immunohistochemical staining showed presence of *H. pylori* in the cholecyst epithelial cells or mesenchymal cells (Figure 4B, C). Numerous IgG4-positive plasmacytes were evident in the cholecystectomy specimen, with a ratio of IgG4/IgG-positive plasmacytes of more than 40%, which met the diagnostic criteria for IgG4-related sclerosing cholecystitis (Figure 4D, E).

On the 3rd day after surgery, the patient was diagnosed with *H. pylori*-positive multiorgan IgG4-RD. He received a first-line therapy (a proton pump inhibitor, clarithromycin and metronidazole) for eradication of *H. pylori* and 40 mg/d of prednisone for seven days without any side effects, and was then discharged with the same steroid dose alone for the following 3 wk. After 4 wk of daily oral prednisone therapy, the patient exhibited no signs of either abdominal distension or body itching. Abnormal liver function test results returned to normal levels. Elevated serum levels of amylase and lipase returned to normal value ranges. ^{14}C-urea breath test was negative for *H. pylori*. The enlarged pancreas returned to its normal size and lobulated form (Figure 1C). The patient received a long-term maintenance dose of 10 mg/d of prednisone after steroid tapering. At 12-mo follow-up, his illness had not recurred, but the serum level of IgG4 was high (15.6 g/L).

DISCUSSION

IgG4-RD is a recently recognized systemic condition characterized by elevated serum IgG4 levels and steroid responsiveness. IgG4-RD shows organ enlargement or nodular lesions consisting of abundant infiltration of lymphocytes and IgG4-positive plasma cells (ratio of IgG4/IgG-positive plasma cells > 40%) and fibrosis in various organs[5]. A histological diagnosis of IgG4-RD requires the presence of at least two of three characteristic histological features including: (1) dense lymphoplasmacytic infiltration; (2) fibrosis arranged at least focally in a storiform pattern; and (3) obliterative phlebitis[5]. The patient in this study fully met the diagnostic criteria for IgG4-related sclerosing cholecystitis and type 1 AIP[5]. Even so, biopsy specimens from the bile duct are difficult to obtain using standard procedures, except cholangiectomy. Diagnostic criteria developed in Japan to establish IgG4-related sclerosing cholangitis include diffuse or segmental narrowing of the introhepatic and/or extrahepatic bile duct associated with the thickening of the bile duct wall and coexistence of AIP/or high serum levels of IgG4[14,15]. Thus, the patient in this study also met the diagnostic criteria for IgG4-related sclerosing cholangitis[1,14,15].

Comprehensive clinical diagnostic criteria for IgG4-related gastric disease have so far not been constituted[5,16]. Recently, Koizumi *et al*[16] suggested that there appeared to be two kinds of IgG4-related gastric disease. One was a gastric lesion showing marked thinking of the wall of the stomach, consisting of dense fibrosis with abundant infiltration of IgG4-positive plasma cells; the other was an IgG4-related pseudotumor in the gastric region, showing polypoid or mass-like lesions. In this study, only sparse and patchy IgG4-positive or IgG-positive plasma cells were identified in the cancer stroma and the gastric

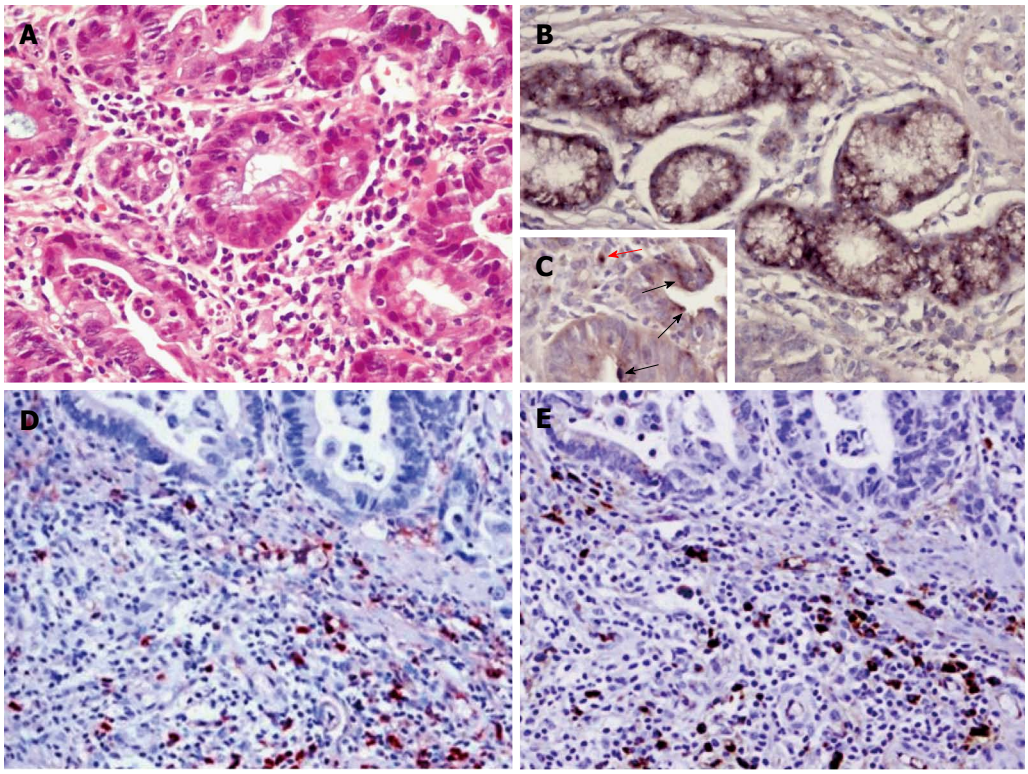


Figure 3 Histological findings of the endoscopic biopsy specimen from the pylorus. HE staining shows a moderately differentiated gastric adenocarcinoma with abundant infiltration of lymphoplasmacytes and eosinophils in stroma (A). Immunostaining reveals *Helicobacter pylori* in gastric epithelial cells (B) or cancer cells (black arrow, C) or mesenchymal cells (red arrow, C), IgG4-positive (D) or IgG-positive plasma cells (E) in the cancer stroma. Original magnification × 400 (A, B and C), × 200 (D and E).

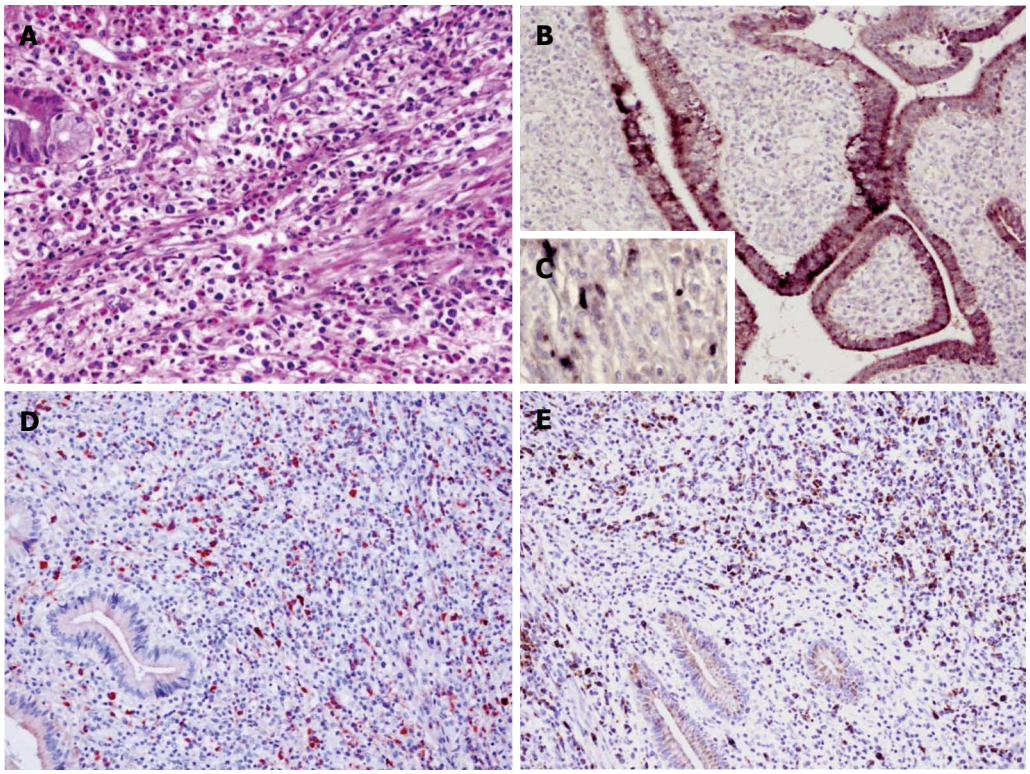


Figure 4 Histological findings of the resected gallbladder specimen. HE staining shows abundant infiltration of lymphoplasmacytes and eosinophils (A). Immunostaining shows *Helicobacter pylori* in the epithelium (B) or mesenchymal cells (C), IgG4-positive (D) or IgG-positive plasma cells (E) in the resected gallbladder sections of the patient. Original magnification × 400 (A and C); × 200 (B, D and E).

submucosa adjacent to the carcinoma (data not shown), and either storiform-type fibrosis or obliterative phlebitis was absent. Thus, our data support a peri-cancerous IgG4 reaction rather than IgG4-related gastric disease.

Since the gallbladder, common bile duct, pancreas and stomach were involved synchronously in this case, we believed there might be a common factor contributing to the pathogenesis seen in the lesions of these adjacent organs. Over the last decade, molecular mimicry of host structures by constituents of *H. pylori* is thought to be connected with the development of autoimmune sequelae in hepatobiliary- or pancreatic-tissue destruction through antibody cross-reactivity[13,17]. The binding motif of the HLA molecule DRB1*0405 was found to be located in the homologous segments between carbonic anhydrase Ⅱ (CAⅡ) of the human pancreas and alpha-carbonic anhydrase of *H. pylori*, and serum anti-CAⅡ Ab levels in AIH were significantly higher than those in chronic pancreatitis and pancreatic adenocarcinoma[18,19]. Additionally, in the case of *H. pylori* infection, T cell-mediated apoptotic signals and granulocyte recruitment as well as activation of the oxidative burst were also thought to contribute to the pathogenesis of AIP[12]. In this study, the patient presented with *H. pylori* infection in the stomach, gallbladder, and pancreas and with abundant infiltration of IgG4-positive plasma cells in the gastric submucosa, gallbladder stroma, and pancreatic tissues. Thus, we speculate that *H. pylori* might act as a trigger *via* direct or indirect action (immune cross-response) in the initiation of onset of multiorgan IgG4-RD and that IgG4 reaction might increase the *H. pylori*-associated risk of developing gastric cancer. However, further identification and characterization of such cases is required to elucidate the mechanism of this association.

High serum IgG4 levels have been proposed as a marker of AIP and also showed good accuracy in distinguishing between AIP and pancreatic cancer and other autoimmune diseases[20]. Additionally, AIP patients with elevated serum IgG4 had higher incidence of jaundice at onset, more frequent diffuse pancreatic enlargement at imaging, and more frequent extrapancreatic lesions compared to those with normal serum IgG4 levels[21]. Over the last decade, the usefulness of serum IgG4 as a marker of the efficacy of steroid treatment has been paid great attention[20]. Recently, response to steroids in AIP patients was recognized regardless of serum IgG4 level, however, a long-term maintenance therapy was required more frequently amongst patients with elevated IgG4 compared to those with normal IgG4[21].

In summary, we report a rare case of *H. pylori*-positive multiorgan IgG4-RD and gastric cancer. This report supports the role of *H. pylori* in the initiation of onset of gastric cancer and multiorgan IgG4-RD. Since the cancer was discovered unexpectedly and resected successfully, a careful examination of IgG4-AIP is required to rule out the presence of gastric malignant tumor before steroid therapy.

COMMENTS

Case characteristics

A 61-year-old male from northeast China presented with a 2-mo history of abdominal distension, pruritus and jaundice.

Clinical diagnosis

The patient was diagnosed with *Helicobacter pylori (H. pylori)*-positive IgG4-related autoimmune pancreatitis (AIP) and sclerosing cholecystocholangitis as well as *H. pylori*-positive gastric cancer.

Differential diagnosis

The differential diagnosis included IgG4-related gastric disease and primary sclerosing cholangitis.

Laboratory diagnosis

The patient was diagnosed with *H. pylori*-positive multiorgan IgG4-related disease (IgG4-RD) as well as *H. pylori*-positive gastric cancer on the basis of the presence of *H. pylori* and IgG4-positive plasmacytes in gastric cancer tissue, pancreatic endoscopic untrasound-guided fine needle (EUS-FN) sample and resected gallbladder specimen, and a high serum IgG4 level.

Imaging diagnosis

A computed tomography scan revealed diffuse gallbladder wall thickening and intrahepatic bile duct dilation, a patchy thickening of the distal common bile duct and diffuse enlargement of the pancreas with loss of lobulation.

Pathological diagnosis

Immunohistochemistry revealed *H. pylori* and IgG4-positive plasmacyte infiltration in resected gastric cancer tissue and gallbladder specimen as well as pancreatic EUS-FN sample.

Treatment

The patient received 30 mg/d of prednisone for 4 wk, and a long-term maintenance dose of 10 mg/d of prednisone.

Related reports

The relationship between *H. pylori* infection and multiorgan IgG4-RD has not yet been clarified.

Term explanation

AIP is a form of pancreatitis with a presumed autoimmune etiology and is currently recognized as two subtypes. Type 1 AIP is a pancreatic lesion of IgG4-related disease, while type 2 AIP is related to granulocytic epithelial lesion.

Experiences and lessons

This report supports the role of *H. pylori* in the initiation of onset of gastric cancer and multiorgan IgG4-RD. Since the gastric cancer was discovered unexpectedly and resected successfully, a careful examination of IgG4-AIP is required to rule out the presence of gastric malignant tumor before steroid therapy.

Peer-review

This is a well-written manuscript about a rare case with *H. pylori*-positive IgG4-related diseases. However, the role of *H. pylori* in the pathogenesis of IgG4-related disease should be more discussed. Please explain why IgG4 levels are still high after treatment and the correlation between the IgG4 levels and the clinical presentation/imaging findings.

REFERENCES

1 **Masaki Y**, Kurose N, Umehara H. IgG4-related disease: a novel lymphoproliferative disorder discovered and established in Japan in the 21st century. *J Clin Exp Hematop* 2011; **51**: 13-20 [PMID: 21628856 DOI: 10.3960/jslrt.51.13]

2 **Kamisawa T**, Notohara K, Shimosegawa T. Two clinicopathologic subtypes of autoimmune pancreatitis: LPSP and IDCP. *Gastroenterology* 2010; **139**: 22-25 [PMID: 20639082 DOI: 10.1053/j.gasto.2010.05.019]

3 **Sah RP**, Chari ST, Pannala R, Sugumar A, Clain JE, Levy MJ, Pearson RK, Smyrk TC, Petersen BT, Topazian MD, Takahashi

N, Farnell MB, Vege SS. Differences in clinical profile and relapse rate of type 1 versus type 2 autoimmune pancreatitis. *Gastroenterology* 2010; **139**: 140-148; quiz e12-e13 [PMID: 20353791 DOI: 10.1053/j.gastro.2010.03.054]

4 **James B**. Electrical stimulation of the brain: JRSM November 1983, p 905. *J R Soc Med* 1984; **77**: 255 [PMID: 20894525 DOI: 10.1007/s10165-011-0520-x]

5 **Deshpande V**, Zen Y, Chan JK, Yi EE, Sato Y, Yoshino T, Klöppel G, Heathcote JG, Khosroshahi A, Ferry JA, Aalberse RC, Bloch DB, Brugge WR, Bateman AC, Carruthers MN, Chari ST, Cheuk W, Cornell LD, Fernandez-Del Castillo C, Forcione DG, Hamilos DL, Kamisawa T, Kasashima S, Kawa S, Kawano M, Lauwers GY, Masaki Y, Nakanuma Y, Notohara K, Okazaki K, Ryu JK, Saeki T, Sahani DV, Smyrk TC, Stone JR, Takahira M, Webster GJ, Yamamoto M, Zamboni G, Umehara H, Stone JH. Consensus statement on the pathology of IgG4-related disease. *Mod Pathol* 2012; **25**: 1181-1192 [PMID: 22596100 DOI: 10.1038/modpathol.2012.72]

6 **Hart PA**, Law RJ, Dierkhising RA, Smyrk TC, Takahashi N, Chari ST. Risk of cancer in autoimmune pancreatitis: a case-control study and review of the literature. *Pancreas* 2014; **43**: 417-421 [PMID: 24622072]

7 **Hart PA**, Smyrk TC, Chari ST. IgG4-related prostatitis: a rare cause of steroid-responsive obstructive urinary symptoms. *Int J Urol* 2013; **20**: 132-134 [PMID: 23075137 DOI: 10.1111/j.1442-2042.2012.03194.x]

8 **Fujimoto M**, Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Koyanagi I, Aini W, Tsuruyama T, Date H, Haga H. Stromal plasma cells expressing immunoglobulin G4 subclass in non-small cell lung cancer. *Hum Pathol* 2013; **44**: 1569-1576 [PMID: 23465276]

9 **Inoue T**, Hayama M, Kobayashi S, Oyaizu T, Nakazato Y, Honma K, Chida M. Lung Cancer Complicated with IgG4-related Disease of the Lung. *Ann Thorac Cardiovasc Surg* 2014; **20** Suppl: 474-477 [PMID: 23574998 DOI: 10.5761/atcs.cr.12.02208]

10 **Figueiredo C**, Garcia-Gonzalez MA, Machado JC. Molecular pathogenesis of gastric cancer. *Helicobacter* 2013; **18** Suppl 1: 28-33 [PMID: 24011242 DOI: 10.1111/hel.12083]

11 **Carrasco G**, Corvalan AH. Helicobacter pylori-Induced Chronic Gastritis and Assessing Risks for Gastric Cancer. *Gastroenterol Res Pract* 2013; **2013**: 393015 [PMID: 23983680 DOI: 10.1155/2013/393015]

12 **Kountouras J**, Zavos C, Chatzopoulos D. Autoimmune pancreatitis, Helicobacter pylori infection, and apoptosis: a proposed relationship. *Pancreas* 2005; **30**: 192-193 [PMID: 15714146]

13 **Kountouras J**, Zavos C, Chatzopoulos D. A concept on the role of Helicobacter pylori infection in autoimmune pancreatitis. *J Cell Mol Med* 2005; **9**: 196-207 [PMID: 15784177 DOI: 10.1111/j.1582-4934.2005]

14 **Okazaki K**, Uchida K, Ikeura T, Takaoka M. Current concept and diagnosis of IgG4-related disease in the hepato-bilio-pancreatic system. *J Gastroenterol* 2013; **48**: 303-314 [PMID: 23417598 DOI: 10.1007/s00535-012-0744-3]

15 **Okazaki K**, Uchida K, Koyabu M, Miyoshi H, Ikeura T, Takaoka M. IgG4 cholangiopathy - current concept, diagnosis, and pathogenesis. *J Hepatol* 2014; **61**: 690-695 [PMID: 24768756 DOI: 10.1016/j.jhep.2014.04.016]

16 **Koizumi S**, Kamisawa T, Kuruma S, Tabata T, Chiba K, Iwasaki S, Endo Y, Kuwata G, Koizumi K, Shimosegawa T, Okazaki K, Chiba T. Immunoglobulin G4-related gastrointestinal diseases, are they immunoglobulin G4-related diseases? *World J Gastroenterol* 2013; **19**: 5769-5774 [PMID: 24124321 DOI: 10.3748/wjg.v19.i35.5769]

17 **Zen Y**, Nakanuma Y. Pathogenesis of IgG4-related disease. *Curr Opin Rheumatol* 2011; **23**: 114-118 [PMID: 21045701 DOI: 10.1097/BOR.0b013e3283412f4a]

18 **Guarneri F**, Guarneri C, Benvenga S. Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry? *J Cell Mol Med* 2005; **9**: 741-744 [PMID: 16202223]

19 **Talar-Wojnarowska R**, Gąsiorowska A, Olakowski M, Dranka-Bojarowska D, Lampe P, Śmigielski J, Kujawiak M, Grzegorczyk J, Małecka-Panas E. Utility of serum IgG, IgG4 and carbonic anhydrase II antibodies in distinguishing autoimmune pancreatitis from pancreatic cancer and chronic pancreatitis. *Adv Med Sci* 2014; **59**: 288-292 [PMID: 25194335 DOI: 10.1016/j.advms.2014.08.003]

20 **Morselli-Labate AM**, Pezzilli R. Usefulness of serum IgG4 in the diagnosis and follow up of autoimmune pancreatitis: A systematic literature review and meta-analysis. *J Gastroenterol Hepatol* 2009; **24**: 15-36 [PMID: 19067780 DOI: 10.1111/j.1440-1746.2008.05676.x]

21 **Matsubayashi H**, Sawai H, Kimura H, Yamaguchi Y, Tanaka M, Kakushima N, Takizawa K, Kadooka M, Takao T, Hebbar S, Ono H. Characteristics of autoimmune pancreatitis based on serum IgG4 level. *Dig Liver Dis* 2011; **43**: 731-735 [PMID: 21515099 DOI: 10.1016/j.dld.2011.03.006]

P- Reviewer: Buzas GM, Hsu JT **S- Editor**: Yu J
L- Editor: Wang TQ **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3435

World J Gastroenterol 2015 March 21; 21(11): 3435-3440
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Gastrointestinal hemorrhage due to ileal metastasis from primary lung cancer

Wei Liu, Wei Zhou, Wei-Lin Qi, Ya-Dan Ma, Yun-Yun Xu

Wei Liu, Wei Zhou, Wei-Lin Qi, Ya-Dan Ma, Yun-Yun Xu, Department of General Surgery, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, Zhejiang Province, China

Author contributions: Zhou W and Liu W performed the operation; Qi WL, Ma YD and Xu YY collected case data and prepared the photos; Liu W wrote the manuscript; Zhou W proofread and revised the manuscript; all authors approved the final version to be published.

Correspondence to: Wei Zhou, MD, Department of General Surgery, Sir Run Run Shaw Hospital, No. 3 East Qingchun Road, Hangzhou 310016, China. nuzwlvran@aliyun.com
Telephone: +86- 571-86090073
Fax: +86- 571-86090073

Received: July 24, 2014
Peer-review started: July 25, 2014
First decision: August 15, 2014
Revised: September 21, 2014
Accepted: November 11, 2014
Article in press: November 11, 2014
Published online: March 21, 2015

Abstract

We report a patient with small intestinal metastasis from lung squamous cell carcinoma. A 66-year-old man who underwent radical lung cancer surgery was admitted to our hospital. Before starting his fifth cycle of chemotherapy, he was found to have a positive fecal occult blood test. Abdominal computed tomography scan revealed an ileal tumor with mesenteric lymph node enlargement. He underwent laparoscopic resection of the involved small intestine and mesentery. Histopathological analysis confirmed metastasis from lung cancer. We conducted a review of the literature and 64 documented cases of small intestinal metastasis from lung cancer were found. The pathologic diagnosis, clinical presentation, site of metastasis, and survival time in these cases were reviewed.

Key words: Lung cancer; Metastasis; Gastrointestinal hemorrhage; Gastrointestinal neoplasms; Small intestine

Core tip: Gastrointestinal tract metastases from lung cancer are relatively rare. We describe a patient with melena due to small intestinal metastasis from lung squamous cell carcinoma. We collected 64 similar documented cases from 2000 to date, and reviewed the pathologic diagnosis, clinical presentation, site of metastasis, treatment, and survival time.

Liu W, Zhou W, Qi WL, Ma YD, Xu YY. Gastrointestinal hemorrhage due to ileal metastasis from primary lung cancer. *World J Gastroenterol* 2015; 21(11): 3435-3440 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3435.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3435

INTRODUCTION

Lung cancer is a malignant tumor with high incidence and mortality[1]. The prognosis of lung cancer is poor as metastases are often present at the time of diagnosis. Distant metastases are usually found in the adrenal glands, bone, liver, brain, and kidney, however, metastases in the gastrointestinal tract are relatively rare[2]. In this report, we present a patient with gastrointestinal bleeding due to ileal metastasis from

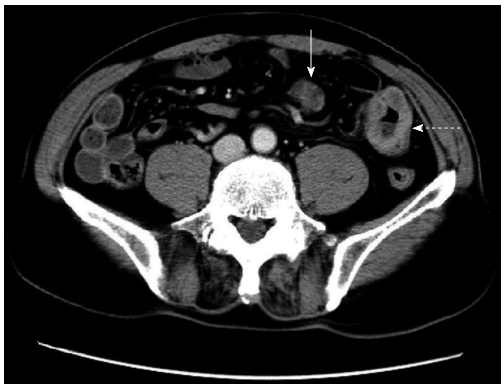


Figure 1 Abdominal computed tomography scan revealed metastatic tumor mass of ileum (solid arrow) and the enlarged lymph node (dotted arrow).

primary lung carcinoma.

CASE REPORT

A 66-year-old male patient who presented with shortness of breath and hemoptysis for 1 wk was admitted to our hospital on May 8, 2013. On chest computed tomography (CT), a mass was seen in the right lower lobe. He underwent a right lower lobectomy with lymph node dissection on May 10, 2013. At surgery, a 3.5 cm mass was found in the right upper lobe near the right lung hilum. In addition, hilum, carina, and mediastinum lymphadenopathy were found. The pathological study confirmed moderately differentiated squamous cell carcinoma with lymph node metastasis (1+/38) (T2N1M0). The patient received four cycles of adjuvant chemotherapy with docetaxel/nedaplatin from June 2013 to September 2013. Just before the fifth cycle of chemotherapy (January 3, 2014), the patient complained of melena. The stool examination for occult blood was strongly positive. On abdominal examination, no abnormalities were found. Colonoscopy also revealed no abnormalities. An abdominal CT scan revealed ileal wall thickening and nearby mesenteric lymph node enlargement, indicating a malignant ileal tumor (Figure 1). Tumor markers were within normal ranges, including CEA which was 2.75 ng/mL. A complete blood count showed anemia with a hemoglobin (Hb) level of 9.6 g/dL. The patient then underwent laparoscopic exploration. A 4.5 cm × 3.0 cm small intestinal tumor with serosal infiltration was found 150 cm from the cecum (Figure 2). Lymph node enlargement was seen near the mesenteric root. The involved small bowel and mesentery were resected. Histopathological analysis confirmed that the tumor was a moderately differentiated squamous cell carcinoma (Figure 3). Immunohistochemically, tumor cells were positive for CKH and P63, but negative for CK20 and CK7 (Figure 4), indicating a metastasis from primary lung carcinoma. One of the two resected mesenteric lymph nodes contained metastasis, and

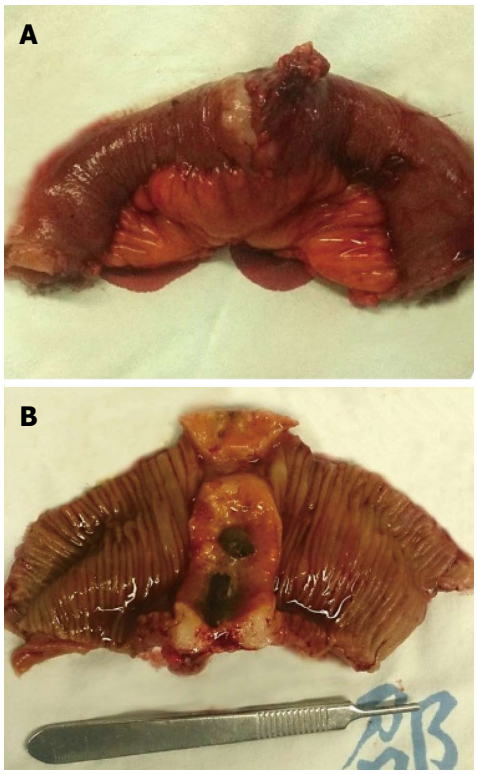


Figure 2 Intraoperative imaging of the resected tumor of the ileum. The tumor was 4.5 cm × 3.0 cm in size, with a clear margin and ulceration on the intraluminal surface.

the surgical margins were negative. The patient had no postoperative complications and was discharged 5 days after surgery. Postoperatively, he underwent four cycles of chemotherapy with gemcitabine/nedaplatin. Brain magnetic resonance imaging and SPECT/CT during chemotherapy revealed no other metastasis. The patient is still alive with no recurrence.

DISCUSSION

We searched the PubMed database for relevant English language publications from 2000 to June 2014 using the MeSH terms "Gastrointestinal Neoplasms/complications", "Gastrointestinal Neoplasms/secondary", and "Lung Neoplasms/pathology". The age and sex of patients, pathologic diagnosis, clinical presentation, site of metastasis, and the survival time of the reported cases were reviewed and analyzed. In total, 64 cases were identified and reviewed[3-33], including 58 (90.6%) male and 6 (9.4%) female patients. The mean age of these patients was 61.4 years (ranging from 36 years to 88 years).

In lung cancer, metastases to the gastrointestinal tract are quite rare and mostly found in the advanced stages of the disease. Yang *et al*[21] reported that the incidence of symptomatic gastrointestinal metastasis from primary lung cancer was 1.77%. However, the prevalence at autopsy is much higher, ranging from 4.7% to 14%[34,35]. This can be explained by the fact that most patients with small bowel metastases have

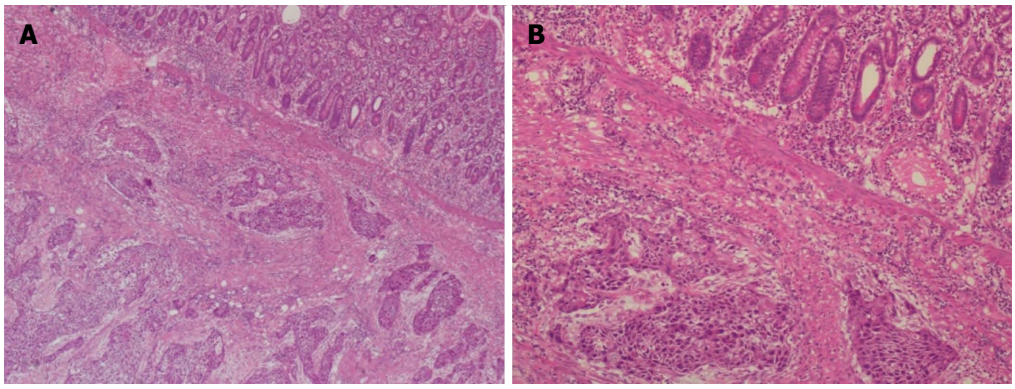


Figure 3 Microscopic findings of metastatic lung squamous cell carcinoma in the ileum.

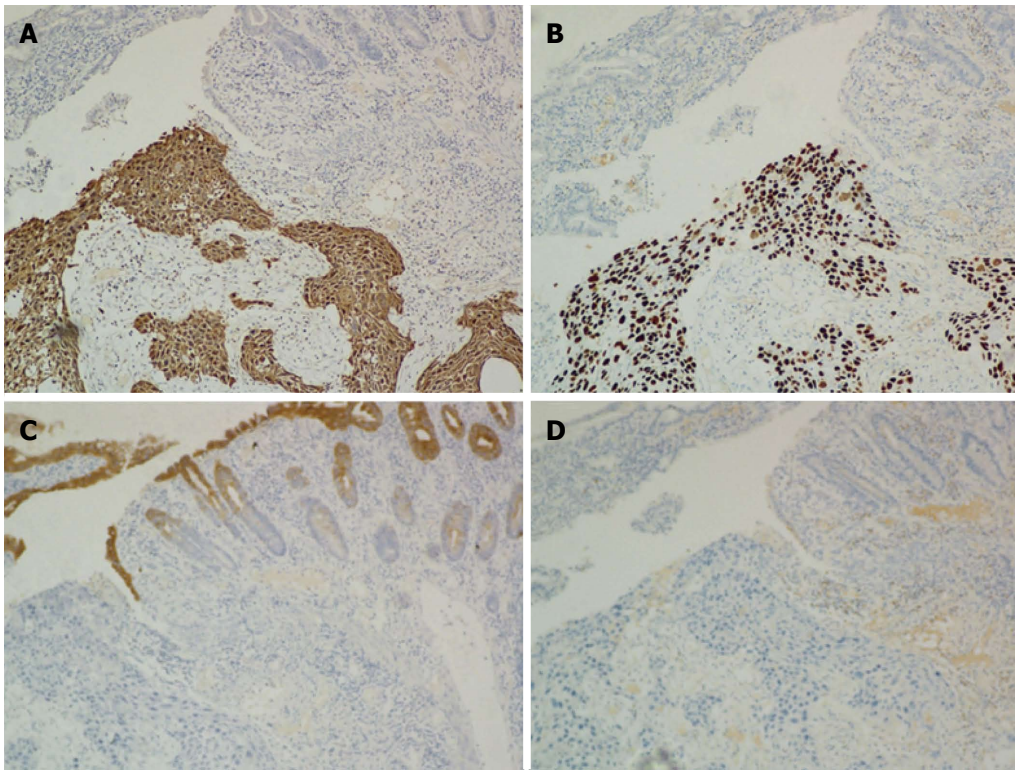


Figure 4 By immunohistochemistry, the tumor cells were found to be positive for CKH (A) and P63 (B), but negative for CK20 (C) and CK7 (D).

no specific symptoms. In the 64 documented cases, 59 patients had certain clinical presentations, of whom 22 (37.3%) had perforation, 19 (32.2%) had obstruction, 10 (16.9%) had hemorrhage and 8 (13.6%) had intussusception. Perforation was the most common symptom in patients with bowel metastases. The toxicity of chemotherapy is likely to be the etiology of bowel perforation[30].

In the 64 documented cases, 57 patients had metastatic sites. The jejunum was the most common site in 50.9% (29/57) of patients, followed by the ileum in 33.3% (19/57), and the duodenum in 15.8% (9/57). The general route of gastrointestinal metastasis has been suggested to be hematogenous metastasis through the spinal veins or lymphogenous metastasis from the mediastinum through the retroperitoneum and mesentery[9]. The difference in incidence between these metastatic sites may be because the jejunum and ileum have a more abundant blood supply and lymphoid tissues.

In lung cancer, all tumor cell types may develop small intestinal metastases. Adenocarcinoma (31.6%, 18/57), squamous cell carcinoma (28.1%, 16/57) and large cell carcinoma (24.6%, 14/57) were most common, followed by other types such as carcinosarcoma, adenosquamous, and neuroendocrine carcinoma (10.5%, 6/57) and small cell carcinoma (5.3%, 3/57). Yoshimoto *et al*[34] reported that patients with large cell carcinoma had a significantly higher rate of gastrointestinal metastases (P = 0.004, OR = 3.524) compared with patients with non-large cell carcinoma. However, the reasons for this are unknown.

Due to the unclear clinical symptoms in patients with small bowel metastases, early diagnosis and treatment are difficult. CT plays an important role in identifying the exact cause of abdominal symptoms in patients with lung cancer[36]. The metastatic lesions were seen on CT scans as wall thickening, an intraluminal polypoid mass or an exophytic mass. PET-CT may be more accurate than CT or other conventional imaging methods for the diagnosis of metastatic malignant sites. However, due to the high cost and the lack of clinical cases, the role of PET-CT in the diagnosis of lung cancer gastrointestinal metastasis is still controversial. Capsule endoscopy has the capability of providing visual images of the bowel and is superior in many aspects of gastrointestinal disease evaluation and management. It can provide help in identifying the presence of small intestinal metastasis when a patient with lung cancer presents with suspected small intestinal bleeding. However, it may have a limited role in patients with bowel perforation and obstruction.

As most lung cancer patients with gastrointestinal metastasis exhibit bowel perforation or an acute abdomen, surgical intervention is required. Dabaja *et al*[37] showed that the 5-year overall survival rate was significantly higher for patients who underwent surgery compared with patients who did not. Laparoscopic surgery not only has the advantages of less trauma, less blood loss and quicker recovery, but also has huge potential in diagnosis. Postoperative chemotherapy and individualized treatment may improve the survival rate for these lung cancer patients. For the lung cancer patient with distant metastasis, the guideline indicates that cisplatin/pemetrexed is recommended for patients with non-squamous non-small cell lung cancer; for patients with squamous cell carcinoma, cisplatin/gemcitabine is an option[38]. The chemotherapy regimen should be changed when there are other metastases present.

Many patients with small bowel metastases had synchronous metastasis in other organs. PAUL reported that 46 patients had small bowel metastasis of 431 autopsies on patients with lung cancer. In addition, all patients with small bowel metastases had at least one other metastatic site with an average of 4.8 sites such as the adrenal gland, liver, and kidney. This indicated that gastrointestinal metastasis is a late symptom of lung cancer and a poor prognostic indicator in the course of lung cancer[39]. In the documented cases, the median survival time was 3 months after detection of small bowel metastases in 48 patients. The poor prognosis was mainly due to late detection, the malignant biological behavior of lung cancer, abdominal infections, surgical complications, and other metastases. Brain and bone are common sites of distant metastases in lung cancer. In contrast, the median survival time of patients with bone metastases was 7 mo. Patients with brain metastases had a median survival of 3-6 mo[40]. Metastases to the small bowel have a worse prognosis than metastases to the brain and bone.

Due to better diagnostic methods and prolonged survival of lung cancer patients, gastrointestinal metastases are more common. When abdominal symptoms are present or persistent positive occult blood tests are found, bowel metastases should be considered. Abdominal CT has high value in diagnosing gastrointestinal metastases. With regard to the treatment of gastrointestinal metastases from lung cancer, surgery can be a curative treatment option. Chemotherapy and supportive care can improve the prognosis.

COMMENTS

Case characteristics

A 66-year-old man with a history of radical lung cancer surgery presented with melena.

Clinical diagnosis

Gastrointestinal hemorrhage; Postoperative lung cancer.

Differential diagnosis

Primary small intestinal carcinoma; colorectal cancer; peptic ulcer.

Laboratory diagnosis

Tumor markers were within normal ranges, including CEA which was 2.75 ng/mL. A complete blood count showed anemia with an Hb level of 9.6 g/dL.

Imaging diagnosis

An abdominal CT scan revealed ileal wall thickening and nearby mesenteric lymph node enlargement, indicating a malignant ileal tumor.

Pathological diagnosis

Histopathological analysis confirmed a moderately differentiated squamous cell carcinoma; immunohistochemistry indicated metastasis from primary lung carcinoma.

Treatment

The patient underwent resection of the involved small bowel and mesentery. Postoperatively, he received four cycles of chemotherapy with gemcitabine/nedaplatin.

Related reports

Gastrointestinal tract metastases from lung cancer are relatively rare, and the clinical symptoms are unclear. The general route of gastrointestinal metastasis and the prognosis are also unclear.

Experiences and lessons

When abdominal symptoms are present or persistent positive occult blood tests are found in patient with lung cancer, bowel metastases should be considered

Peer-review

The authors reported a rare and interesting case of a patient with small intestine metastasis from lung squamous cell carcinoma, and they reviewed 64 documented cases with small intestine metastasis from lung cancer.

REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]

2 **Hoffman PC**, Mauer AM, Vokes EE. Lung cancer. *Lancet* 2000; **355**: 479-485 [PMID: 10841143 DOI: 10.1016/S0140-6736(00)82038-3]

3 **Song Y**, Li M, Shan J, Ye X, Tang S, Fang X, Ding K, Yuan Y. Acute small bowel obstruction: a rare initial presentation for the metastasis of the large-cell carcinoma of the lung. *World J Surg Oncol* 2012; **10**: 26 [PMID: 22284720 DOI: 10.1186/1477-7819-10-26]

4 **Salemis NS**, Nikou E, Liatsos C, Gakis C, Karagkiouzis G, Gourgiotis S. Small bowel perforation secondary to metastatic non-small cell lung cancer. A rare entity with a dismal prognosis.

J Gastrointest Cancer 2012; **43**: 391-395 [PMID: 22033892 DOI: 10.1007/s12029-011-9329-2]
5 **Mulder MC**, Kist JW, Consten EC, Verheijen PM. Gastrointestinal metastasis as the first presentation of lung carcinoma. *Int J Colorectal Dis* 2012; **27**: 839-840 [PMID: 22006495 DOI: 10.1007/s00384-011-1326-1]
6 **Jung LY**, Jeon SY, Kim SR, Chung MJ, Lee YC. Pulmonary sarcomatoid carcinoma accompanying duodenal involvement. *Am J Respir Crit Care Med* 2012; **185**: 899-900 [PMID: 22505760 DOI: 10.1164/ajrccm.185.8.899]
7 **Guner A**, Karyagar S, Livaoglu A, Kece C, Kucuktulu U. Small Bowel Intussusception due to Metastasized Sarcomatoid Carcinoma of the Lung: A Rare Cause of Intestinal Obstruction in Adults. *Case Rep Surg* 2012; **2012**: 962683 [PMID: 23346451 DOI: 10.1155/2012/962683]
8 **Nishizawa Y**, Kobayashi A, Saito N, Nagai K, Sugito M, Ito M, Nishizawa Y. Surgical management of small bowel metastases from primary carcinoma of the lung. *Surg Today* 2012; **42**: 233-237 [PMID: 22045233 DOI: 10.1007/s00595-011-0005-8]
9 **Yamada H**, Akahane T, Horiuchi A, Shimada R, Shibuya H, Hayama T, Nozawa K, Ishihara S, Matsuda K, Watanabe T. A case of lung squamous cell carcinoma with metastases to the duodenum and small intestine. *Int Surg* 2011; **96**: 176-181 [PMID: 22026313]
10 **Lee PC**, Lo C, Lin MT, Liang JT, Lin BR. Role of surgical intervention in managing gastrointestinal metastases from lung cancer. *World J Gastroenterol* 2011; **17**: 4314-4320 [PMID: 22090788 DOI: 10.3748/wjg.v17.i38.4314]
11 **Bugiantella W**, Cavazzoni E, Graziosi L, Valiani S, Franceschini MS, Donini A. Small bowel metastasis from lung cancer: a possible cause of acute abdomen. Case report and literature review. *G Chir* 2011; **32**: 120-122 [PMID: 21453601]
12 **Sayilir A**, Oztaş E, Onal IK, Kurt M, Kekıllı M, Akdoğ G, Ibış M. Small bowel obstruction: a presenting symptom of squamous cell carcinoma of the lung. *Turk J Gastroenterol* 2011; **22**: 440-442 [PMID: 21948581 DOI: 10.4318/tjg.2011.0292]
13 **Kim SY**, Ha HK, Park SW, Kang J, Kim KW, Lee SS, Park SH, Kim AY. Gastrointestinal metastasis from primary lung cancer: CT findings and clinicopathologic features. *AJR Am J Roentgenol* 2009; **193**: W197-W201 [PMID: 19696259 DOI: 10.2214/AJR.08.1907]
14 **Chiu WK**, Lin YC, Wang LT, Chen JH, Yu JC, Hsieh CB. Jejunojejunal intussusception secondary to metastasis from adenocarcinoma of the lung--a case report. *Acta Chir Belg* 2009; **109**: 519-522 [PMID: 19803270]
15 **Huang YJ**, Wu MH, Lin MT. Multiple small-bowel intussusceptions caused by metastatic malignant melanoma. *Am J Surg* 2008; **196**: e1-e2 [PMID: 18513686 DOI: 10.1016/j.amjsurg.2007.05.062]
16 **Kagohashi K**, Kadono K, Satoh H, Ohtsuka M. Intussusception due to intestinal metastasis from lung cancer. *Lung Cancer* 2007; **57**: 247-248 [PMID: 17602784 DOI: 10.1016/j.lungcan.2007.05.008]
17 **Goh BK**, Yeo AW, Koong HN, Ooi LL, Wong WK. Laparotomy for acute complications of gastrointestinal metastases from lung cancer: is it a worthwhile or futile effort? *Surg Today* 2007; **37**: 370-374 [PMID: 17468816 DOI: 10.1007/s00595-006-3419-y]
18 **Kostakou C**, Khaldi L, Flossos A, Kapsoritakis AN, Potamianos SP. Melena: a rare complication of duodenal metastases from primary carcinoma of the lung. *World J Gastroenterol* 2007; **13**: 1282-1285 [PMID: 17451216]
19 **Gómez-Patiño JA**, Almaraz CS, Sánchez MI. [Primary lung carcinoma with intestinal metastases: 3 cases in a series of 420 patients]. *Arch Bronconeumol* 2007; **43**: 472-473 [PMID: 17692250 DOI: 10.1016/S1579-2129(07)60106-2]
20 **Yuksel O**, Uyar P, Sahin TT, Demirhan B. Small bowel perforation due to metastatic lung squamous cell carcinoma. *Saudi Med J* 2007; **28**: 631-633 [PMID: 17457493]
21 **Yang CJ**, Hwang JJ, Kang WY, Chong IW, Wang TH, Sheu CC, Tsai JR, Huang MS. Gastro-intestinal metastasis of primary lung carcinoma: clinical presentations and outcome. *Lung Cancer* 2006; **54**: 319-323 [PMID: 17010474 DOI: 10.1016/j.lungcan.2006.08.007]
22 **Kanemoto K**, Kurishima K, Ishikawa H, Shiotani S, Satoh H, Ohtsuka M. Small intestinal metastasis from small cell lung cancer. *Intern Med* 2006; **45**: 967-970 [PMID: 16974060 DOI: 10.2169/internalmedicine.45.1651]
23 **Alvarez Laso C**, Paredes Aracil E, Azcano González E, Ots Gutiérrez JR, Bernabeu Miralles M, Compañ Rosique A. Small bowel occlusion due to intussusception of a single metastasis of lung cancer. *Clin Transl Oncol* 2006; **8**: 833-834 [PMID: 17134974]
24 **Yilmaz S**, Dursun M, Canoruç F, Bayan K, Büyükbayram H. Upper gastrointestinal bleeding caused by small-cell lung cancer: a case report. *Dig Dis Sci* 2006; **51**: 788-790 [PMID: 16615004 DOI: 10.1007/s10620-006-3207-y]
25 **Tomas D**, Ledinsky M, Belicza M, Kruslin B. Multiple metastases to the small bowel from large cell bronchial carcinomas. *World J Gastroenterol* 2005; **11**: 1399-1402 [PMID: 15761985]
26 **Burnette RE**, Ballard BR. Metastatic pleomorphic carcinoma of lung presenting as abdominal pain. *J Natl Med Assoc* 2004; **96**: 1657-1660 [PMID: 15622698]
27 **Felsher J**, Brodsky J, Brody F. Laparoscopic small bowel resection of metastatic pulmonary carcinosarcoma. *J Laparoendosc Adv Surg Tech A* 2003; **13**: 397-400 [PMID: 14733704 DOI: 10.1089/109264203322656478]
28 **Sakorafas GH**, Pavlakis G, Grigoriadis KD. Small bowel perforation secondary to metastatic lung cancer: a case report and review of the literature. *Mt Sinai J Med* 2003; **70**: 130-132 [PMID: 12634905]
29 **Testini M**, Trabucco S, Di Venere B, Piscitelli D. Ileal intussusception due to intestinal metastases from primary malignant melanoma of the lung. *Am Surg* 2002; **68**: 377-379 [PMID: 11952250]
30 **Yuen JS**, Chow PK, Ahmed Q. Metastatic lung cancer causing bowel perforations: spontaneous or chemotherapy-related? *ANZ J Surg* 2002; **72**: 245-246 [PMID: 12071466 DOI: 10.1046/j.1445-2197.2002.02236.x]
31 **Cremon C**, Barbara G, De Giorgio R, Salvioli B, Epifanio G, Gizzi G, Stanghellini V, Corinaldesi R. Upper gastrointestinal bleeding due to duodenal metastasis from primary lung carcinoma. *Dig Liver Dis* 2002; **34**: 141-143 [PMID: 11926559 DOI: 10.1016/S1590-8658(02)80245-6]
32 **Lee KA**, Lee SK, Seo DW, Kim MH. Duodenal metastasis from lung cancer presenting as obstructive jaundice. *Gastrointest Endosc* 2001; **54**: 228 [PMID: 11474398 DOI: 10.1067/mge.2001.116896]
33 **Chagpar A**, Moyana TN, Chappell EW. Isolated metachronous jejunal metastases after resection of bronchogenic carcinoma. *Can J Surg* 2000; **43**: 57-58 [PMID: 10714260]
34 **Yoshimoto A**, Kasahara K, Kawashima A. Gastrointestinal metastases from primary lung cancer. *Eur J Cancer* 2006; **42**: 3157-3160 [PMID: 17079136 DOI: 10.1016/j.ejca.2006.08.030]
35 **Antler AS**, Ough Y, Pitchumoni CS, Davidian M, Thelmo W. Gastrointestinal metastases from malignant tumors of the lung. *Cancer* 1982; **49**: 170-172 [PMID: 6274500]
36 **Rossi G**, Marchioni A, Romagnani E, Bertolini F, Longo L, Cavazza A, Barbieri F. Primary lung cancer presenting with gastrointestinal tract involvement: clinicopathologic and immunohistochemical features in a series of 18 consecutive cases. *J Thorac Oncol* 2007; **2**: 115-120 [PMID: 17410025]
37 **Dabaja BS**, Suki D, Pro B, Bonnen M, Ajani J. Adenocarcinoma of the small bowel: presentation, prognostic factors, and outcome of 217 patients. *Cancer* 2004; **101**: 518-526 [PMID: 15274064 DOI: 10.1002/cncr.20404]
38 **Ettinger DS**, Akerley W, Borghaei H, Chang AC, Cheney RT, Chirieac LR, D'Amico TA, Demmy TL, Ganti AK, Govindan R, Grannis FW, Horn L, Jahan TM, Jahanzeb M, Kessinger A, Komaki R, Kong FM, Kris MG, Krug LM, Lennes IT, Loo BW, Martins R, O'Malley J, Osarogiagbon RU, Otterson GA, Patel JD, Pinder-Schenck MC, Pisters KM, Reckamp K, Riely GJ, Rohren E, Swanson SJ, Wood DE, Yang SC, Hughes M, Gregory KM. Non-small cell lung cancer. *J Natl Compr Canc Netw* 2012; **10**: 1236-1271 [PMID: 23054877]

39 **McNeill PM**, Wagman LD, Neifeld JP. Small bowel metastases from primary carcinoma of the lung. *Cancer* 1987; **59**: 1486-1489 [PMID: 3028602]
40 **D'Antonio C**, Passaro A, Gori B, Del Signore E, Migliorino MR, Ricciardi S, Fulvi A, de Marinis F. Bone and brain metastasis in lung cancer: recent advances in therapeutic strategies. *Ther Adv Med Oncol* 2014; **6**: 101-114 [PMID: 24790650 DOI: 10.1177/1758834014521110]

P- Reviewer: Tsuchiya A, Herszenyi L, Keishiro A **S- Editor**: Yu J
L- Editor: A **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3441

World J Gastroenterol 2015 March 21; 21(11): 3441-3446
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Icotinib plus gemcitabine for metastatic pancreatic cancer: A case report

Jing Zhao, Hong Shen, Han-Guang Hu, Jian-Jin Huang

Jing Zhao, Hong Shen, Han-Guang Hu, Jian-Jin Huang, Department of Medical Oncology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, Zhejiang Province, China

Author contributions: Zhao J and Shen H wrote the manuscript; Hu HG participated in the clinical management of the patient; Huang JJ was in charge of the patient; and all authors approved the final manuscript.

Supported by National Natural Science Foundation of China, No. 81201640; Department of Education of Zhejiang Province of China, No. Y201225802; and Medical and Technology Plan of Zhejiang Province of China, No. 2013KYA093.

Correspondence to: Jian-Jin Huang, MD, Department of Medical Oncology, The Second Affiliated Hospital, Zhejiang University School of Medicine, 88 Jie-Fang Road, Hangzhou 310009, Zhejiang Province, China. hhjj@medmail.com.cn
Telephone: +86-571-87784795
Fax: +86-571-87767088

Received: August 8, 2014
Peer-review started: August 9, 2014
First decision: September 15, 2014
Revised: October 5, 2014
Accepted: November 19, 2014
Article in press: November 19, 2014
Published online: March 21, 2015

Abstract

A large majority of patients diagnosed with pancreatic cancer have advanced metastatic disease with unresectable malignancies. Despite treatment advances, the survival benefit from chemotherapeutic regimens and targeted drugs is limited. Moreover, their application is limited in China because of high toxicity and cost. Recently, inhibitors of epidermal growth factor receptor activity have shown promise for the treatment of solid cancers when used in combination with standard therapy. However, these drugs have not been evaluated extensively for the treatment of pancreatic cancer. Here, we report the treatment of a 64-year-old male with metastatic pancreatic cancer using a novel regimen of icotinib with gemcitabine. Marked shrinkage of the mass was observed after two treatment cycles, and partial remission was achieved. The abdominal pain was relieved. The adverse effects were tolerable and treatment cost was acceptable. This is the first reported case for the treatment of advanced pancreatic cancer with icotinib plus gemcitabine and demonstrates a promising therapeutic alternative.

Key words: Epidermal growth factor receptor tyrosine kinase inhibitor; Gemcitabine; Icotinib; Metastatic pancreatic cancer; Regimen

Core tip: Patients with advanced metastatic pancreatic cancer have a poor prognosis. Currently, treatments available in China are accompanied by high toxicity and cost, with minimal benefit. Icotinib is a newly developed oral epidermal growth factor receptor tyrosine kinase inhibitor, which was combined with standard gemcitabine therapy for treatment of metastatic pancreatic cancer in a 64-year-old male patient. This case report demonstrates that the novel regimen produced partial remission with marked shrinkage of the pancreatic mass. Thus, icotinib combined with gemcitabine may be a promising therapeutic alternative for metastatic pancreatic cancer.

Zhao J, Shen H, Hu HG, Huang JJ. Icotinib plus gemcitabine for metastatic pancreatic cancer: A case report. *World J Gastroenterol*

2015; 21(11): 3441-3446 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3441.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3441

INTRODUCTION

Pancreatic cancer is one of the most aggressive and lethal malignancies. The mortality rate of pancreatic cancer is extremely high, with a 25% one-year survival rate, and < 5% five-year survival[1]. According to the 2012 China Annual Report of Cancer Registration, pancreatic cancer incidence was ranked seventh in China, and mortality ranked sixth[2]. Early detection is difficult, thus 80% of patients have locally advanced or distant metastases when diagnosed[3]. Consequently, these patients need chemotherapy, radiotherapy, or palliative surgery to prolong their survival, control symptoms and improve their quality of life.

Gemcitabine has been the standard treatment for patients with locally advanced or metastatic pancreatic cancer since 1997. Although it improves survival compared with fluorouracil[4], it provides only marginal survival benefits. Recent evidence implicates the epidermal growth factor receptor (EGFR) signaling pathway in the development, progression, and metastasis of pancreatic cancer[5-7], and blockade of EGFR suppresses tumor proliferation[8]. Icotinib is a recently developed oral EGFR tyrosine kinase inhibitor that suppresses tumor growth and improves survival of Chinese patients with non-small-cell lung cancer[9]. However, there are no reports concerning its use for treatment of pancreatic cancer. We present the case of a patient diagnosed with metastatic pancreatic cancer who was treated with icotinib and gemcitabine.

CASE REPORT

A 64-year-old Asian male patient was admitted to our hospital in January 2014 complaining of epigastric pain that radiated toward the back, which had been present for one month. The pain was accompanied by abdominal distension, but without nausea, vomiting, or jaundice. The Karnofsky Performance Status score was 90, and the pain score-assessed with a numerical rating scale-was a 3. Laboratory examinations revealed normal liver, biliary, and pancreatic enzymes, and a normal complete blood count. The markers for hepatitis A, B, and C were negative. Screening for tumor markers showed elevated levels of carcinoembryonic antigen (CEA) (33.9 ng/mL), carbohydrate antigen (CA) 19-9 (> 12000.0 U/mL), and CA 242 (> 500.0 U/mL). Magnetic resonance imaging revealed a 3.6 cm × 3.4 cm mass in the head of the pancreas (Figure 1A) and multiple nodules in the liver (Figure 2A), which were also observed by positron emission tomography-computerized tomography (Figure 3). An ultrasound-guided percutaneous needle biopsy of the pancreatic

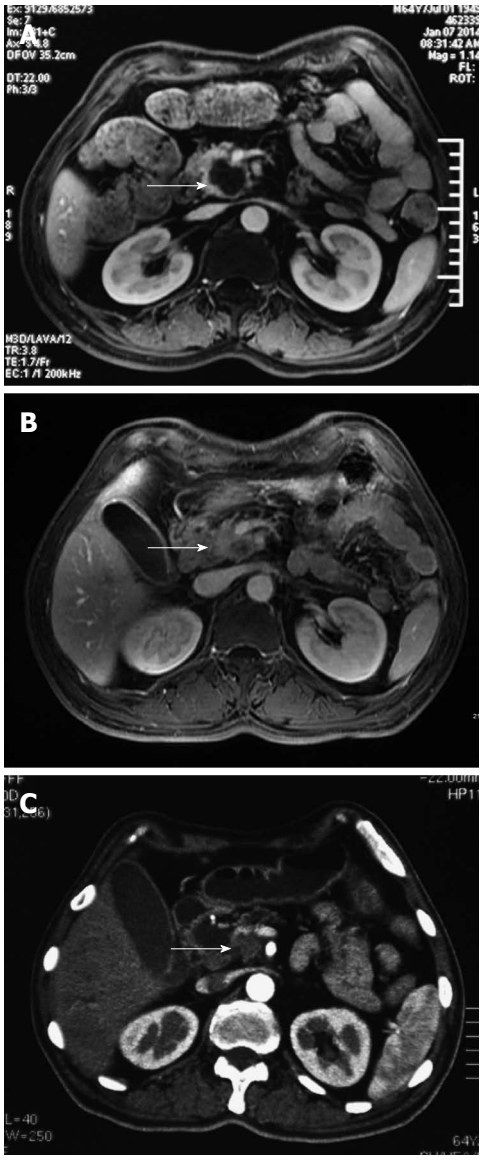


Figure 1 Contrast-enhanced magnetic resonance imaging of the pancreas. A: Before treatment, a mass was observed (arrow) in the head of pancreas; B: Marked shrinkage of the mass was seen after two treatment cycles; C: Mass size remained stable after four treatment cycles.

mass was performed, and pathology indicated that it was an adenocarcinoma (Figure 4). The patient was diagnosed with stage Ⅳ pancreatic cancer with liver metastasis.

Because the mass was unresectable, the patient was given palliative therapy with 125 mg tid icotinib (Conmana; Betta Pharmaceuticals Co. Ltd., Zhejiang, China), combined with 1000 mg/m^2 gemcitabine on days 1, 8, and 15 every four weeks. The patient complained of abdominal pain (score 3), which was treated with a non-steroidal anti-inflammatory (celecoxib). The patient developed a grade 1 acne-like rash and grade 2 myelosuppression in response to the chemotherapy, which was well tolerated and recovered quickly with supportive care.

After two cycles of therapy, serum tumor markers

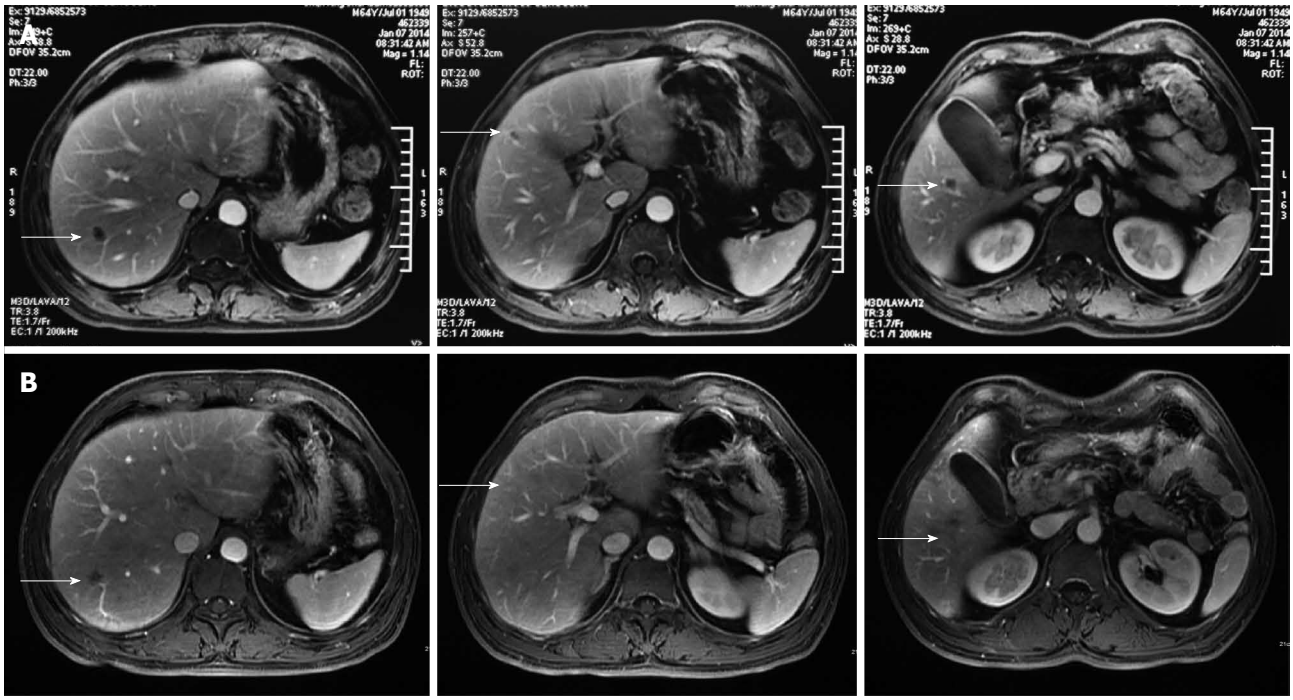


Figure 2 Contrast-enhanced magnetic resonance imaging of the liver. A: Before treatment, multiple metastatic nodules (arrows) were observed in the liver at three different levels; B: Marked shrinkage of the nodules was seen after two treatment cycles.

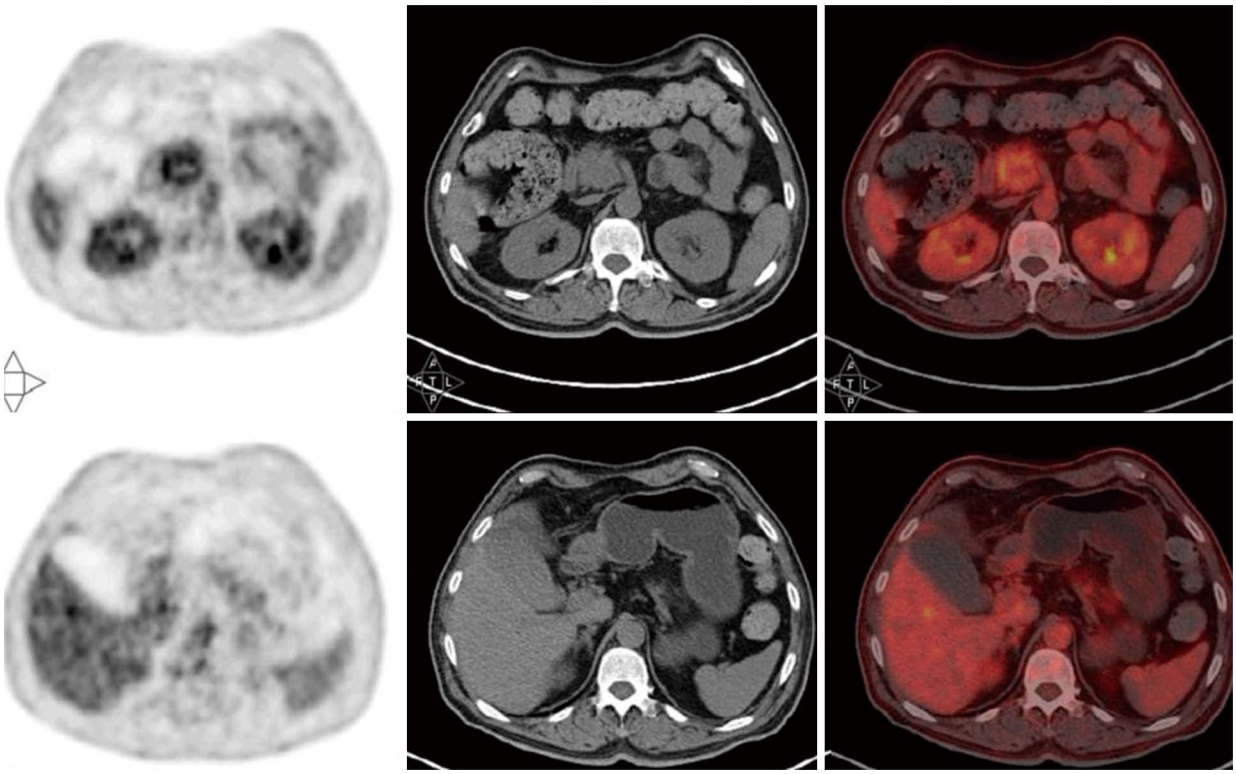


Figure 3 Positron emission tomography-computerized tomography. Masses in the pancreas and liver were observed before treatment.

were decreased: CEA 4.9 ng/mL; CA 19-9, 2369.9 U/mL; CA 242, 329.1 U/mL. Because the pain decreased to a score of 0-1, the celecoxib was eliminated from the treatment regimen. Magnetic resonance imaging revealed marked shrinkage of the masses in the pancreas and liver (Figure 1B, 2B), with partial remission according to the Response Evaluation Criteria In Solid Tumors 1.1 criteria[10].

On day 8 of the third cycle, the patient suffered a grade 3 liver injury. A liver panel screen showed

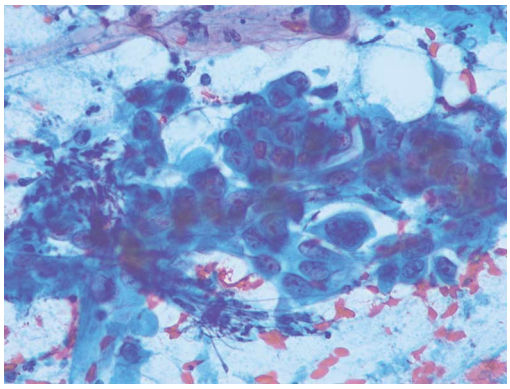


Figure 4 Cytological examination of the pancreatic mass. Hematoxylin and eosin staining indicated the mass was an adenocarcinoma (magnification × 200).

alanine transaminase at 400 U/L and aspartate transaminase at 210 U/L. The patient did not complain of nausea, vomiting, anorexia, or fatigue, however the chemotherapy regimen was suspended. Hepatic protective therapy was initiated, and chemotherapy was resumed once the liver enzymes decreased to within the normal range.

After four treatment cycles, the patient developed jaundice. The liver panel showed elevated biliary enzymes and serum bilirubin, and serum CA 19-9 was again elevated (> 12000.0 U/mL). A repeat abdominal computed tomography scan revealed no change in the pancreatic mass (Figure 1C) with significant enlargement of the intrahepatic biliary duct. Treatment was terminated because of the obstructive jaundice, which resolved with endoscopic retrograde biliary drainage. The patient refused further chemotherapy. He remains under supportive care in lieu of further treatment.

DISCUSSION

Survival of patients with pancreatic cancer can be improved by combining standard treatment with novel therapies. Combining gemcitabine with nab-paclitaxel or treatments containing folinic acid, fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX), improves the progression-free and median overall survivals, and overall response rate[11-13]. However, these regimens have many limitations. For example, Conroy *et al*[11] showed increased toxicity with FOLFIRINOX, including grade 3/4 neutropenia, febrile neutropenia, and diarrhea. Similarly, Von Hoff *et al*[12] found that grade 3/4 neutropenia and leukopenia occurred more frequently in patients receiving gemcitabine combined with nab-paclitaxel. In addition, these treatments are expensive and are not covered by medical insurance in China. Therefore, an effective, economical, and safe regimen for the treatment of advanced pancreatic cancer is urgently needed.

Inhibition of EGFR by monoclonal antibodies, which competitively inhibit ligand binding, and tyrosine kinase inhibitors has provided an additional strategy for cancer treatment[14]. A phase Ⅱ trial showed that nimotuzumab with gemcitabine improved the progression-free and overall survivals of advanced pancreatic cancer patients, though one-third of patients experienced grade 3 adverse events[13]. The therapeutic value of cetuximab remains unclear[15]. EGFR tyrosine kinase inhibitors, such as erlotinib, gefitinib, and icotinib, may provide a more economical alternative. A Canadian phase Ⅲ trial indicates that supplementing treatment with erlotinib significantly improved the progression-free, overall, and one-year survivals of pancreatic cancer patients compared with gemcitabine alone[16]. Treatment with erlotinib was well tolerated in these patients, with only a slight increase in the incidence of grade 3/4 rash and diarrhea, and no difference in the quality of life score. Erlotinib has since been approved for treatment of locally advanced and metastatic pancreatic cancer by the Food and Drug Administration and the European Union.

Icotinib is a highly specific and selective EGFR tyrosine kinase inhibitor. It is a small quinazoline compound with a chemical backbone structure similar to erlotinib, with a shorter halftime[17]. Icotinib has been shown to regulate the transcription and expression of a gene associated with cell proliferation and differentiation, and showing antiproliferative and antiangiogenic effects[18,19]. As icotinib was approved by the State Food and Drug Administration of China for the treatment of non-small-cell lung cancer in 2011, it is covered by medical insurance in Zhejiang province and represents an affordable treatment option for many patients. Icotinib has been evaluated for the treatment of solid tumors[20], and a phase Ⅲ clinical trial demonstrated effects equivalent to gefitinib in patients with non-small-cell lung cancer, with a better safety profile and lower cost[9]. The adverse effects reported included rash, diarrhea, nausea, and abdominal distention.

The patient in the case presented here agreed to combination treatment with icotinib after refusing erlotinib and nimotuzumab because of the high cost, and FOLFIRINOX because of the adverse effects. The treatment effectively relieved the abdominal pain, reduced the size of the pancreatic mass after two treatment cycles and achieved partial remission, with markedly reduced serum CA 19-9, CEA, and CA 242 levels. The progression-free survival was 3.73 mo, similar to the gemcitabine and erlotinib regimen and better than gemcitabine alone[16]. The patient experienced low-grade adverse events, and recovered rapidly from the liver injury experienced during the third treatment cycle. To our knowledge, this is the first reported case of icotinib with gemcitabine for the treatment of advanced pancreatic cancer, and demonstrates that this regimen is a promising alternative treatment option.

In conclusion, the combination of icotinib with gemcitabine may be a viable option for the treatment of metastatic pancreatic cancer, with acceptable adverse effects and cost. However, large prospective studies should be carried out to further clarify the efficiency, safety, and overall survival benefit.

COMMENTS

Case characteristics

A 64-year-old Asian male patient with epigastric pain radiating toward the back that had been present for one month.

Clinical diagnosis

Pancreatic cancer with liver metastasis.

Differential diagnosis

Pancreatic cyst; islet cell tumor; liver cyst.

Laboratory diagnosis

Carcinoembryonic antigen, 33.9 ng/mL; carbohydrate antigen 19-9, > 12000.0 U/mL; carbohydrate antigen 242, > 500.0 U/mL; normal liver, biliary, and pancreatic enzymes; normal complete blood count.

Imaging diagnosis

Contrast-enhanced magnetic resonance and positron emission tomography-computerized tomography showed a mass in the head of pancreas (3.6 cm × 3.4 cm) and multiple nodules in the liver.

Pathological diagnosis

Hematoxylin and eosin staining indicated adenocarcinoma.

Treatment

Icotinib (125 mg, *tid*) with gemcitabine (1000 mg/m^2) on days 1, 8, and 15 every four weeks.

Related reports

This is the first case for the treatment of advanced pancreatic cancer with icotinib combined with gemcitabine.

Term explanation

Icotinib is an epidermal growth factor receptor tyrosine kinase inhibitor. Gemcitabine is a nucleoside analog used for chemotherapy.

Experiences and lessons

Combination therapy comprised of icotinib and gemcitabine is an alternative choice for treatment of metastatic pancreatic cancer with tolerated adverse effects and acceptable cost.

Peer-review

An interesting case study of response to a newer combination therapy in patients with advanced metastatic cancer. The authors would have done well documenting the PET/CT response after therapy. The case is nicely documented, except that the manuscript looks to be corrected for grammar and spellings.

REFERENCES

1 **Li D**, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet* 2004; **363**: 1049-1057 [PMID: 15051286 DOI: 10.1016/S0140-6736(04)15841-8]

2 **He J**, Zhao P, Chen WQ. Chinese cancer registry annual report. Beijing: Military medical science press, 2012: 56-58

3 **Zervos EE**, Rosemurgy AS, Al-Saif O, Durkin AJ. Surgical management of early-stage pancreatic cancer. *Cancer Control* 2004; **11**: 23-31 [PMID: 14749620]

4 **Burris HA**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413 [PMID: 9196156]

5 **Tobita K**, Kijima H, Dowaki S, Kashiwagi H, Ohtani Y, Oida Y, Yamazaki H, Nakamura M, Ueyama Y, Tanaka M, Inokuchi S, Makuuchi H. Epidermal growth factor receptor expression in human pancreatic cancer: Significance for liver metastasis. *Int J Mol Med* 2003; **11**: 305-309 [PMID: 12579331]

6 **Bruns CJ**, Solorzano CC, Harbison MT, Ozawa S, Tsan R, Fan D, Abbruzzese J, Traxler P, Buchdunger E, Radinsky R, Fidler IJ. Blockade of the epidermal growth factor receptor signaling by a novel tyrosine kinase inhibitor leads to apoptosis of endothelial cells and therapy of human pancreatic carcinoma. *Cancer Res* 2000; **60**: 2926-2935 [PMID: 10850439]

7 **Troiani T**, Martinelli E, Capasso A, Morgillo F, Orditura M, De Vita F, Ciardiello F. Targeting EGFR in pancreatic cancer treatment. *Curr Drug Targets* 2012; **13**: 802-810 [PMID: 22458527 DOI: 10.2174/138945012800564158]

8 **Ng SS**, Tsao MS, Nicklee T, Hedley DW. Effects of the epidermal growth factor receptor inhibitor OSI-774, Tarceva, on downstream signaling pathways and apoptosis in human pancreatic adenocarcinoma. *Mol Cancer Ther* 2002; **1**: 777-783 [PMID: 12492110]

9 **Shi Y**, Zhang L, Liu X, Zhou C, Zhang L, Zhang S, Wang D, Li Q, Qin S, Hu C, Zhang Y, Chen J, Cheng Y, Feng J, Zhang H, Song Y, Wu YL, Xu N, Zhou J, Luo R, Bai C, Jin Y, Liu W, Wei Z, Tan F, Wang Y, Ding L, Dai H, Jiao S, Wang J, Liang L, Zhang W, Sun Y. Icotinib versus gefitinib in previously treated advanced non-small-cell lung cancer (ICOGEN): a randomised, double-blind phase 3 non-inferiority trial. *Lancet Oncol* 2013; **14**: 953-961 [PMID: 23948351 DOI: 10.1016/S1470-2045(13)70355-3]

10 **Eisenhauer EA**, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**: 228-247 [PMID: 19097774 DOI: 10.1016/j.ejca.2008.10.026]

11 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

12 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]

13 **Su D**, Jiao SC, Wang LJ, Shi WW, Long YY, Li J, Bai L. Efficacy of nimotuzumab plus gemcitabine usage as first-line treatment in patients with advanced pancreatic cancer. *Tumour Biol* 2014; **35**: 2313-2318 [PMID: 24142531 DOI: 10.1007/s13277-013-1306-x]

14 **Ciardiello F**, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med* 2008; **358**: 1160-1174 [PMID: 18337605 DOI: 10.1056/NEJMra0707704]

15 **Philip PA**, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, Khorana AA, Goldman B, Fenoglio-Preiser CM, Abbruzzese JL, Blanke CD. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. *J Clin Oncol* 2010; **28**: 3605-3610 [PMID: 20606093 DOI: 10.1200/JCO.2009.25.7550]

16 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677 DOI: 10.1200/JCO.2006.07.9525]

17 **Guan YS**, He Q, Li M. Icotinib: activity and clinical application in Chinese patients with lung cancer. *Expert Opin Pharmacother*

2014; **15**: 717-728 [PMID: 24588695 DOI: 10.1517/14656566.2014.890183]

18 **Zhao Q**, Shentu J, Xu N, Zhou J, Yang G, Yao Y, Tan F, Liu D, Wang Y, Zhou J. Phase I study of icotinib hydrochloride (BPI-2009H), an oral EGFR tyrosine kinase inhibitor, in patients with advanced NSCLC and other solid tumors. *Lung Cancer* 2011; **73**: 195-202 [PMID: 21144613 DOI: 10.1016/j.lungcan.2010.11.007]

19 **Yang C**, Yan J, Yuan G, Zhang Y, Lu D, Ren M, Cui W. Icotinib inhibits the invasion of Tca8113 cells via downregulation of nuclear factor κB-mediated matrix metalloproteinase expression. *Oncol Lett* 2014; **8**: 1295-1298 [PMID: 25120710 DOI: 10.3892/ol.2014.2311]

20 **Chen H**, Ren SH, Pang HM, Wang RL, Shi GG, Ge HT, Zhang XY, Wang L. Clinic Research on Icotinib and Capecitabine in the Treatment of Senile Advanced Colorectal Cancer (in Chinese). *Zhongguo QuanKe Yixue* 2013; **16**: 3960-3962

P- Reviewer: Nakano H, Sikora SS, Smith RC **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i11.3447

World J Gastroenterol 2015 March 21; 21(11): 3447-3448
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Cobalamin deficiency as an extra intestinal manifestation of *Helicobacter pylori* infection

Emmanuel Andrès

Emmanuel Andrès, Department of Internal Medicine, University Hospital of Strasbourg, 67000 Strasbourg, France
Author contributions: Andrès E wrote the paper.
Conflict-of-interest: Professor Emmanuel Andrès has received fees for serving as a speaker, as a consultant and as an advisory board member for the Laboratories: BMS, Pfizer, GSK, Ferring. Professor Emmanuel Andrès has received research funding from the French Research Agency.

Correspondence to: Emmanuel Andrès, Professor of Internal Medicine, Department of Internal medicine, University Hospital of Strasbourg, 67000 Strasbourg,
France. emmanuel.andres@chru-strasbourg.fr
Telephone: +33-388115066
Fax: +33-388116262
Received: September 15, 2014
Peer-review started: September 19, 2014
First decision: November 18, 2014
Revised: December 5, 2014
Accepted: December 22, 2014
Article in press: December 22, 2014
Published online: March 21, 2015

Abstract

We read with great interest the excellent review by Wong *et al* on extra intestinal manifestations of *Helicobacter pylori* (*H. pylori*) infection published in the journal. This is a well-documented and structured review. However, I believe that Wong *et al* failed to report the relationship between *H. pylori* infection and cobalamin.

Key words: Cobalamin deficiency; *Helicobacter pylori*; Food-cobalamin deficiency; Oral cobalamin therapy

Core tip: We read with great interest the excellent review by Wong *et al* on extra intestinal manifestations of *Helicobacter pylori* (*H. pylori*) infection published in the journal. This is a well-documented and structured review. However, I believe that Wong *et al* failed to report the relationship between *H. pylori* infection and cobalamin.

Andrès E. Cobalamin deficiency as an extra intestinal manifestation of *Helicobacter pylori* infection. *World J Gastroenterol* 2015; 21(11): 3447-3448 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3447.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3447

TO THE EDITOR

We read with great interest the excellent review by Wong *et al*[1] on extra intestinal manifestations of *Helicobacter pylori* (*H. pylori*) infection published in the journal. This is a well-documented and structured review. However, I believe that Wong *et al*[1] failed to report the relationship between *H. pylori* infection and cobalamin. In fact, in our opinion, cobalamin [vitamin (B_{12})] deficiency related to *H. pylori* infection is a well-studied hematological manifestation, comparable to iron deficiency. This is supported clinically by current guidelines[2], several clinical studies on cobalamin deficiency[3,4] and a therapeutic study[5]. This latter study probably provides the most convincing arguments to support the role of *H. pylori* in the genesis of vitamin B_{12} deficiency. The study of Andrès *et al*[5] showed a correction between vitamin B_{12} deficiency and an eradication treatment of *H.*

pylori (without any supplementation of cobalamin). *H. pylori* causes vitamin B_{12} deficiency and related manifestations (macrocytic anemia or neurological manifestations) by food-cobalamin malabsorption[3,4]. Pangastritis leads to decreased gastric acid, which impairs the release of vitamin B_{12} from haptocorrin to the intrinsic factor. Thus, oral cobalamin therapy (not only intramuscular therapy) may be used to treat cobalamin deficiency related to *H. pylori* infection.

REFERENCES

1 **Wong F**, Rayner-Hartley E, Byrne MF. Extraintestinal manifestations of Helicobacter pylori: a concise review. *World J Gastroenterol* 2014; **20**: 11950-11961 [PMID: 25232230 DOI: 10.3748/wjg.v20.i34.11950]

2 **Carmel R**, Sarrai M. Diagnosis and management of clinical and subclinical cobalamin deficiency: advances and controversies. *Curr Hematol Rep* 2006; **5**: 23-33 [PMID: 16537043]

3 **Andrès E**, Perrin AE, Demangeat C, Kurtz JE, Vinzio S, Grunenberger F, Goichot B, Schlienger JL. The syndrome of food-cobalamin malabsorption revisited in a department of internal medicine. A monocentric cohort study of 80 patients. *Eur J Intern Med* 2003; **14**: 221-226 [PMID: 12919836 DOI: 10.1016/S0953-6205(03)00074-8]

4 **Andrès E**, Loukili NH, Noel E, Kaltenbach G, Abdelgheni MB, Perrin AE, Noblet-Dick M, Maloisel F, Schlienger JL, Blicklé JF. Vitamin B12 (cobalamin) deficiency in elderly patients. *CMAJ* 2004; **171**: 251-259 [PMID: 15289425]

5 **Andrès E**, Fothergill H, Mecili M. Efficacy of oral cobalamin (vitamin B12) therapy. *Expert Opin Pharmacother* 2010; **11**: 249-256 [PMID: 20088746 DOI: 10.1517/14656560903456053]

P- Reviewer: Quadros EV, Treiber G **S- Editor**: Yu J
L- Editor: Stewart G **E- Editor**: Zhang DN

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327