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

World Journal of *Gastroenterology*

World J Gastroenterol 2016 December 28; 22(48): 10477-10686

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 1375 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 (25), Chile (4), China (165), Croatia (2), Cuba (1), Czech (6), Denmark (2), Egypt (9), Estonia (2), Finland (6), France (20), Germany (58), Greece (31), Guatemala (1), Hungary (14), Iceland (1), India (33), Indonesia (2), Iran (10), Ireland (9), Israel (18), Italy (194), Japan (149), 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).

Gordon Stanley Howarth, *Roseworthy*
Seungha Kang, *Brisbane*
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*
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*
Yan-Chang Lei, *Hangzhou*
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*
Di Qu, *Shanghai*
Ju-Wei Mu, *Beijing*
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*

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*
Ferenc Sipos, *Budapest*
Miklós Tanyi, *Debrecen*
Tibor Wittmann, *Szeged*

Iceland

Tryggvi Bjorn Stefánsson, *Reykjavík*

Indiad

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*
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*
Naoaki Sakata, *Sendai*
Ken Sato, *Maebashi*
Toshiro Sato, *Tokyo*
Tomoyuki Shibata, *Toyoake*
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*
Letiţia 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*
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*

World Journal of Gastroenterology

Contents

Weekly Volume 22 Number 48 December 28, 2016

Randomized Controlled Trial

CASE REPORT

World Journal of Gastroenterology
Volume 22 Number 48 December 28, 2016

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
Yuan Qi, Vice Director
World Journal of Gastroenterology
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
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: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
December 28, 2016

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/bpg/gerinfo/204

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.v22.i48.10477

World J Gastroenterol 2016 December 28; 22(48): 10477-10481
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Protons pump inhibitor treatment and lower gastrointestinal bleeding: Balancing risks and benefits

Alberto Lué, Angel Lanas

Alberto Lué, Angel Lanas, Digestive Diseases Service, University Clinic Hospital Lozano Blesa, Avenida San Juan Bosco, 50009 Zaragoza, Spain

Alberto Lué, Angel Lanas, IIS Aragon, Avenida San Juan Bosco, 50009 Zaragoza, Spain

Angel Lanas, University of Zaragoza, Calle de Pedro Cerbuna, 50009 Zaragoza, Spain

Angel Lanas, CIBERehd, Av. Monforte de Lemos, 28029 Madrid, Spain

Author contributions: Lué A and Lanas A contributed to this paper.

Conflict-of-interest statement: Lué A and Lanas A declare no conflict of interest related to this publication.

Manuscript source: Invited manuscript

Correspondence to: Angel Lanas, MD, DSc, Professor, Chairman, Digestive Diseases Service, University Clinic Hospital Lozano Blesa, Avenida San Juan Bosco, 50009 Zaragoza, Spain. alanas@unizar.es
Telephone: +34-97-6768886
Fax: +34-97-6768846

Received: August 27, 2016
Peer-review started: August 31, 2016
First decision: September 20, 2016
Revised: September 28, 2016
Accepted: November 12, 2016
Article in press: November 13, 2016
Published online: December 28, 2016

Abstract

Proton pump inhibitors (PPIs) represent a milestone in the treatment of acid-related diseases, and are the mainstay in preventing upper gastrointestinal bleeding in high-risk patients treated with nonsteroidal anti-inflammatory drugs (NSAIDs) or low-dose aspirin. However, this beneficial effect does not extend to the lower gastrointestinal tract. PPIs do not prevent NSAID or aspirin-associated lower gastrointestinal bleeding (LGB). PPIs may increase both small bowel injury related to NSAIDs and low-dose aspirin treatment and the risk of LGB. Recent studies suggested that altering intestinal microbiota by PPIs may be involved in the pathogenesis of NSAID-enteropathy. An increase in LGB hospitalization rates may occur more frequently in older patients with more comorbidities and are associated with high hospital resource utilization, longer hospitalization, and increased mortality. Preventive strategies for NSAID and aspirin-associated gastrointestinal bleeding should be directed toward preventing both upper and lower gastrointestinal damage. Future research should be directed toward identifying patients at low-risk for gastrointestinal events associated with the use of NSAIDs or aspirin to avoid inappropriate PPI prescribing. Alternatively, the efficacy of new pharmacologic strategies should be evaluated in high-risk groups, with the aim of reducing the risk of both upper and lower gastrointestinal bleeding in these patients.

Key words: Proton pump inhibitor; Small bowel; Small bowel; Lower gastrointestinal bleeding; Nonsteroidal anti-inflammatory drugs

Core tip: Proton pump inhibitors (PPIs) reduce the risk of upper, but not lower gastrointestinal bleeding (LGB) in patients receiving nonsteroidal anti-inflammatory drugs (NSAIDs) or low-dose aspirin. PPIs could

exacerbate small bowel damage related to NSAIDs and low-dose aspirin, which contributes to an increased risk of LGB possibly related to pathological modifications of small bowel microbiota. LGB is a life-threatening condition, especially in older patients with comorbidities treated with NSAIDs, aspirin, or anticoagulants. No accepted treatments exist for decreasing the risk of LGB in these patients. Future research is needed on reducing inappropriate PPI use and evaluating possible pharmacologic interventions to decrease the risk of gastrointestinal bleeding.

Lué A, Lanas A. Protons pump inhibitor treatment and lower gastrointestinal bleeding: Balancing risks and benefits. *World J Gastroenterol* 2016; 22(48): 10477-10481 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10477.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10477

INTRODUCTION

Proton pump inhibitors (PPIs) are one of the most prescribed drugs worldwide. PPIs are used to treat acid-related disorders like gastro-esophageal reflux disease peptic ulcer, peptic ulcer bleeding, and *Helicobacter pylori* infection when combined with antibiotics. PPIs should also be considered in any patient with risk factors for gastrointestinal bleeding who receives treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) or prophylaxis for cardiovascular events with aspirin or other antiplatelet agents[1].

Treatment with NSAIDs, low-dose aspirin, other antiplatelet drugs, and anticoagulation are associated with an increased risk of both upper and lower gastrointestinal bleeding (LGB). Concomitant treatment with these compounds and PPIs has been associated with a decreased risk of upper gastrointestinal bleeding (UGB) but, obviously, not with an increased risk of LGB. The beneficial effect of PPIs is not expected to occur beyond the duodenum because NSAID-gastropathy, but not NSAID-enteropathy, is a pH-dependent phenomenon and gastroduodenal mucosal protection by PPIs is due mainly to their antisecretory effects[2].

PROTON PUMP INHIBITOR TREATMENT AND RISK OF LOWER GASTROINTESTINAL BLEEDING

In a recent case-control study that included 1008 patients hospitalized for gastrointestinal bleeding, treatment with NSAIDs, low-dose aspirin, other antiplatelet agents, and anticoagulants increased the risk of both UGB and LGB. In this work, concomitant use of PPI was associated with a reduced risk of UGB but not LGB[3]. In fact, there was a significant increase in the risk of LGB with PPI use, which has also been detected by other authors[3-8]. Due to the intrinsic nature of observational studies, the finding was explained as probably due to a "confounding by indication" bias. PPI use could be a marker of a group of patients that have an increased risk of LGB because of their clinical characteristics rather than the PPI use itself[3]. In a previous study, we observed that among patients on dual antiplatelet treatment who received concomitant PPI treatment, gastrointestinal bleeding events were more frequent in the lower gastrointestinal tract (74% *vs* 26%)[9]. There are several potential reasons explaining this change in the pattern of gastrointestinal events, which include (1) a very high protective effect of PPIs on the gastroduodenal mucosa associated with a profound decrease in the rate of UGB and a relative (but not absolute) increase in the proportion of LGB in low-dose ascorbic acid users; (2) an absence of the mucosal protective effect of PPIs beyond the duodenum; (3) a direct harmful effect of low-dose aspirin on the small bowel; (4) promotion of bleeding of pre-existing lesions by these compounds; and (5) exacerbation of NSAID- and aspirin-enteropathy by PPI treatment[10-14].

Lately, a hypothesis proposed that PPIs exacerbate NSAID and low-dose aspirin-associated small bowel injury by inducing changes in the intestinal microbiota[15-17]. A multicenter, cross-sectional study including data collected from endoscopic capsule explorations from 205 patients treated with low-dose aspirin for 3 mo showed that 57.6% of patients had at least one mucosal lesion. In the multivariate analysis, concomitant PPI use (OR = 2.04; 95%CI: 1.05-3.97) and use of enteric-coated aspirin (OR = 4.05; 95%CI: 1.49-11.0) were independent risk factors for the presence of mucosal injury[8]. Similar results were observed in patients receiving NSAIDs. In a recent randomized clinical trial, 57 healthy volunteers were allocated to receive celecoxib plus placebo or rabeprazole for 2 wk. The patients were evaluated by capsule endoscopy at the start and end of treatment. In the PPI group, the authors observed an increased rate of small bowel injury (44.7% *vs* 16.7%, $P = 0.04$)[6].

Some authors suggest that acid gastric suppression induced by PPI treatment is related to changes in small bowel microbiota and explain, at least in part, NSAID-induced enteropathy. It is well known that in addition to bile acids[18] and inhibition of cyclooxygenase activity[19], bacterial mucosal translocation and the consequent activation of the innate inflammatory cascade are important factors in the pathogenesis of NSAID-enteropathy[20,21]. In an animal model, Wallace *et al*[17] demonstrated that concomitant treatment with PPIs and NSAIDs resulted in a higher rate of small bowel mucosal ulceration and bleeding. The authors observed that PPI treatment was related to a modification in the small bowel microbiota, consisting of a decrease in jejunal Actinobacteria and Bifidobacteria spp. In this study, the restoration of physiologic microbiota with probiotic treatment prevented intestinal injury[17].

Similar findings have been observed in human subjects. A small randomized clinical trial, including 25

patients treated with low-dose enteric-coated aspirin and omeprazole randomized to either placebo or probiotic treatment with *Lactobacillus casei* for 3 mo, observed that patients in the probiotic group had a significant decrease in the number of mucosal breaks when evaluated by capsule endoscopy ($P = 0.039$)[15].

All these data suggest that PPI treatment not only does not protect the lower gastrointestinal tract from NSAID- and aspirin-related injury, but that they may increase the intestinal mucosal damage and the risk of LGB. We observed that the LGB hospitalization rate has increased significantly in the past years[9,12,13]. These LGB events occur more frequently in older patients, who usually have a higher number of comorbidities, and are associated with a longer hospital stay and higher mortality rates[12]. These events are associated with greater use of hospital resources and also complicate the management of patients at discharge[12]. A considerable percentage of patients did not resume prophylactic treatment with low-dose aspirin or anticoagulants after an acute episode of LGB, increasing the risk of serious cardiovascular events[22]. A recent retrospective analysis including 295 patients with a previous diagnosis of LGB and low-dose aspirin observed that discontinuing low-dose aspirin is related to a decreased risk in LGB recurrence (6.9% *vs* 18.9%; $P = 0.007$), but increased the rates of mortality (26.7% *vs* 8.2%, $P = 0.001$) and cardiovascular events (36.5% *vs* 22.8%, $P = 0.017$)[23]. This study is a clear example of how difficult it is to find the right balance between risks and benefits in these patients. On one side, there is a need to reduce or prevent cardiovascular events and on the other side there is an increased risk of LGB.

BALANCING RISK AND BENEFITS OF PROTON PUMP INHIBITOR TREATMENT

Current clinical practice faces several dilemmas in patients who need NSAIDs or aspirin, since damage to the upper gastrointestinal tract must be prevented without injuring the lower gastrointestinal tract. This clinical dilemma is especially important in patients who need, for example, preventive treatments against cardiovascular disease and who have had a previous LGB episode. Alternative therapeutic approaches with non-aspirin antiplatelet agents poses a similar risk of LGB as aspirin and many not be a valid option[3]. The same is true for patients who need NSAIDs, although COX-2 selective NSAIDs have been shown to be less damaging to the lower gastrointestinal tract than traditional NSAIDs[2,11,24]. The addition of a PPI to celecoxib increases intestinal mucosal damage[6,10,17]. Therefore, future efforts must concentrate on finding strategies that help clinicians in the decision making process. The first relevant issue is to improve overall PPI prescribing and avoid use of PPIs in patients at low risk of gastrointestinal complications who take NSAIDs, aspirin, other antiplatelet agents, or anticoagulants. PPIs are prescribed too often in routine clinical practice, where at least 50% of prescriptions are for non-approved indications[25]. Therefore, the first step should be to promote the proper use of these drugs. Identifying patients at low risk of gastrointestinal events who may not benefit from treatment with PPIs is essential. Tools that could help physicians in the decision making process are now available[26]. On the other hand, we have to reconsider whether current guidelines are valid or respond to problems in daily clinical practice. For example, the PPI treatment indication in patients receiving NSAIDs is based on data from clinical trials of chronic NSAID users. However, observational studies suggest that the risk of gastrointestinal bleeding may be higher when the drug use is recent, and most patients take these drugs for short periods of time[11]. We also need more evidence to evaluate the risks of upper and lower gastrointestinal bleeding with new antiplatelet drugs, new oral direct anticoagulants, and the multiple combinations available in order to better assess the risks and benefits.

On the other hand, in patients at high risk for LGB, preventive strategies are needed. This group includes older patients, with a great number of comorbidities who need more complex management[9,12,23]. As mentioned above, in these patients LGB represents a serious and life threatening complication. Currently, there is no clear effective pharmacological treatment to prevent LGB in NSAID, aspirin, or anticoagulant users. Some studies have evaluated the effect of the prostaglandin analogue misoprostol on small bowel injury induced by NSAIDs[7,27]. Other mucosal protectants such as rebamipide[28-31], irsogladine[32,33], or geranylgeranylacetone[34,35] have been tested and are only available in the Asia-Pacific region. Their potential beneficial effects in these patients still need to be evaluated in new studies.

In the same way, some studies have evaluated the efficacy of probiotics in preventing lower gastrointestinal tract injury among patients treated with NSAIDs or low-dose aspirin[15,16]. The probiotic administered in these two trials was *Lactobacillus casei* in patient treated with low-dose aspirin, while patient treated with NSAIDs received the probiotic mixture VSL#3®, that includes *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii subsp. Bulgaricus*, *Lactobacillus acidophilus* and *Lactobacillus plantarum*[15,16]. The results are promising and suggest that pharmacological prophylaxis could be provided by this strategy. However, more studies with a larger number of patients are also needed. At present, there is not sufficient evidence to recommend a specific probiotic strain to prevent lower gastrointestinal injury. However some bacteria have demonstrated antiinflammatory activity and a protective effect on intestinal mucosa. For example: (1) *Lactobacillus GG* protect mucosal cells from apoptosis and increase mucosal integrity; (2) *Lactobacillus plantarum*

promotes mucine from epithelial cells; (3) *Lactobacillus reuterii* and *Lactobacillus casei* have demonstrated to suppress tumor necrosis factor production *in vivo*; and (4) *Streptococcus thermophillus* and *Lactobacillus acidophilus* could prevent bacterial translocation[36].

CONCLUSION

In conclusion, PPIs represent a milestone in the treatment of acid-related disease. Moreover, PPIs significantly reduce the risk of UGB in patients treated with NSAIDs, low-dose aspirin, other antiplatelet agents, or anticoagulants. This beneficial effect is not observed in the lower gastrointestinal tract. Furthermore, recent studies suggest that PPIs may in fact increase small bowel injury and contribute to the observed increase of LGB in the past years; however, results are controversial. Clinicians very often face clinical dilemmas and need to balance the risks and benefits of treatment. The use of aspirin in preventing cardiovascular disease and the risk of gastrointestinal bleeding is one of these situations. The dilemma is even more difficult to resolve when one of the drugs (PPI) can have a dual and opposite effect (beneficial to the upper GI tract but damaging to the lower GI tract). Clinicians have to evaluate carefully the risk of upper gastrointestinal bleeding in patients that have a previous episode of LGB in order to discontinue inappropriate administration of PPI. Future research should be focused on identifying patients at low risk for upper gastrointestinal bleeding to avoid inappropriate PPI treatment and to evaluate the efficacy of new pharmacologic strategies in high-risk groups. The aim of this strategy is to reduce the risk of LGB in these patients while at the same time providing the maximal beneficial effect to the upper gastrointestinal tract.

REFERENCES

1 **Bhatt DL**, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, Johnson DA, Mahaffey KW, Quigley EM. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. *Circulation* 2008; **118**: 1894-1909 [PMID: 18836135 DOI: 10.1161/circulationaha.108.191087]

2 **Scarpignato C**, Lanas A, Blandizzi C, Lems WF, Hermann M, Hunt RH. Safe prescribing of non-steroidal anti-inflammatory drugs in patients with osteoarthritis--an expert consensus addressing benefits as well as gastrointestinal and cardiovascular risks. *BMC Med* 2015; **13**: 55 [PMID: 25857826 DOI: 10.1186/s12916-015-0285-8]

3 **Lanas Á**, Carrera-Lasfuentes P, Arguedas Y, García S, Bujanda L, Calvet X, Ponce J, Perez-Aísa Á, Castro M, Muñoz M, Sostres C, García-Rodríguez LA. Risk of upper and lower gastrointestinal bleeding in patients taking nonsteroidal anti-inflammatory drugs, antiplatelet agents, or anticoagulants. *Clin Gastroenterol Hepatol* 2015; **13**: 906-912.e2 [PMID: 25460554 DOI: 10.1016/j.cgh.2014.11.007]

4 **Lanas A**, Sostres C. PPI therapy. The small bowel, low-dose aspirin and PPIs--should we be concerned? *Nat Rev Gastroenterol Hepatol* 2014; **11**: 458-460 [PMID: 25001974 DOI: 10.1038/nrgastro.2014.120]

5 **Nagata N**, Niikura R, Aoki T, Sakurai T, Moriyasu S, Shimbo T, Sekine K, Okubo H, Watanabe K, Yokoi C, Yanase M, Akiyama J, Uemura N. Effect of proton-pump inhibitors on the risk of lower gastrointestinal bleeding associated with NSAIDs, aspirin, clopidogrel, and warfarin. *J Gastroenterol* 2015; **50**: 1079-1086 [PMID: 25700638 DOI: 10.1007/s00535-015-1055-2]

6 **Washio E**, Esaki M, Maehata Y, Miyazaki M, Kobayashi H, Ishikawa H, Kitazono T, Matsumoto T. Proton Pump Inhibitors Increase Incidence of Nonsteroidal Anti-Inflammatory Drug-Induced Small Bowel Injury: A Randomized, Placebo-Controlled Trial. *Clin Gastroenterol Hepatol* 2016; **14**: 809-815.e1 [PMID: 26538205 DOI: 10.1016/j.cgh.2015.10.022]

7 **Watanabe T**, Sugimori S, Kameda N, Machida H, Okazaki H, Tanigawa T, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Higuchi K, Arakawa T. Small bowel injury by low-dose enteric-coated aspirin and treatment with misoprostol: a pilot study. *Clin Gastroenterol Hepatol* 2008; **6**: 1279-1282 [PMID: 18995219 DOI: 10.1016/j.cgh.2008.06.021]

8 **Endo H**, Sakai E, Taniguchi L, Kessoku T, Komiya Y, Ezuka A, Kawamura H, Taguri M, Higurashi T, Ohkubo H, Yamada E, Takahashi H, Inamori M, Maeda S, Sakaguchi T, Hata Y, Nagase H, Nakajima A. Risk factors for small-bowel mucosal breaks in chronic low-dose aspirin users: data from a prospective multicenter capsule endoscopy registry. *Gastrointest Endosc* 2014; **80**: 826-834 [PMID: 24830581 DOI: 10.1016/j.gie.2014.03.024]

9 **Casado Arroyo R**, Polo-Tomas M, Roncalés MP, Scheiman J, Lanas A. Lower GI bleeding is more common than upper among patients on dual antiplatelet therapy: long-term follow-up of a cohort of patients commonly using PPI co-therapy. *Heart* 2012; **98**: 718-723 [PMID: 22523056 DOI: 10.1136/heartjnl-2012-301632]

10 **Goldstein JL**, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. *Clin Gastroenterol Hepatol* 2005; **3**: 133-141 [PMID: 15704047]

11 **Lanas A**, García-Rodríguez LA, Arroyo MT, Gomollón F, Feu F, González-Pérez A, Zapata E, Bástida G, Rodrigo L, Santolaria S, Güell M, de Argila CM, Quintero E, Borda F, Piqué JM. Risk of upper gastrointestinal ulcer bleeding associated with selective cyclo-oxygenase-2 inhibitors, traditional non-aspirin non-steroidal anti-inflammatory drugs, aspirin and combinations. *Gut* 2006; **55**: 1731-1738 [PMID: 16687434 DOI: 10.1136/gut.2005.080754]

12 **Lanas A**, García-Rodríguez LA, Polo-Tomás M, Ponce M, Alonso-Abreu I, Perez-Aisa MA, Perez-Gisbert J, Bujanda L, Castro M, Muñoz M, Rodrigo L, Calvet X, Del-Pino D, Garcia S. Time trends and impact of upper and lower gastrointestinal bleeding and perforation in clinical practice. *Am J Gastroenterol* 2009; **104**: 1633-1641 [PMID: 19574968 DOI: 10.1038/ajg.2009.164]

13 **Lanas A**, García-Rodríguez LA, Polo-Tomás M, Ponce M, Quintero E, Perez-Aisa MA, Gisbert JP, Bujanda L, Castro M, Muñoz M, Del-Pino MD, Garcia S, Calvet X. The changing face of hospitalisation due to gastrointestinal bleeding and perforation. *Aliment Pharmacol Ther* 2011; **33**: 585-591 [PMID: 21205256 DOI: 10.1111/j.1365-2036.2010.04563.x]

14 **Smecuol E**, Pinto Sanchez MI, Suarez A, Argonz JE, Sugai E, Vazquez H, Litwin N, Piazuelo E, Meddings JB, Bai JC, Lanas A. Low-dose aspirin affects the small bowel mucosa: results of a pilot study with a multidimensional assessment. *Clin Gastroenterol Hepatol* 2009; **7**: 524-529 [PMID: 19249402 DOI: 10.1016/j.cgh.2008.12.019]

15 **Endo H**, Higurashi T, Hosono K, Sakai E, Sekino Y, Iida H, Sakamoto Y, Koide T, Takahashi H, Yoneda M, Tokoro C, Inamori M, Abe Y, Nakajima A. Efficacy of Lactobacillus casei treatment on small bowel injury in chronic low-dose aspirin users: a pilot randomized controlled study. *J Gastroenterol* 2011; **46**: 894-905 [PMID: 21556830 DOI: 10.1007/s00535-011-0410-1]

16 **Montalto M**, Gallo A, Curigliano V, D'Onofrio F, Santoro L, Covino M, Dalvai S, Gasbarrini A, Gasbarrini G. Clinical trial: the effects of a probiotic mixture on non-steroidal anti-inflammatory drug enteropathy - a randomized, double-blind, cross-over, placebo-

controlled study. *Aliment Pharmacol Ther* 2010; **32**: 209-214 [PMID: 20384610 DOI: 10.1111/j.1365-2036.2010.04324.x]
17 **Wallace JL**, Syer S, Denou E, de Palma G, Vong L, McKnight W, Jury J, Bolla M, Bercik P, Collins SM, Verdu E, Ongini E. Proton pump inhibitors exacerbate NSAID-induced small intestinal injury by inducing dysbiosis. *Gastroenterology* 2011; **141**: 1314-1322 [PMID: 21745447 DOI: 10.1053/j.gastro.2011.06.075]
18 **Petruzzelli M**, Vacca M, Moschetta A, Cinzia Sasso R, Palasciano G, van Erpecum KJ, Portincasa P. Intestinal mucosal damage caused by non-steroidal anti-inflammatory drugs: role of bile salts. *Clin Biochem* 2007; **40**: 503-510 [PMID: 17321514 DOI: 10.1016/j.clinbiochem.2007.01.015]
19 **Takeuchi K**, Tanaka A, Kato S, Amagase K, Satoh H. Roles of COX inhibition in pathogenesis of NSAID-induced small intestinal damage. *Clin Chim Acta* 2010; **411**: 459-466 [PMID: 20074562 DOI: 10.1016/j.cca.2009.12.026]
20 **Blackler RW**, Gemici B, Manko A, Wallace JL. NSAID-gastroenteropathy: new aspects of pathogenesis and prevention. *Curr Opin Pharmacol* 2014; **19**: 11-16 [PMID: 24929967 DOI: 10.1016/j.coph.2014.05.008]
21 **Lanas A**, Scarpignato C. Microbial flora in NSAID-induced intestinal damage: a role for antibiotics? *Digestion* 2006; **73** Suppl 1: 136-150 [PMID: 16498262 DOI: 10.1159/000089789]
22 **Sostres C**, Lanas A. Epidemiology of Low Dose Aspirin Damage in the Lower Gastrointestinal Tract. *Curr Pharm Des* 2015; **21**: 5094-5100 [PMID: 26369682]
23 **Chan FK**, Leung Ki EL, Wong GL, Ching JY, Tse YK, Au KW, Wu JC, Ng SC. Risks of Bleeding Recurrence and Cardiovascular Events With Continued Aspirin Use After Lower Gastrointestinal Hemorrhage. *Gastroenterology* 2016; **151**: 271-277 [PMID: 27130815 DOI: 10.1053/j.gastro.2016.04.013]
24 **Chan FK**, Lanas A, Scheiman J, Berger MF, Nguyen H, Goldstein JL. Celecoxib versus omeprazole and diclofenac in patients with osteoarthritis and rheumatoid arthritis (CONDOR): a randomised trial. *Lancet* 2010; **376**: 173-179 [PMID: 20638563 DOI: 10.1016/s0140-6736(10)60673-3]
25 **Lanas A**. We Are Using Too Many PPIs, and We Need to Stop: A European Perspective. *Am J Gastroenterol* 2016; **111**: 1085-1086 [PMID: 27166129 DOI: 10.1038/ajg.2016.166]
26 **Lanas A**, Polo-Tomás M, Casado-Arroyo R. The aspirin cardio-vascular/gastrointestinal risk calculator--a tool to aid clinicians in practice. *Aliment Pharmacol Ther* 2013; **37**: 738-748 [PMID: 23413984 DOI: 10.1111/apt.12240]
27 **Kojima Y**, Takeuchi T, Ota K, Harada S, Edogawa S, Narabayashi K, Nouda S, Okada T, Kakimoto K, Kuramoto T, Inoue T, Higuchi K. Effect of long-term proton pump inhibitor therapy and healing effect of irsogladine on nonsteroidal anti-inflammatory drug-induced small-intestinal lesions in healthy volunteers. *J Clin Biochem Nutr* 2015; **57**: 60-65 [PMID: 26236102 DOI: 10.3164/jcbn.15-32]
28 **Niwa Y**, Nakamura M, Ohmiya N, Maeda O, Ando T, Itoh A, Hirooka Y, Goto H. Efficacy of rebamipide for diclofenac-induced small-intestinal mucosal injuries in healthy subjects: a prospective, randomized, double-blinded, placebo-controlled, cross-over study. *J Gastroenterol* 2008; **43**: 270-276 [PMID: 18458842 DOI: 10.1007/s00535-007-2155-4]
29 **Kurata S**, Nakashima T, Osaki T, Uematsu N, Shibamori M, Sakurai K, Kamiya S. Rebamipide protects small intestinal mucosal injuries caused by indomethacin by modulating intestinal microbiota and the gene expression in intestinal mucosa in a rat model. *J Clin Biochem Nutr* 2015; **56**: 20-27 [PMID: 25834302 DOI: 10.3164/jcbn.14-67]
30 **Kurokawa S**, Katsuki S, Fujita T, Saitoh Y, Ohta H, Nishikawa K, Sato Y, Sato Y, Ohira K, Yamada M, Kato M. A randomized, double-blinded, placebo-controlled, multicenter trial, healing effect of rebamipide in patients with low-dose aspirin and/or non-steroidal anti-inflammatory drug induced small bowel injury. *J Gastroenterol* 2014; **49**: 239-244 [PMID: 23595613 DOI: 10.1007/s00535-013-0805-2]
31 **Watanabe T**, Takeuchi T, Handa O, Sakata Y, Tanigawa T, Shiba M, Naito Y, Higuchi K, Fujimoto K, Yoshikawa T, Arakawa T. A multicenter, randomized, double-blind, placebo-controlled trial of high-dose rebamipide treatment for low-dose aspirin-induced moderate-to-severe small intestinal damage. *PLoS One* 2015; **10**: e0122330 [PMID: 25874951 DOI: 10.1371/journal.pone.0122330]
32 **Isomura Y**, Yamaji Y, Yamada A, Watanabe Y, Suzuki H, Kobayashi Y, Yoshida S, Watabe H, Hirata Y, Yoshida H, Koike K. Irsogladine improves small-intestinal injuries in regular users of nonsteroidal anti-inflammatory drugs. *Gastrointest Endosc* 2014; **80**: 118-125 [PMID: 24518124 DOI: 10.1016/j.gie.2013.12.030]
33 **Kuramoto T**, Umegaki E, Nouda S, Narabayashi K, Kojima Y, Yoda Y, Ishida K, Kawakami K, Abe Y, Takeuchi T, Inoue T, Murano M, Tokioka S, Higuchi K. Preventive effect of irsogladine or omeprazole on non-steroidal anti-inflammatory drug-induced esophagitis, peptic ulcers, and small intestinal lesions in humans, a prospective randomized controlled study. *BMC Gastroenterol* 2013; **13**: 85 [PMID: 23672202 DOI: 10.1186/1471-230x-13-85]
34 **Umegaki E**, Kuramoto T, Kojima Y, Nouda S, Ishida K, Takeuchi T, Inoue T, Tokioka S, Higuchi K. Geranylgeranylacetone, a gastro-mucoprotective drug, protects against NSAID-induced esophageal, gastroduodenal and small intestinal mucosal injury in healthy subjects: A prospective randomized study involving a comparison with famotidine. *Intern Med* 2014; **53**: 283-290 [PMID: 24531083]
35 **Xiong L**, Huang X, Li L, Yang X, Liang L, Zhan Z, Ye Y, Chen M. Geranylgeranylacetone protects against small-intestinal injuries induced by diclofenac in patients with rheumatic diseases: a prospective randomized study. *Dig Liver Dis* 2015; **47**: 280-284 [PMID: 25660821 DOI: 10.1016/j.dld.2015.01.005]
36 **Ganesh BP**, Versalovic J. Luminal Conversion and Immunoregulation by Probiotics. *Front Pharmacol* 2015; **6**: 269 [PMID: 26617521 DOI: 10.3389/fphar.2015.00269]

P- Reviewer: Hreinsson JP, Lakatos PL, Rabago L, Souza JLS, Tan HJ
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Liu WX

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.v22.i48.10482

World J Gastroenterol 2016 December 28; 22(48): 10482-10501
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Does pressure cause liver cirrhosis? The sinusoidal pressure hypothesis

Sebastian Mueller

Sebastian Mueller, Department of Medicine and Center for Alcohol Research, Salem Medical Center, University of Heidelberg, Zeppelinstr, 69121 Heidelberg, Germany

Author contributions: Mueller S solely contributed to the conception and design, writing the paper, critical reading and final approval.

Conflict-of-interest statement: No conflict of interest to report.

Manuscript source: Invited manuscript

Correspondence to: Sebastian Mueller, MD, PhD, Professor of Medicine, Vice Head, Co-Director, Department of Medicine and Center for Alcohol Research, Salem Medical Center, University of Heidelberg, Zeppelinstraße 11 - 33, 69121 Heidelberg, Germany. sebastian.mueller@urz.uni-heidelberg.de
Telephone: +49-6221-483210
Fax: +49-6221-484494

Received: July 27, 2016
Peer-review started: August 1, 2016
First decision: September 28, 2016
Revised: October 10, 2016
Accepted: November 23, 2016
Article in press: November 28, 2016
Published online: December 28, 2016

Abstract

Independent of their etiology, all chronic liver diseases ultimately lead to liver cirrhosis, which is a major health problem worldwide. The underlying molecular mechanisms are still poorly understood and no efficient treatment strategies are available. This paper introduces the sinusoidal pressure hypothesis (SPH), which identifies an elevated sinusoidal pressure (SP) as cause of fibrosis. SPH has been mainly derived from recent studies on liver stiffness. So far, pressure changes have been exclusively seen as a consequence of cirrhosis. According to the SPH, however, an elevated SP is the major upstream event that initiates fibrosis *via* biomechanic signaling by stretching of perisinusoidal cells such as hepatic stellate cells or fibroblasts (SPH part Ⅰ: initiation). Fibrosis progression is determined by the degree and time of elevated SP. The SPH predicts that the degree of extracellular matrix eventually matches SP with critical thresholds > 12 mmHg and > 4 wk. Elevated arterial flow and final arterialization of the cirrhotic liver represents the self-perpetuating key event exposing the low-pressure-organ to pathologically high pressures (SPH part Ⅱ: perpetuation). It also defines the "point of no return" where fibrosis progression becomes irreversible. The SPH is able to explain the macroscopic changes of cirrhotic livers and the uniform fibrotic response to various etiologies. It also opens up new views on the role of fat and disease mechanisms in other organs. The novel concept will hopefully stimulate the search for new treatment strategies.

Key words: Liver stiffness; Stretch force; Sinusoidal pressure hypothesis; Liver cirrhosis; Hepatic arterial buffer response; Biomechanics; Arterialization; Hepatic stellate cells; Fibroblasts; Cellular and intercellular mechano-signaling

Core tip: This paper introduces the sinusoidal pressure hypothesis, which identifies an elevation of sinusoidal pressure (SP) as cause of fibrosis/cirrhosis. Accordingly,

elevated SP is the major upstream event that initiates fibrosis progression *via* biomechanic signaling by stretching of perisinusoidal cells. Fibrosis progression is determined by the degree and time of elevated SP. The cirrhotic extracellular matrix eventually matches the degree of pressure. Arterialization of the stiff cirrhotic liver represents the final self-perpetuating key event exposing the low-pressure-organ to pathologically high pressures. It also defines the "point of no return" where fibrosis progression becomes irreversible.

Mueller S. Does pressure cause liver cirrhosis? The sinusoidal pressure hypothesis. *World J Gastroenterol* 2016; 22(48): 10482-10501 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10482.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10482

INTRODUCTION

Liver cirrhosis is the final stage of all liver diseases and associated with a high mortality worldwide[1]. Nevertheless, the pathophysiology is poorly understood and, consequently, no targeted treatment options exist despite intensive research activities over many decades. This paper introduces the sinusoidal pressure hypothesis (SPH), which identifies an elevation of sinusoidal pressure (SP) as the cause of fibrosis. SPH is an integrative concept derived from various biophysical, cellular, hemodynamic and clinical findings. It has been mainly stimulated by observations using the recently developed transient elastography (TE) to measure liver stiffness (LS)[2]. Although TE was primarily introduced to the liver community as diagnostic screening tool for liver fibrosis, it has been rapidly learnt that pressure-associated conditions are important confounding factors of elevated LS[3]. Moreover and although long term data are still scarce, it has increasingly become evident that elevated LS *per se* is a prognostic unfavorable condition and a predictor of liver-related mortality. In contrast, a normal LS rules out manifest chronic liver disease and fibrosis. Based on a first article published in 2010[3], a much more detailed concept is presented here which has been encouraged by novel preliminary findings as well as the resonance at various meetings including the conferences of the German-Romanian Society of Gastroenterology in Temeswar 2016 and the EASL monothematic conference on fibrosis in Porto in 2016.

The term pressure hypothesis has now been specified more precisely "SPH". SPH is divided into two parts: While part Ⅰ refers to pressure-mediated fibrosis progression, the novel part Ⅱ encompasses "arterialization" as an important step of self-perpetuation leading to continued pressure elevation in the low-pressure-organ and defining the "point of no return" with irreversible fibrosis progression. SPH could provide a major framework for a better understanding of disease formation based on biomechanics. It will hopefully lead to the design of novel experiments and studies to undergo the meticulous process of verification and falsification. Pressure as a driving force of fibrogenesis could be used to better understand the genetics, proteomics and metabolomics that modulate pressure-mediated biomechanical processes instead of focusing on the search of target genes in *e.g.*, genome wide studies. A major methodological challenge is still the fact that SP *per se* cannot be directly addressed by *e.g.*, micro-sensors but it rather requires the interpretation of indirect data combined with common sense and logical reasoning. In the long term perspective, it is hoped that SP may be addressable directly with the recent development of molecular "mechanic force sensors" still *in status nascendi*[4]. In addition, SPH draws a closer attention to often overlooked mechanic aspects in biological tissues that not only include hydrostatic pressure but also the mechanic energy transferred by the cardiac pulse wave and its absorption by hepatic tissue and fat. Moreover, the SPH also opens up new views on the mechanic role of fat and it may stimulate studies of disease mechanisms in other organs.

Introducing pressure to the pathology of liver fibrosis, SPH naturally addresses the important issue of how to lower and modulate SP to prevent disease progression. It is hoped that the concept of the SPH may lead to a better individual patient selection and optimized therapeutic concepts in the future. In the following, SPH will be explained after providing a short background and the description of hitherto unexplained observations in the research area of cirrhosis. Cardiac cirrhosis will be discussed in more detail since it represents a rarely discussed non-inflammatory form of pressure-associated cirrhosis. At the end, specific consequences of SPH are discussed, their relation to various clinical and preliminary findings and potential future therapeutic directions.

BACKGROUND OF LIVER CIRRHOSIS: DEFINITION AND CLINICAL IMPORTANCE

Chronic liver diseases frequently lead to scarring (cirrhosis), a process in which the architectural organization of functional liver units becomes disrupted. Liver cirrhosis is the result of excessive accumulation of extracellular matrix (ECM) with increased LS. This is often accompanied by a progressive loss of organ function despite the use of immunosuppressive, anti-viral or anti-inflammatory agents[5,6]. Excess ECM deposition also causes progressive elevation of the hepatic vascular resistance with important hemodynamic consequences including portal hypertension, the formation of vascular collaterals and the so-called hyperdynamic circulation with elevated cardiac output and lowered arterial

pressure[7]. Moreover, liver cirrhosis is an important pre-cancerogenic lesion finally resulting in hepatocellular cancer (HCC). At present, HCC shows the second fastest growth rate worldwide and ranks at third place in cancer-related mortality[8,9]. More than 90% of HCCs develop in cirrhotic livers mostly due to alcoholic liver disease (ALD) or chronic hepatitis C[10,11]. Currently, progression of fibrosis to cirrhosis is most efficiently blocked by treating the underlying disease but no satisfying anti-fibrotic treatment regimen exists to directly attack the fibrotic processes. Finally, it should be mentioned that only a minority typically progress towards cirrhosis while many patients with chronic liver disease will never end up with cirrhosis. This variety is largely affected by disease-related factors (for example, viral genotype) or host-related factors (*e.g.*, genetic/epigenetic)[12].

BACKGROUND OF MOLECULAR MECHANISMS OF HEPATIC FIBROGENESIS: PRESENT UNDERSTANDING AND UNEXPLAINED OBSERVATIONS

The mechanisms of hepatic fibrosis are very complex and so far not fully understood. A variety of adverse stimuli such as hepatotoxins, viruses, bile acids and hypoxia can trigger fibrogenesis and so-called reactive oxygen species seem to play an important role in fibrosis progression[12]. The major proteins of the ECM are collagens forming important scaffolds and barriers. Collagen type Ⅰ, Ⅲ and Ⅳ are the most abundant ECM components in the liver and their relative content increases up to tenfold in cirrhosis[13,14]. In the acute phase of liver disease fibrosis is a dynamic process, in which fibrogenesis is usually counterbalanced by fibrolysis, *i.e.*, the removal of excess ECM by proteolytic enzymes, most importantly by matrix metalloproteinases (MMPs). With repeated injury or sufficient severity, fibrogenesis prevails over fibrolysis, resulting in excess ECM synthesis and deposition, a downregulation of MMP synthesis, secretion and activity along with an increase of the tissue inhibitors of MMPs (TIMPs, especially TIMP-1). ECM components, MMPs and TIMPs are mainly produced by activated hepatic stellate cells (HSCs) and fibroblasts[15]. Activated macrophages (Kupffer cells) but also other cells are a major source for fibrogenic cytokines such as TGF-β, also called the master cytokine of fibrosis development, that further stimulate HSCs and fibroblasts to transdifferentiate into activated myofibroblasts, the main cell type responsible for excess matrix deposition at sites of tissue repair. Figure 1A depicts the conventional course of events ultimately leading to fibrosis. In this conventional concept, increased matrix deposition results in elevated LS that is the final consequence of liver fibrosis (discussed below). Despite the enormous progress in understanding mechanisms of fibrogenesis, however, several key observations in patients with liver cirrhosis cannot be explained sufficiently so far:

(1) It is not clear why different liver etiologies ranging from inflammatory, infectious, biliary, metabolic or even non-inflammatory causes such as congestion ultimately lead to histologically almost identical forms of liver cirrhosis. Especially non-inflammatory causes such as cardiac cirrhosis remain poorly understood although they can develop the full scale of complications ranging from portal hypertension to liver cancer. This also refers to other rare hemodynamic causes of fibrosis such as experimental portal ligation or the Budd-Chiari syndrome. For a better overview, Supplemental Table 1 represents a list of different etiologies that all cause fibrosis. This table also provides current information on LS elevation and AST/ALT ratio that is relevant for the discussions below.

(2) At present, typical macroscopic features of cirrhosis such as large fibrous septa spanning over several centimeters through the organ (Figure 1B) cannot be explained *e.g.*, by the action of local humoral factors or profibrogenic cytokines such as TGFbeta. It is also not clear why fibrous septa during conversion of micro-nodular to macro-nodular cirrhosis may fuse to very large septa. These septa may partly resolve during regression of fibrosis after *e.g.*, viral clearance.

(3) It is known that fibrosis can partly or even fully reverse at earlier stages while end-stage cirrhosis will further progress even in the absence of the initial cause *e.g.*, after abstaining from alcohol or successful HCV treatment. This so-called "point of no return" is not well understood nor the underlying mechanisms. In addition, this critical time point cannot be exactly defined in individual patients, an important draw back for prognosis evaluation and treatment initiation.

(4) So far, various systemic search strategies either based on genetics, proteomics or metabolomics have not been able to provide a clear understanding of the molecular mechanisms of fibrosis[16-18].

(5) For decades, hepatic steatosis has been considered a mandatory prerequisite for fibrosis (Figure 1A). However, this mandatory role of steatosis for fibrosis progression is increasingly questioned[19,20]. So, although steatosis is abundant in patients who consume alcohol, only a minority of ca. 20% progress to fibrosis[21]. In fact, many subjects with overweight show "benign" fat accumulation without lipotoxicity and inflammation. In addition, segmental steatosis can often be observed by ultrasound imaging of the liver but no segmental fibrosis or cirrhosis has been reported so far[22].

(6) It remains unclear why typical etiologies of human liver cirrhosis such as ALD and NAFLD are difficult to reproduce in small standard animal models such as mice and rats despite inflammation and

Table 1 Part Ⅰ (initiation) and Ⅱ (perpetuation) of sinusoidal pressure hypothesis

SPH Part Ⅰ: Initiation of a pro-fibrogenic response by elevated sinusoidal pressure
1. All liver diseases cause SP elevation. SP is the combined result of dynamic and static components that include the hepatic inflow/outflow balance, intra- and extrahepatic shunts as well as vascular filling by water retention and osmotic pressure.
2. LS represents the sum of matrix deposition (fibrosis) and SP. In non-cirrhotic livers, LS corresponds to SP.
3. Dosage and time of elevated SP/LS determine fibrosis progression (biomechanic signaling). Matrix deposition ultimately matches SP (force = counter force).
4. At the cellular level, SP elevation causes stretch forces on perisinusoidal cells that ultimately lead to collagen (matrix) deposition *via* inter- and intracellular biomechanic signaling.
SPH Part Ⅱ: Continued pressure-elevation by arterialization of the fibrotic liver (perpetuation)
1. At a LS of ca. 12 kPa/SP of 12 mmHg, arterial blood supply becomes essential ultimately leading to arterialization of the liver (*via* hypoxia-signaling including HABR, VEGF *etc.*).
2. Arterial supply is ultimately not reversible causing loss of endothelial fenestrae, capillarization and sustained SP and LS elevation.
3. Arterialization initiates a vicious cycle leading to further matrix deposition, eventual complete disconnection of hepatocytes from blood supply and ischemia with subsequent arterialization and nodular regeneration.
4. Finally, the arterialized liver (high oxygen, high pressure) combined with cell death and enhanced regeneration will cause a pro-cancerogenic environment and HCC.

SPH: Sinusoidal pressure hypothesis; HABR: Hepatic arterial buffer response; HCC: Hepatocellular carcinoma; LS: Liver stiffness; SP: Sinusoidal pressure; VEGF: Vascular endothelial growth factor.

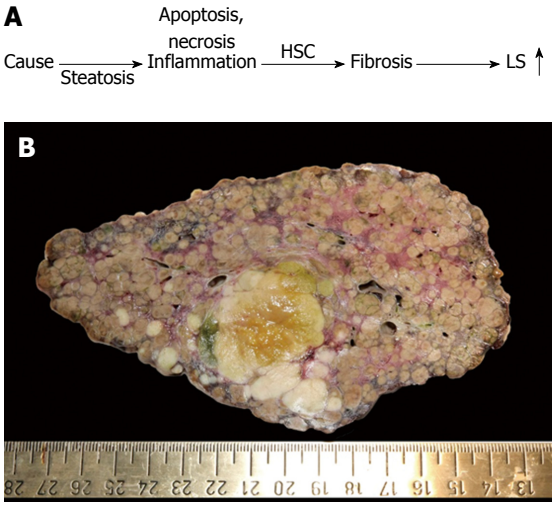

Figure 1 Liver fibrosis. A: Conventional sequence of fibrosis progression. Here, elevated liver stiffness (LS) is primarily regarded as correlate of matrix deposition (fibrosis stage); B: Macroscopic aspect of a cirrhotic liver in a patient with alcoholic liver disease (courtesy of C. Lackner, University of Graz). Note the large fibrous septa spanning through the whole organ which are clearly visible at the macroscopic level. In addition, a primary liver cancer (HCC) can be seen (green area). HCC: Hepatocellular carcinoma; HSC: Hepatic stellate cells.

steatosis. Robust cirrhosis is only generated in very toxic models such as CCl_4 or TAA treatment[23].

OBSERVATIONS LEADING TO SPH: THE ROLE OF MATRIX AND PRESSURE IN MODULATING LS

Measurement of LS to assess liver fibrosis has been introduced more than 10 years ago and is now increasingly used worldwide for fibrosis screening[2]. During fibrosis progression, LS increases continuously from ca. 4 kPa up to 75 kPa (upper detection limit of the Fibroscan device). A threshold of 12.5 kPa is widely considered as cut-off value of histological F4 cirrhosis stage[24]. The data from more than 500 clinical studies can be briefly summarized as follows with regard to their relevance for SPH[3]: (1) LS highly correlates with histological fibrosis stage independent of the underlying liver disease ($r > 0.8$)[3]. A normal LS (< 6 kPa) excludes liver pathology and liver fibrosis[3]; (2) irrespective of cirrhosis, LS can be drastically but reversibly elevated under conditions such as inflammation, cholestasis and congestion[3] (Figures 2 and 3). In the long term perspective, these conditions are all able to cause cirrhosis and they are typically associated with intra-hepatic pressure changes. Moreover, as shown in Figure 4B, LS correlates directly with SP in non-cirrhotic livers[25]; (3) pressure-related elevation of LS precedes the development of fibrosis[3,26,27]. Vice versa, LS improves after elimination of liver pathology *e.g.*, after clearance of HCV[28], water elimination by diuretics in patients with heart failure and liver congestion[25] or alcohol withdrawal[29] (Figure 3); and (4) LS is an independent predictor of liver-related mortality[30,31]. Genetic risk factors of liver disease such as some PNPLA3 variants are also known to cause LS elevation *e.g.*, in the presence of alcohol consumption[32].

Initially, elevated LS was solely regarded as a consequence of fibrosis progression. Especially the observation that an increase of the central venous pressure as well as the intra-ductal biliary pressure are able to drastically and reversibly elevate LS without any other confounders such as inflammation[25,33-35] suggested an important role of the SP in mediating fibrosis[3].

In this context, it is important to conceive that inflammatory liver diseases are also associated with pressure change like in any other tissue (*e.g.*, skin induration and swelling in patients with skin abscesses or furuncle). It is well established in the field of pathology that inflamed tissues are hyper-vascularized (*rubor*, *calor*, *tumor*, *functio laesa*). The reasons for this are manifold and include infiltration of inflammatory

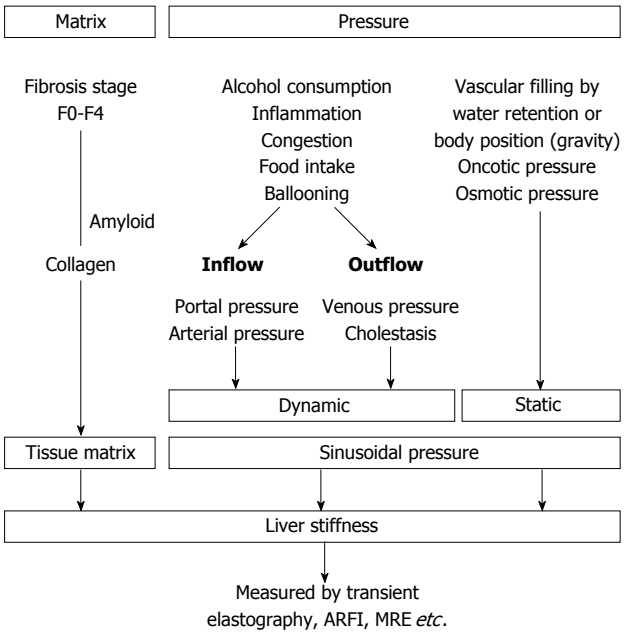

Figure 2 Liver stiffness is modulated both by matrix and pressure-associated conditions. Both dynamic and static components affect the sinusoidal pressure. MRE: Magnetic resonance elastography; ARFI: Acoustic radiation force imaging.

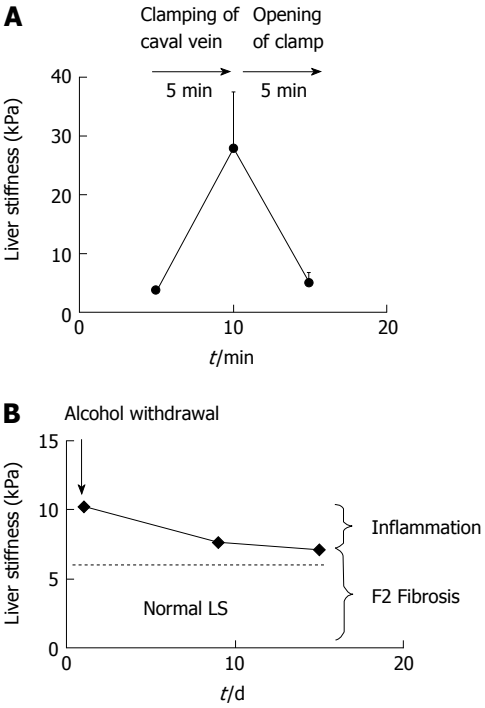

Figure 3 Examples of reversible pressure-mediated changes of liver stiffness. A: Reversible drastic increase of liver stiffness (LS) after clamping of the caval vein in narcotized landrace pigs (modified from Ref. [25]); B: Decrease of LS after alcohol detoxification in a heavy female drinker unmasks the inflammation-related LS from the fibrosis-related LS. F2 fibrosis was confirmed histologically. Modified from Ref. [29].

cells or enhanced arterial perfusion through the action of vasodilating agents and cytokines. The development of inflammatory edema and the swelling of cells

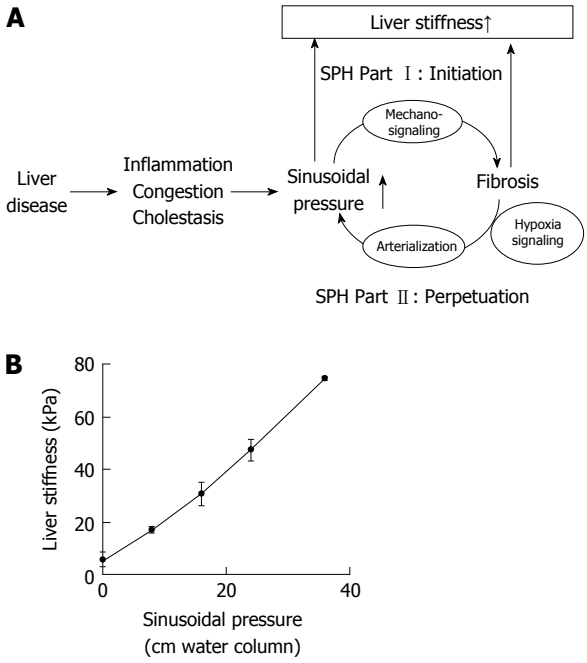

Figure 4 The sinusoidal pressure hypothesis and the role of liver stiffness. A: Sinusoidal pressure hypothesis at the whole organ level. SP is the driving force of matrix deposition. Irrespective of the etiology, all liver pathologies (shown in the left) increase the SP that initiates matrix deposition *via* specific inter- and intracellular biomechanic signaling pathways (SPH Part Ⅰ, Initiation). LS should be regarded as the combined read-out of elevated pressure and fibrosis. Both SP elevation and matrix deposition increase vascular resistance that ultimately lead to elevated hepatic arterial flow and finally complete arterial blood supply. The arterial response is mainly driven by hypoxia signaling and metabolic demand. Depending on dosage (> 12 mmHg) and time (> 4 wk), this vicious cycle will ultimately cause a complete arterialization leading to irreversible cirrhosis by exposing the low pressure organ to permanent high pressure (SPH Part Ⅱ, Perpetuation); B: LS almost linearly depends on sinusoidal pressure in an isolated pig liver. In this experiment (modified from [25]), all vessels (caval and portal vein, hepatic artery and common bile duct) were ligated. The isolated organ was increasingly filled with isotonic sodium chloride solution and put under pressure. Under these conditions, according to the physical law of communicating pipes, the pressure within the caval or portal vein directly matches the SP. Similar to compliance studies in lungs, LS will show a slower increase at higher SP levels (not shown). LS: Liver stiffness; SP: Sinusoidal pressure; SPH: Sinusoidal pressure hypothesis.

may further contribute to an elevated tissue stiffness (*tumor*) but it will always mainly be caused by hyper-perfusion requiring a well-functioning blood circulation. This fact has been rather put aside in many previous studies on fibrosis progression normally focusing on humoral, inflammatory, genetic and many other conditions but not hemodynamic consequences and pressure. It is also the reason why *in vitro* studies on the molecular mechanisms of fibrosis progression may have missed the role of pressure for tissue stiffness.

SPH PART Ⅰ: INITIATION OF A PROFIBROGENIC RESPONSE BY ELEVATED SP

The above mentioned chain of thoughts has led to

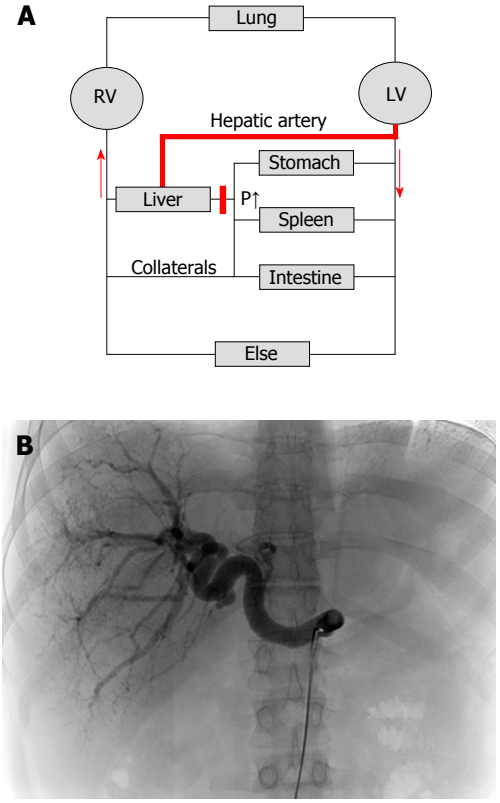

Figure 5 Cirrhotic livers are primarily supplied with blood by the hepatic artery (arterialization). A: Hemodynamics of the low-pressure organ liver in the context of systemic circulation. Cirrhosis causes an increased vascular resistance, collateral formation and increased hepatic arterial flow to maintain hepatic perfusion. Elevated hepatic arterial flow can be observed already before the onset of fibrosis. It eventually leads to a complete arterialization of the cirrhotic liver. In some cases, portal flow completely reverses (so called hepatofugal flow) and hepatic blood exits the liver both via hepatic and portal veins. Note that the blood circulation (red arrows) is functionally maintained by two serial pumps (RV and LV). A dysbalance of these two pumps such as observed during right heart failure can also cause higher SP (congestion) and ultimately cardiac liver cirrhosis; B: Cirrhotic livers are characterized by predominant arterial blood supply. CT angiography of a patient with liver cirrhosis showing a prominent hepatic artery in 31 years old female patient with cryptogenic liver cirrhosis Child A (courtesy of Dr. B. Radeleff, University of Heidelberg). Under such conditions, the hepatic artery supplies the liver with more than 80% of blood. RV: Right ventricle; LV: Left ventricle; CT: Computed tomography.

the key principles of part Ⅰ of SPH that are shown in Figure 4A and Table 1. They can be summarized in the following four key points:

(1) According to SPH, all potential causes of cirrhosis whether of inflammatory or non-inflammatory origin ultimately lead to an elevated SP. In contrast to conventional concepts in which pressure changes such as portal hypertension are merely seen as a consequence of cirrhosis, SP is the primary cause for matrix deposition. SP consists of dynamic and static components such as hepatic inflow and outflow balances or water retention (Figure 2). Even minimal increases of SP seem to be critical for the low-pressure organ liver which is typically exposed to no more than ca. 6 mm of mercury *via* the portal vein. SP elevation may first develop in portal or central areas depending on the localization of the underlying disease (*e.g.*, portal-tract disease such as HCV *vs* perivenular disease such as ALD).

(2) In contrast to conventional concepts (Figure 1A), elevated LS is the consequence of both elevated SP and increased matrix deposition. This also means that LS almost exclusively mirrors SP in the absence of fibrosis (Figure 4B).

(3) At the cellular level and as will be discussed below, SP is the actual driving force for the production of ECM by stretching of perisinusoidal cells *e.g.*, HSCs, fibroblasts and liver endothelial cells. It remains open whether these cells simply "feel" the surrounding pressure-mediated stiffness by dedicated sensing mechanisms[36] or whether they directly sense pressure-mediated stretch forces. So far, stiffness-mediated activation of HSC has not been linked to pressure or SP[14,37]. According to the physics of mechanics, it is easily conceivable that pressure-induced stretch forces will overlay at the whole organ levels leading to regions with high trajectory forces and consequent large septa formation.

SP-mediated stretch forces and matrix are in continuous equilibrium. Dosage and time of elevated SP/LS determine fibrosis progression (biomechanic signaling) eventually leading to a degree of matrix deposition that "matches" the pressure. Experimental and common clinical observations suggest that a SP > 12 mmHg and a time period > 4 wk are critical thresholds to be validated. It is needless to add that all of these pressure-mediated processes will be modulated by other environmental and genetic factors *e.g.*, the sensitivity of the liver tissues in responding towards pressure changes.

SPH PART Ⅱ: PERPETUATION BY ARTERIALIZATION OF THE FIBROTIC LIVER

The hepatic artery is directly connected to the sinusoidal bed *via* arteriole inlets and provides about 20% of blood in a normal healthy liver. The stiffer the liver becomes due to inflammation or fibrosis the more pressure is required to maintain sufficient blood flow. Although the elevation of portal pressure (portal hypertension > 12 mmHg) can partly maintain some portal flow it will hardly reach values higher than 35 mmHg. Under these conditions, the hepatic artery will be the only vessel with sufficiently high pressure to maintain hepatic blood supply (Figure 5A). An example (CT angiography) of a patient with a manifest cirrhosis and a strong hepatic artery with an almost exclusive arterial perfusion is demonstrated in Figure 5B. This arterialization is an important hallmark of cirrhotic livers[38]. It is also part of the daily experience of liver sonographers that cirrhotic livers are characterized by large hepatic arteries with a strong perfusion signal. In contrast, the hepatic artery is almost invisible in normal

livers[22]. Even after cirrhosis has been established, the hepatic arterial flow velocity and volume will further increase when progressing from Child-Pugh stage A to C stage[39], respectively. This has been recently confirmed in whole-liver perfusion enhanced CT imaging scan studies[40]. Enhanced angiogenesis has also been observed before the manifestation of fibrosis in small animal models using contrast-enhanced micro CT[41]. Taken together, all these established clinical and experimental findings allow us to conclude that stiff cirrhotic livers are ultimately exposed to predominant arterial perfusion.

Elevation of hepatic arterial flow and subsequent arterialization is mainly driven by the HABR[42] and hypoxia signaling[43]. SPH postulates that this arterialization defines the so-called "point of no return". It provides a pressure-based rationale to explain the self-perpetuation of fibrosis progression and the uniform, etiology-independent progression of fibrosis. Arterialization of the fibrotic liver ultimately leads to a sustained exposure of the low-pressure organ liver (typically < 6 mmHg) to higher pressures (Figure 5). In ca. 7% of patients with cirrhosis, extreme flow changes can be observed such as complete reversal of the portal flow (so called hepatofugal portal flow)[44]. Part Ⅱ of SPH is summarized in Table 1 and depicted in Figure 4A. At the end, the arterialized liver (high oxygen, high pressure) together with massive matrix deposition will cause self-inflicted ischemia. The combination of these events stimulates the formation of regenerative nodule finally causing the typical nodular aspect of cirrhotic livers. High pressure in combination with cell death and enhanced regeneration ultimately provides an ideal environment of genetic instability and formation of cancer (HCC). It is also postulated that the typical laboratory finding of cirrhotic livers, an increased AST/ALT ratio and a slight GGT elevation (see Supplemental Table 1)[45] is indicative for the stage of arterialization.

CARDIAC CIRRHOSIS - AN EXAMPLE OF NON-INFLAMMATORY, PRESSURE-INDUCED FIBROSIS?

Before the concept of SPH, pressure-associated fibrosis formation in the absence of inflammation has not been appreciated very much. Indeed, most liver diseases seen in daily practice are more or less related to inflammation. However, cardiac cirrhosis is a typically non-inflammatory disease developing in patients with right heart failure and liver congestion. It is mostly but not always seen in the elderly and seems to be solely related to pressure. Notably, cardiac cirrhosis has not been in the focus of studies performed both by cardiologists and hepatologists. A major reason may be that these patients are usually > 70 years old, often present to the hospital in life threatening conditions which impose ethical restrictions for study recruitment. On the other side, decompensated heart failure is one of the most common causes of hospitalization and death in the elderly[46]. First profound insights have been already provided in a now classical work by Sherlock[47] in 1951. Up to date, pressure, hypoxia or nutritional aspects have been discussed as causative underlying factors and its existence has even been questioned by some authors. However, in one of the standard text books on liver histology written by Lefkowitch *et al*[48], cardiac cirrhosis is described in detail with all its histological features. Cardiac cirrhosis can develop rapidly even in young patients with congenital malformation of the heart after the Fontan procedure. These patients will develop portal hypertension, esophageal varices and they can even die from primary liver cancer[49,50]. Figure 6 shows a typical finding of bridging cirrhosis both in an elderly patient with chronic heart failure (Figure 6A-F) and in a young patient after Fontan operation (Figure 6G and H). Hepatic hypoxia can be generally ruled out as cause of cardiac cirrhosis since real ischemia typically leads to dramatic increases of transaminase levels (higher than 1000 U/L with AST > ALT) seen *e.g.*, during acute heart failure or resuscitation. To learn more about the principal development of fibrosis during congestion, we recently explored an experimental model in Wistar rats of liver congestion by clamping the caval vein subphrenically over 4 mo[27]. LS was measured invasively using the novel Fibroscan platform (Echosens, Paris). As shown in Figure 7, congestion significantly and immediately increased LS from mean 6.0 to 10.7 kPa ($P < 10^{-12}$). Of note, the semiquantitative Chevallier fibrosis score significantly increased from 0 to 7. At the mRNA level, profibrogenic markers TGF-β and α-SMA were significantly upregulated[51]. Importantly, detailed histological analysis ruled out inflammation, necrosis or liver injury in this model. In addition, transaminases were not elevated. These findings indicate that increased venous pressure and LS are associated with a pronounced pro-fibrogenic response and histological fibrosis progression in the absence of inflammation. Taken together, patients with longstanding liver congestion during heart failure provide typical examples of pressure-associated fibrosis in the absence of inflammation.

HOW TO ASSESS SP?

Pressure in the context of liver disease is usually discussed with regard to portal pressure as a consequence of cirrhosis. Portal pressure can be assessed directly during TIPS implantation or indirectly *via* wedge pressure measurements of a wedged hepatic vein. So far, SP cannot be determined experimentally *in vivo* since no miniaturized catheters exist without pressure perturbation. Although SP cannot be assessed *in vivo*, we recently measured SP in an isolated pig liver with clamped inflow and outflow vessels[25] (Figure

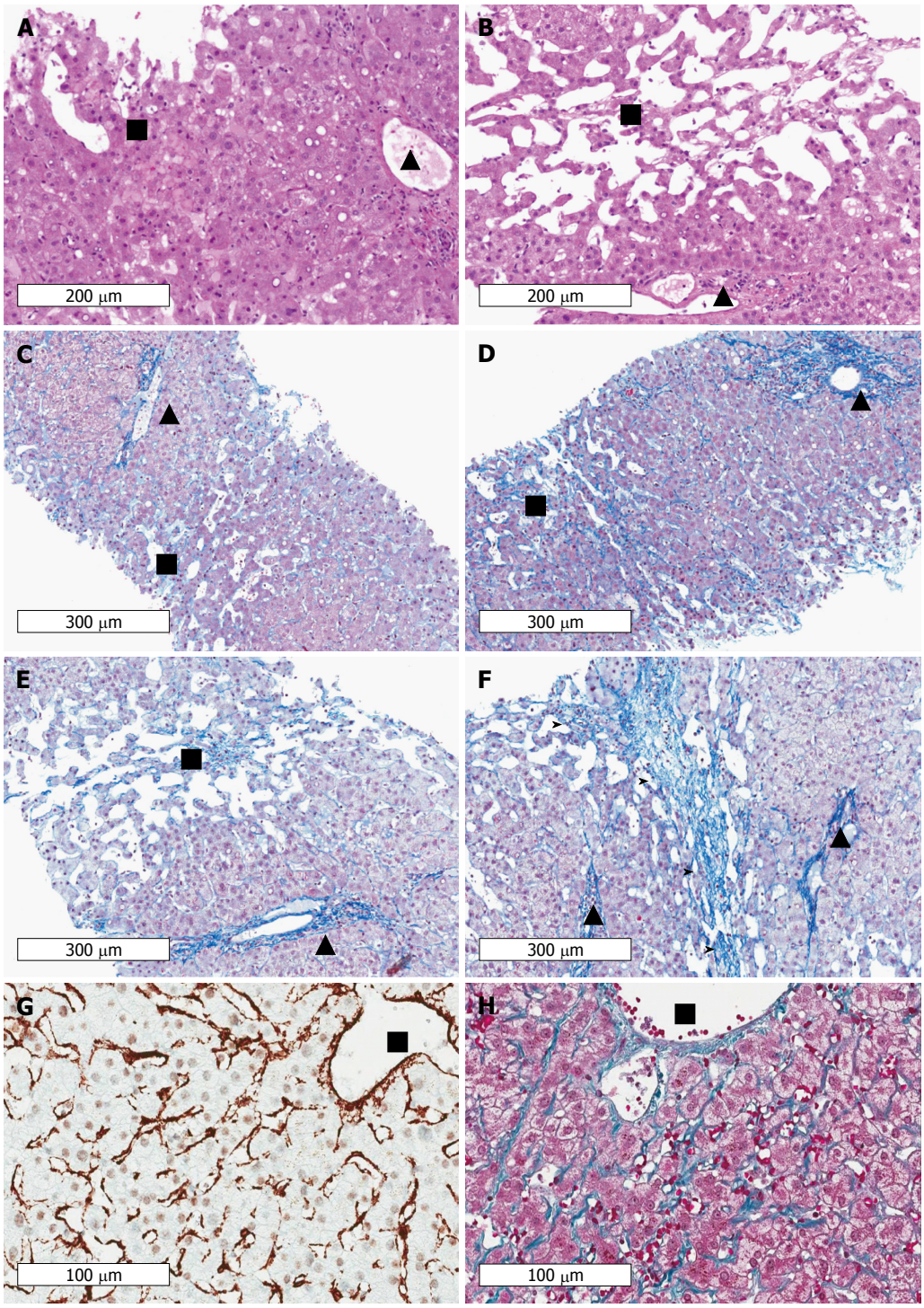

Figure 6 Cardiac cirrhosis as example of a pressure-associated cirrhosis in the absence of notable inflammation. Patients with congestive heart failure may even die from complications of cirrhosis such as variceal bleeding or liver cancer. Note the absence of inflammation in areas with sinusoidal dilation and congestion in long-lasting congestion characterized by marked sinusoidal dilation and atrophy of liver cell plates. Early (A) and advanced (B) stages of congestive heart failure stained by hematoxyline and eosin. Portal tracts and centrilobular areas are marked with black triangles and squares, respectively. Inflammation is also not a feature in areas with sinusoidal dilation and congestion in intermediate and central portions of the lobules. C: Chromotrope aniline blue stain (fibrosis) of an early stage of congestive hepatopathy in a case with congestive heart failure. The portal tract and its structures is regular whereas in central and intermediate portions of the hepatic lobulus mild sinusoidal dilation, slight atrophy of liver cell plates and minimal perisinusoidal fibrosis are seen. D: If venous outlaw obstruction persists perisinusoidal fibrosis and atrophy of liver cell plates in centrilobular areas become more pronounced, (E) which is then followed by loss of liver cell plates and centrilobular fibrosis extending towards neighbouring central veins (F) finally resulting in fibrous septa (marked by arrow heads). Notably, portal-central relations are mostly preserved. Stain for αSMA (G) and fibrosis (Masson trichrome) (H) indicating septal and perisinusoidal fibrosis from a liver biopsy of a 31 years-old male patient with Fontan circulation. The images show diffuse activation of hepatic stellate cells in the absence of any inflammation. Fontan intervention was performed early around birth because of an unilateral ventricle. HVPG was 1 mmHg, LS was 19 kPa. (Images A-F: Courtesy of Dr. C. Lackner, University of Graz; images G-H: Courtesy of Dr. P. Bedossa, Hôpital Beaujon, Université Paris Diderot). HVPG: Hepatic venous pressure gradient; LS: Liver stiffness.

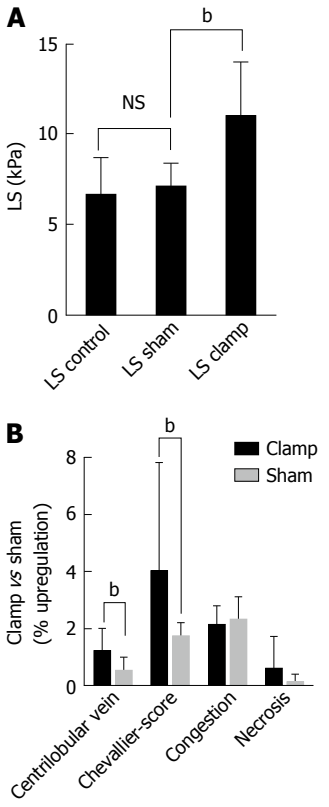

Figure 7 Example of a purely pressure-driven fibrosis: Experimental liver congestion over 4 mo causes significant non-inflammatory liver fibrosis. The caval vein was subphrenically partly clamped in Wistar rats to cause hepatic congestion. A: Illustrates LS in male Wistar rats (n = 6) in control, sham operated and clamped group immediately after onset of congestion; B: Histological analysis of liver tissue sections shows a significant development of fibrosis after 4 mo of congestion in the clamped but not sham operated animals. (data from [27]), $^{b}P < 0.01$. LS: Liver stiffness.

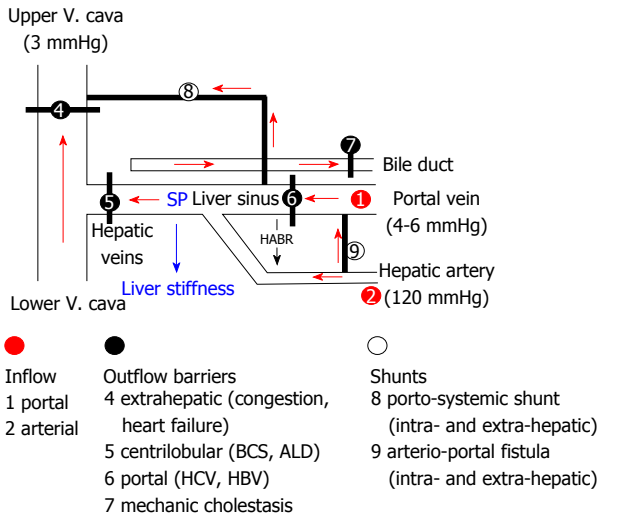

Figure 8 Sinusoidal pressure hypothesis at the vascular level. Simplified scheme of the hepatic vascular architecture and conditions that result in elevated sinusoidal pressure (SP) and liver stiffness (LS). SP and LS are shown as consequence of the various inflow (red circles) and outflow balances (black circles) in a schematized vascular architecture of the liver. Intrahepatic or extrahepatic shunts (white circles) will also affect SP in a complex manner and, according to SPH, will also have an important impact on the development of portal hypertension and liver function. According to SPH, M1 (stiff livers with good liver function) and M2 (soft liver with poor liver function) can be postulated for patients with liver cirrhosis based on intrahepatic shunt formation. Red arrows: Flow direction; HABR: Hepatic arterial buffer response; SPH: Sinusoidal pressure hypothesis.

4B). We then loaded the isolated liver with iso-osmotic sodium chloride solution and measured the hydrostatic pressure in the hepatic vein. In such an isolated and clamped organ, pressures of all communicating vessels are equal according to the physical law of communicating pipes. In the depicted pressure range of up to ca. 40 cm water column, LS correlates almost linearly with SP. For these reasons, LS can be used as an indirect and non-invasive estimate of SP in the absence of cirrhosis. It can be assumed, however, that it will reach a non-linear saturation plateau at higher pressures comparable with the compliance curve of the lung.

SPH AT THE HEMODYNAMIC LEVEL: SP AS CONSEQUENCE OF THE HEPATIC INFLOW/OUTFLOW BALANCE AND STATIC/DYNAMIC COMPONENTS

The liver is generally a low-pressure organ with the portal vein entering the liver with a pressure of ca. 5 mmHg while ca. 3 mm of mercury are measured in the caval vein[52-54]. Close to the right atrium, this pressure can even reach negative values. Despite this low hepatic venous pressure gradient (HVPG) of ca. 3-6 mmHg, the liver is supplied with ca. 25% of the total cardiac output[54]. This also demonstrates the very low vascular resistance of the healthy liver according to Ohm's law of streaming fluids that easily adapts to flow changes *e.g.*, from the splanchnic side[42]. Consequently, the hepatic blood velocity within the sinusoidal bed is very slow which minimizes shear forces causing a predominance of the static pressure and stretch forces of the perisinusoidal cells. This will be discussed later.

Figure 8 shows a simplified scheme of the vascular and biliary architecture of the liver to better illustrate the role of the various inflow, outflow and shunt factors on SP.

Based on the physics of liquids, SP will be mainly determined by static and dynamic components (Figure 2). The static part of the SP is determined by the intravasal pressure and the elastic properties of the vessels walls and also exists in the absence of a functioning blood circulation. Osmotic, oncotic pressure as well as gravitational forces related to the body positioning further contribute to this component. In contrast, the dynamic component is represented by the kinetic energy of the blood flow and becomes only relevant under conditions of an operating blood circulation. Here, the flow resistance constituted by the liver and the blood flow rate generated by the heart will

both affect SP. Moreover, the dynamic part of SP will depend on the localization of *e.g.*, the inflammation. Inflammatory components such as cellular swelling or infiltration of inflammatory cells will all increase the vascular resistance locally either in the portal or central areas. It explains why both a rapid increase of arterial[55] or portal[22] inflow or outflow barriers within the venous outflow tract (congestion)[25], bile ducts (mechanic cholestasis)[35] or the sinusoidal bed[56] are able to increase LS.

In clinical practice, both the static and dynamic components contribute to the overall size of SP in a complex manner. For instance, heart failure causes water retention early on through the activation of the renin angiotensin aldosterone system ultimately causing vascular overfilling with edema. LS will increase simply due to the increase of this static SP. Consequently, treatment with diuretics will decrease LS in such patients[25]. We recently demonstrated that the decrease of LS correlates best with the decrease of weight in patients with decompensated heart failure[57]. Besides water retention, however, the dynamic component also contributes to SP elevation in patients with heart failure. Especially in patients with predominant right heart failure, the left heart is strong enough to increase the pressure before the right heart ultimately causing liver congestion. Taken together, the introduction of pressure into the pathology of fibrosis allows various novel insights to understand fibrogenesis at the hemodynamic level.

ROLE OF HABR WITHIN THE CONCEPT OF SPH

The hepatic artery is connected to the sinusoidal bed via arteriole inlets and an elevated hepatic arterial flow can be seen already before the establishment of fibrosis[58] further increasing with the progression to cirrhosis[39]. Moreover, the liver harbors an autonomous regulatory circuit by which the arterial perfusion is upregulated in response to decreased portal flow, the so-called hepatic arterial buffer response (HABR). HABR has been established many years ago and it is mainly explained by the pharmacological vaso-dilating effects of adenosine (adenosine wash out theory)[59]. Importantly, the HABR does only work in an unidirectional fashion since no elevation of portal flow can be seen in response to decreased arterial flow (Figure 8). This unidirectional aspect of the HABR appears to be highly relevant for the concept of SPH. While an increased inflow via the portal vein (*e.g.*, during food intake) will be "buffered" by the HABR, an increase of the arterial inflow will be directly transmitted to the sinusoidal bed without "buffering". These assumptions have been confirmed indirectly. Thus, a rapid injection of an isotonic solution (volume charge) into the portal vein does not result in LS elevation[55]. Moreover, patients with an arterio-portal fistula of the spleen show portal hypertension but not elevated LS. In contrast, in patients with cirrhosis, LS increases more drastically in response to food intake and alcohol consumption[45,55,60]. This underlines the fact that the buffering-response of HABR will be partly or completely lost in the course of an elevated arterial flow during inflammation or a complete arterialization in the cirrhotic liver. It also means that a predominant arterial flow will be more detrimental to the liver since flow changes will not be "buffered" any longer. Thus, within the concept of SPH, arterialization and loss of the HABR will cause further pressure elevation and enhance pressure-mediated fibrosis.

ROLE OF SHUNTS WITHIN THE CONCEPT OF SPH

Both arterioportal shunts and portosystemic shunts have been described in- and outside the liver and they constitute about one third of the portal flow[61]. Moreover, surgical shunt interventions have been explored for many years and shunt implantations such as TIPS still remain an important option in patients with severe complications of portal hypertension. According to SPH and as shown in Figure 8, these shunts will drastically modulate intra- and extrahepatic pressures. Whether and how they affect SP, fibrosis progression and liver function is still poorly understood. Shunts *per definition* bypass blood and, thus, they will efficiently lower pressure gradients. Recent findings on the association between LS and portal pressure seem to be related to shunt formation and collaterals. Thus, LS only seems to correlate well with portal pressure at a HVPG < 12 mmHg both in human and animal studies[55,62]. In the cirrhotic liver, LS continuously increases while portal pressure stays at lower levels due to the formation of porto-systemic collaterals such as esophageal varices[62]. On the other side, arterio-portal hepatic fistulas have been occasionally described even further complicating the hemodynamic effects of shunts[63,64]. Such arterio-portal fistulas or shunts will decrease arterial perfusion but increase portal pressure. According to SPH, intrahepatic shunts should efficiently lower SP and thus halt pressure-mediated fibrosis progression (Figure 8). Unfortunately, the decreased SP may have detrimental effects on the nutritional and oxygen supply of the liver tissue. Taken together and as will be discussed below, SPH offers a new look at the pressure-modulating role of intrahepatic shunts not only on portal hypertension but also the progression of fibrosis.

SPH AT THE CELLULAR LEVEL

Myofibroblasts are regarded as the major matrix and collagen-producing cells in the liver but also in other tissues. Neo-expression of the alpha isoform of smooth muscle actin (α-SMA) is used as marker

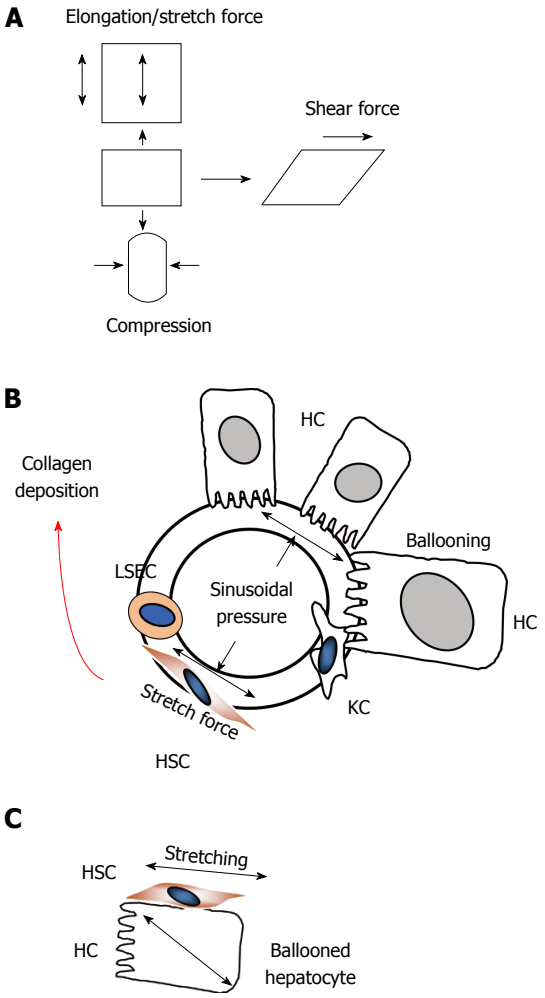

Figure 9 Sinusoidal pressure hypothesis at the cellular level. A: Potential mechanic forces (stretch, compressing and shear forces) are shown that can act on cells; B: According to SPH, SP predominantly translates into mechanic stretch forces within the perisinusoidal bed. Hepatocyte cell death, inflammation or congestion all lead to increased SP that causes stretching of *e.g.*, hepatic stellate cells (HSC), liver sinus endothelial cells (LSEC) or hepatocytes (HC); C: Moreover, intracellular pressure such as seen in ballooned hepatocytes can also cause stretch forces on the hepatocellular membrane and aligned HSC finally causing pericellular fibrosis.

for activated myofibroblasts[65]. Most studies have focused on fibroblasts, which form focal adhesions (FA) during mechanic stress that link the cell's actin cytoskeleton and plasma membrane to the underlying ECM[37,66]. It should be noted that biomechanics has been intensively studied and discussed previously with regard to liver fibrosis[14,37]. Mechanic forces or stress can act in various manners and directions on tissues and cells either by compression, elongation/stretch or shear forces (Figure 9A). Cell surface integrins, which connect to cytoplasmic actins at the site of FA, bind to latency-associated peptides (LAP)[37]. On soft surfaces there is minimal resistance to cell generated tension and the complex remains latent. On stiff surfaces there is significant resistance to cell-generated tension, this tension increases, and the LAP is pulled open, releasing active TGF-β.

However, it has been less appreciated so far that intra-vascular pressure such as SP could be the typical physiological signal of cellular/tissue stiffness. Pressure is not directly visible in histological sections and can only be seen when looking for indirect morphological signs *e.g.*, dilatation of the liver sinus (Figure 6). At the cellular level, SP translates into specific mechanic forces that mainly include stretch forces/stress in contrast to shear forces (Figure 9A). This is due to the enormous broad vascular bed of the liver with a rather slow sinusoidal blood flow (static and dynamic stretch forces, Figure 2). Like other cells, fibroblasts and HSCs are known to contract and to respond to mechanic forces[36]. The SPH puts SP as major mechanistic upstream event of fibrogenesis (cause of fibrosis). Increased SP will result in well-defined stretch forces of perisinusoidal cells *e.g.*, of HSCs and ultimately cause stretch-induced collagen deposition (Figure 9B). SPH may also explain the so-called pericellular fibrosis as is commonly observed in patients suffering from ALD but also other liver diseases. Pericellular fibrosis describes collagen deposition around single ballooned hepatocytes (Figure 9C). Here, intracellular pressure causes stretch forces from inside the hepatocyte that will be also detected by aligned stellate cells and finally lead to mechano-mediated collagen deposition. Thus, both intravascular and intracellular pressure can cause stretch forces at the hepatocyte membrane with consequent stretching of HSC and/or elevation of cellular stiffness.

FURTHER CLINICAL, ANIMAL AND PRELIMINARY OBSERVATIONS IN SUPPORT OF SPH

SPH cannot be proven in one simple experiment or one clinical study, but integrates many established and preliminary observations. In the following, several important examples will be discussed in more detail in addition to the initial observations mentioned above. A detailed list of further arguments in favor of the SPH concept is provided in Supplemental Table 2 within the Appendix (supplemental material file):

(1) Pressure whether static (oncotic, hydrostatic) or dynamic generally determines tissue stiffness. It is well known that *e.g.*, arteriovenous shunt implantation in renal failure patients undergoing dialysis rapidly causes an induration and thickening of the vessel wall. In this operation, a vein is used for the shunt formation and the induration is popularly known as "arterialization" of the shunt. In addition, it can be commonly seen in patients with prolonged non-inflammatory edema of the lower extremities *e.g.*, during chronic heart failure that skin induration remains despite the elimination of the edema or the underlying cause[22]. These long known and general observations point towards an evolutionary-conserved principle of "pressure-

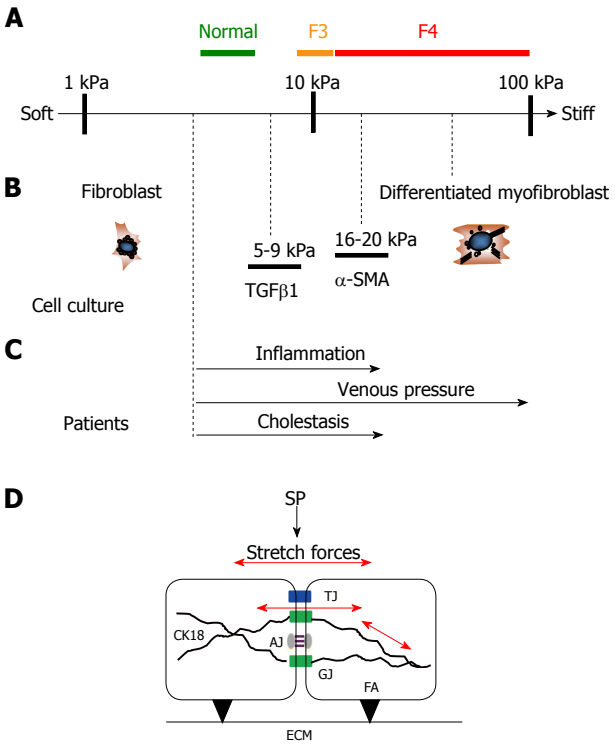

Figure 10 Similar stiffness values are found under pro-fibrogenic conditions in human and cellular studies. A: Stiffness scale with cut off values for normal, F3 and F4 fibrosis (cirrhosis) in humans; B: Known stiffness conditions to activate fibroblasts using atomic force microscopy in cellular studies (modified from Ref. [37]); C: Known LS values in various pathological conditions from human studies that ultimately cause liver fibrosis (modified from Ref. [3]); D: Potential intracellular and intercellular mechano-signaling via intercellular junctions through stretch forces caused by SP elevation. Besides interactions of focal adhesions (FA) with the extracellular matrix (ECM), SP causes intra- and intercellular stretch forces (red arrows) of perisinusoidal cells which are important for matrix production. Several intercellular junctions are schematically shown that may play an important role in biomechanic stretch signaling such as tight junctions (TJ), gap junctions (GJ) and adherence junctions (AJ). Intermediate filaments such as cytokeratin 18 (CK18) play a critical role in liver disease. CK18 is interacting with intercellular junctions and, hence, is most likely important for biomechanic signaling. LS: Liver stiffness; SP: Sinusoidal pressure.

mediated wall thickening of vessels". It seems that the connective tissue at morphological boundaries of *e.g.*, the blood compartment follows a "program" in order to resist and withstand pressure. According to these considerations, the liver would be especially vulnerable to small pressure challenges since the liver is generally exposed to the low incoming pressure of the portal vein of less than 6 mmHg. In fact and in contrast to other organs, the liver is anatomically completely embedded into the venous drainage system of the splanchnic organs. It can be easily imagined that wall-thickening occurs at small SP elevation and that loss of fenestrae and capillarization will result in a loss of liver function. It will also drastically impair the enormous hepatic exchange of metabolites.

(2) We have increasingly learnt that elevated LS *per se* seems to be a risk factor for cirrhosis progression (*e.g.*, LS elevation in response to alcohol or upon HCV infection). It appears that patients who progress to fibrosis show early elevation of LS and LS elevation precedes fibrosis progression[3,26,27]. In contrast, only ca. 30% of patients with ALD and elevated transaminases show LS elevation while the remaining 70% have normal LS despite inflammation[45]. Moreover, successful HCV treatment lowers or even normalizes LS. Likewise, autoimmune hepatitis causes significant LS elevation that can be rapidly normalized if treated timely[22]. More examples could be given with regard to other liver diseases. In contrast, no fibrosis progression has been observed in patients that did not show an LS elevation in response to a viral disease or toxin[22]. If LS is regarded as measure/correlate of SP (in non-cirrhotic livers, Figure 4B), it consequently means that a lack of SP elevation prevents fibrosis progression.

(3) It clearly appears that a normal LS excludes chronic liver disease and liver pathology. So far, no exceptions have been observed from this "rule of thumb". On the other side, there is an enormous variation of LS in response to fibrogenic stimuli with some patients progressing faster to "stiff livers" than others. More details about the relation of LS and liver function will be discussed below.

(4) It is quite striking to see that comparable stiffness values have been observed in patients with various liver diseases and confounders and in cellular studies analyzing the pro-fibrogenic response of HSC and fibroblasts under culturing conditions with exactly defined stiffness as assessed by atomic force microscopy (for details see Figure 10A-C). The identical levels of stiffness and profibrogenic conditions both in clinical and cellular studies are a strong argument for the role of pressure and pressure-mediated stiffness elevation in fibrosis progression.

(5) In contrast, short term LS elevation during acute congestion or acute alcohol exposure (Figure 3) does not cause fibrosis pointing towards the time of pressure exposure as critical parameter. In confirmation, acute forms of hepatitis with short flairs of transaminase elevation (*e.g.*, acute hepatitis A or acute autoimmune hepatitis) do not develop liver cirrhosis and typically show a limited time of LS elevation of 3-5 wk. Therefore, the time of LS elevation of 4 wk seems to be critical for the development and reversal of fibrosis.

(6) In about 7% of patients with liver cirrhosis, a so-called hepatofugal portal flow is observed[44]. In these patients, portal flow has completely reversed. Concordantly, the wedged hepatic venous pressure is higher than the portal pressure which clearly points towards a primarily arterial cause of hepatic perfusion in such patients.

IMPORTANT CONSEQUENCES OF SPH

Any novel hypothesis should offer new insights into poorly explainable observations. In addition, novel studies and experiments should be designable to

verify, falsify or optimize the hypothesis. The following seven major topics have been identified where SPH could provide novel perspectives and initiate new studies:

Awareness of mechanic forces and explanation of macroscopic organization of liver cirrhosis

SPH helps to explain why fibrous septa of bridging cirrhosis may span through the whole organ over large distances. In this concept, SP increases stretch forces that will superimpose over larger distances without the presence of additional *e.g.*, humoral "pro-fibrogenic factors" since mechanic forces will simply add up according to the laws of mechanical physics. These resulting stretch forces are not only confronted with the liver capsule but all elastic and connective tissue within the liver. As a result, fibrous tissue will span over several layers of liver tissue in the centimeter range. The concept of SPH opens up novel targeted studies that address in more detail the role of fluid mechanics in the liver which has unique features as compared to other organs. First, as already mentioned above, the liver is an organ exposed to a low-pressure environment (4-6 mmHg) which can be optionally put under high pressure by the hepatic artery. Second, capillary, adhesion, shear and other forces are insufficiently understood in the liver and they all could contribute to SP. Moreover, the stretching forces do not only put endothelial cells on stage but also all other liver cells with intra- and intercellular filaments and junctions (Figure 10D). In summary, SPH increases the awareness of mechanic forces in the pressure-sensitive liver. It also links to specific forces at the cellular level (stretch *vs* shear forces) and could explain the uniform responses of various cells to pressure (*e.g.*, HSC *vs* fibroblasts) avoiding the necessity to search for specific "profibrogenic" cells.

Uniform formation of end-stage liver cirrhosis by different etiologies

SPH could also explain the uniform response of fibrosis formation to very different and heterogeneous liver pathologies and their combinations. All liver pathologies are related to changes of the SP whether they are of vascular, inflammatory, non-inflammatory or other origin. Within this concept, SP is a complex result of inflow and outflow activities. Supplemental Figure 3 shows schematically the consequences of a periportal (HCV) or pericentral (ALD) inflammation, initially causing periportal or pericentral fibrosis and ultimately bridging fibrosis. Naturally enough, pressure-mediated fibrogenesis will be modulated individually by many genetic and environmental factors at various levels.

Role of hepatic shunt formation and consequences for a novel typology of liver cirrhosis

SPH strongly draws attention to the modulation of intra- and extrahepatic pressure modulation by intrahepatic shunts and extrahepatic collaterals. Shunts per definition bypass blood and thus will efficiently lower pressure gradients. Although microscopic intrahepatic portosystemic shunts have been recognized for many years they are difficult to study[61]. In contrast to arteriovenous shunts, they are present in almost every patient with established cirrhosis and they shunt about 36% of the portal flow[61]. On the other side, arterio-portal hepatic fistulas have been occasionally described[63,64]. As a logical consequence of SPH, especially intrahepatic shunts should efficiently lower SP and thus halt pressure-mediated fibrosis progression (Figure 8). Shunt formation, however, would bypass hepatocytes and, hence, decrease liver function. Thus, the liver could only escape fibrosis progression for the cost of decreased liver function. It would be highly attractive to test whether such mechanisms are indeed an essential part of the liver's physiology to escape high pressures. It could also explain recently observed different types of patients with liver cirrhosis, those with high stiffness but excellent liver function (type M1) and those with rather low LS but very limited liver function (type M2)[21,22,67]. A broad spectrum of all kinds of variations can be observed daily and the biology behind is not well understood nor has it been implemented for risk stratification or transplantation. These considerations could eventually lead to a novel typology of liver cirrhosis.

Molecular and genetic basis of pressure-associated fibrogenesis

SPH with pressure as major driving force could help to better design, delineate and interpret the so-far overwhelming and confusing data obtained by high through-put screening technologies including the many and still rapidly improving genetic or OMICS approaches. It is quite conceivable that pressure is a very complex, highly controlled and evolutionary conserved vital parameter in all mammals. It also could help to better integrate still poorly understood findings *e.g.*, on the role of genetic risk factors such as certain polymorphisms of the *PNPLA3* gene (adiponutrin) for fibrosis progression. It seems quite clear that pressure-mediated processes that ultimately cause fibrosis will be strongly modulated by genes. It is also easily conceivable that SP with all confounders discussed herein are co-modulated by genes *e.g.*, transporters that effect water and electrolyte metabolism, the vessel boundaries, pressure-controlling hormones but, of course, also the response of the biomechanical signal transduction cascade in response to elevated pressure.

Role of arterial pulse wave energy and energy absorption in the liver

The high kinetic energy of the heart-beat is preserved in the elastic aorta and then propagated to peripheral tissues *via* the pulse wave. Due to the stiffer peripheral

arterioles, arterial pressure ultimately increases in the periphery being highest in the arteries of the lower extremities[54]. Almost nothing is known about the consequences of pulse-wave energy propagation in the liver. It is easily conceivable that stiffening of the liver during fibrosis progression but also by pressure-related confounders (Figure 3) could cause a much stronger, Tsunami-like release of mechanic energy in the liver. Moreover and as discussed below, liver fat could be an unexpected and very efficient "sound energy absorbing" factor such as commonly observed in abdominal ultrasound. It will be of high interest to study in more detail the mechanic role of pulse wave energy and hepatic steatosis for SP elevation and fibrosis progression.

Mechanic role of steatosis

The role of steatosis is increasingly and controversially discussed. However, a major physical property of fat is the absorption of mechanic forces or sound energy. Here, SPH could provide an alternative to partly interpret the role of fat on a mechanic basis. Whether the sum of export, elimination, import and synthesis of hepatic fat has mechanic consequences remains to be elucidated. Preliminary data in patients with ALD suggest that steatosis is not correlated with LS which fulfills the general perception that fat tissue is soft[32]. It has also been occasionally observed that LS decreases while steatosis drastically increases (as measured by non-invasive CAP) during improvement of acute hepatitis of various etiology[22]. On the other side, multiple studies on NAFLD patients found a correlation of elevated LS and steatosis but it remains to be clarified whether this is due to co-existing inflammation in these patients. Moreover, SPH would provide a better explanation why *e.g.*, segmental steatosis not necessarily translates into segmental cirrhosis. SPH also draws more attention to the shock wave absorption of the pulsatile heart beat that will drastically increase *e.g.*, at the interface of an artery to the cirrhotic liver. A fatty liver should better attenuate these forces and thus help to at least halt the deleterious effects of mechanic energy exerted by the pulse wave. Taken together, a mechanic approach towards hepatic steatosis could provide a completely novel view on liver pathology.

Role of the intrahepatic localization of inflammation for fibrosis progression (portal vs lobular)

Liver diseases with a more pronounced SP elevation throughout the whole organ are more likely and more rapidly to progress to cirrhosis (see also the simplified vascular architecture of the liver in Figure 8). Thus, certain Zone III localized hepatic diseases such as Budd Chiari syndrome and Schistosomiasis are known to rapidly produce cirrhosis and they are known to strongly increase LS[68-70]. SPH may offer novel possibilities to explain such clinical observations. It is also known that *e.g.*, ALD primarily starts in the region of the central vein causing so-called perivenular fibrosis[71]. In a recent large multicenter study on 2068 patients with biopsy-proven HCV and ALD we could show that inflammation in the lobular zone (ALD) translated into higher LS elevation as compared to a portal-tract-pronounced inflammation such as HCV infection[45] (see also Supplemental Figure 2). According to SPH, a pericentral liver disease will cause a more efficient SP elevation with consequent fibrosis progression. Supplemental Figure 3 provides a simplified scheme of the sequential events of fibrosis formation in ALD and HCV according to SPH. Thus, the localization of the inflammation determines the initial elevation of SP. Infiltration of inflammatory cells and the increase of vascular resistance due to cellular swelling and edema will cause the typical inflammatory hyper-perfusion which can be detected by elevated LS. A prolonged too intensive and too long inflammation will ultimately cause predominant hepatic arterial perfusion with permanent SP elevation. Taken together, SPH could provide a novel concept to better comprehend fibrosis progression based on the localization of the inflammatory disease.

WHAT ARE POTENTIAL LIMITATIONS OF THE SPH?

If the SP is important for fibrosis progression why then has it not been addressed so far? There are several plausible reasons: First, SP cannot be measured directly without affecting the pressure itself for technical reasons. Second, pressure changes are typically accompanied by changes of other important parameters such as blood flow or oxygen supply and it is an experimental challenge to dissect these confounders. Third, SP is not easily identified by popular molecular screening techniques (gene sequencing, RNA microarrays, proteomics, metabolomics) that are favored at present to identify novel target molecules. Fourth, histology does not provide direct and immediate information on pressure except some indirect features shown in Figure 6. In fact, pressure can only be studied *in vivo* within intact organisms with integral boundaries and functioning blood circulation requiring cumbersome detection technologies. Although there are good arguments in favor of SPH it remains unclear to what extent SP contributes to fibrosis and whether known (*e.g.*, TGFβ) or additional mediators of fibrosis significantly modulate pressure-induced fibrosis. Future studies and innovative research tools will hopefully be able to further dissect pressure changes from changes of other important conditions such as hypoxia. It can be also assumed that many pressure-relevant aspects of the liver are genetically inherited in a complex manner. SPH hopefully will help to better design and interpret the findings from genetic screening studies.

Table 2 Potential studies to elucidate sinusoidal pressure-mediated mechanisms of fibrosis

Potential studies to validate SPH
1. Matrix-modulating effects of pressure-lowering or modulating drugs.
2. Physical aspects of pressure formation in biological tissues including the role of cardiac pulse wave energy and its mechanic absorption by fat.
3. Effect of water metabolism, water channels (aquaporins), electrolyte transporters and other transporters and osmotic pressure on matrix formation.
4. Role of pressure-mediated biomechanic signaling for matrix formation including genetics, proteomics and metabolomics.
5. Role of ECM, cellular and inter-cellular junctions on pressure-mediated matrix formation.
6. Role of SP on gap junctions and matrix formation[78].
7. Role of vasoactive systems/substances, such as nitric oxide, cyclooxygenase-derivatives, carbon monoxide and endogenous cannabinoids on SP and fibrosis[79].
8. Role of vasoconstrictor systems, such as the sympathetic nervous system, vasopressin, angiotensin and endothelin-1 on SP and fibrosis[80,81].
9. Optimization of pressure sensors *e.g.*, for the liver sinus including the development of molecular stretch force measuring sensors[4].
10. Association of pressure, tissue/cellular stiffness and matrix formation at various organizational levels (cell, organ and whole organism).
11. Interplay of organ systems involved in water and pressure regulation (*e.g.*, heart, brain, kidney and liver) for pressure regulation and matrix development.
12. Role of liver size and globularization of liver in various species in order to better sustain stretch forces of SP elevation.
13. Mechanisms and modulation of vessel and shunt formation in the liver.

ECM: Extracellular matrix; SP: Sinusoidal pressure; SPH: Sinusoidal pressure hypothesis.

HOW TO DISSECT SP FROM HYPOXIA-MEDIATED LIVER PATHOLOGIES?

A specific challenge for SPH is the dissection of pressure-related effects from hypoxia-mediated consequences. The individual hepatocyte is predominantly exposed to ca. 2% oxygen while intravascular oxygen levels vary dependent on localization (venous *vs* portal) and range between 8% to 16%[72,73]. It is well established that the portal zone 1 is exposed to ca. 16% oxygen while the precentral zone 3 see significantly less oxygen of ca. 8%. Already the pioneering article of Sherlock[47] on cardiac cirrhosis pointed out the difficulties to dissect pressure-associated changes from hypoxia-related aspects. In fact, since pressure changes are always and immediately accompanied by flow and oxygen changes, it may be almost impossible to rule out directly the distinctive role of both important parameters. In addition, as mentioned in the chapter above, it is still not possible to directly assess SP and oxygen levels in the liver sinus without disturbance of these factors for technical reasons. However, in my opinion, some general considerations and indirect conclusions are in favor of the SPH concept. First, hepatocytes and other liver cells tolerate quite low oxygen levels[72]. In fact, real hepatic ischemia is rarely observed. It typically leads to drastic elevation of transaminases up to several thousands of units *e.g.*, during cardiac arrest. Thus, as long as no such drastic transaminase elevations are observed, the involvement of hypoxia seems to be less likely. Second, although the portal tract is much more efficiently exposed to highly oxygenated blood from the hepatic artery, portal-tract localized liver diseases such as chronic HCV also develop fibrosis as compared to zone III disease such as ALD. Finally, namely the development of large fibrous septa at the whole organ level are difficult to comprehend based on oxygen levels but rather point to trajectory forces caused by pressure elevation. Nevertheless, it will remain an open and highly interesting area of research to further dissect the role of SP and hypoxia and even more likely the role of hypoxia in modulating pressure-induced fibrosis.

POTENTIAL FUTURE EXPERIMENTAL STRATEGIES TO VALIDATE SPH

As mentioned above, pressure itself is a physiological key process of mammals which is controlled by many cellular, nerval and hormonal conditions that all link to matrix formation. In fact, the epithelial boundaries are critical for pressure maintenance and they put all aligning cells of the vascular system whether they are veins, arteries, capillaries or specialized vascular entities such as the hepatic sinusoidal bed on stage. For instance, while FA and ECM-cell mechano-signaling have been intensively studied[14,37], intercellular mechanotransduction (intercellular junctions of parenchymal cells and intermediate filaments) and its relation to pressure is largely unknown and would require adequate animal models for validation. Therefore, future studies should address these molecular mechanisms. A list of such potential studies is provided in Table 2. One potential strategy is the use of well-established *in vitro* models under pressure-mimicking conditions with varying stiffness using viscoelastic gels (*e.g.*, polyacrylamide)[74,75]. The stiffness of these gels should be comparable to human fibrosis stages and validated using the Fibroscan or atomic force microscopy[74]. The various liver-associated cells could be studied independently, individually or in combination using co-culture approaches. Important cells should not be restricted to HSC and fibroblasts, but also endothelial cells, hepatocytes and macrophages. The role of intercellular junctions and important molecules responsible for mechano-signaling could be examined

Table 3 Direct clinical consequences of sinusoidal pressure hypothesis

Potential clinical impact of SPH	Ref.
1. Therapeutic effects of pressure lowering drugs. Optimization of timing, patient selection, dosage and duration. Risk balancing of side affects to other organs (kidneys, arterial underfilling).	[77]
2. Long-term therapy with diuretics as causal/fibrosis-blocking treatment.	
3. Testing of an optimized risk stratification of cirrhotics on outcome according to liver stiffness (M1 *vs* M2 type, see paragraph "Important consequences of SPH and critical discussion", point 3) in addition to liver function scores such as Child-Pugh score or MELD score.	[25]
4. Liver disease as cause and consequence in the systemic context with other organs such as kidney and heart failure.	[57]
5. Test whether GGT elevation and an AST/ALT ratio > 1 at low AST and ALT levels is related with arterialization of liver and, consequently, with manifestation of liver cirrhosis.	
6. Study water retention in cirrhosis, pregnancy, renal and heart failure and its consequences on hydrostatic SP.	[82]
7. Implementation of osmotic stress, water channels (aquaporines) and transporters.	
8. Therapeutic approaches to lower SP by targeting mechano-signaling: mechanic conditioning and pharmacotherapy acting on mechano-signaling.	
9. Role of biomembrane composition, lipid composition and potential protective role of steatosis on pressure-induced fibrosis.	
10. Non-invasive LS measurements to monitor and optimize treatment of liver diseases.	
11. Implementation of liquid physics to better understand the dynamic component of SP and its role on fibrosis progression.	
12. Understanding of pulse wave energy and its consequences on the liver tissue.	

ALT: Alanine aminotransferase; AST: Asparagin aminotransferase; GGT: Gamma-glutamyl transpeptidase; LS: Liver stiffness; SP: Sinusoidal pressure; SPH: Sinusoidal pressure hypothesis.

in 2D *vs* 3D cell cultures. As mechanistic proof of principle, hepatic mechano-conditioning using stretch chambers and fat loading (in the absence of lipotoxicity) to lower cellular stiffness could be studied. Such studies could then be translated to *in vivo* animal models. For instance, the time of onset of LS as compared to fibrosis development, invasive pressure measurements in different compartments (portal, central venous, systolic and diastolic arterial pressure), mechano-signaling and the expression of intercellular junction molecules could be studied in well-established inflammatory and non-inflammatory fibrosis model (TAA *vs* congestion). The animal models would allow to study the onset of arterialization in cirrhotic livers using vascular immunostaining and the role of intercellular mechano-signaling. The new insights could help to identify novel treatment strategies *e.g.*, to lower LS or, second, to better identify patient sub-cohorts that are at increased risk to develop fibrosis *via* mechano-signaling due to SP. Another important issue will be the interplay of SP with hypoxia and angiogenesis and how this affects vascularization of the sinusoidal bed including shunt formation.

FUTURE CLINICAL IMPACT OF SPH

SPH could boost and stimulate basic and clinical research activities not only restricted to liver disease but also the bidirectional role of liver-diseases within the whole organism and its relation to other organs such as the heart, kidney, or lung. Some potential therapeutic consequences are listed in Table 3. It may further lead to a re-evaluation or optimization of established supportive standard therapies in cirrhotics. Thus, SPH could help to explain the predominantly and widely discussed beneficial effects of pressure-lowering drugs such as NSBB and it could help to optimize treatment regimens, patient selection and a better understanding of their mechanisms[76,77]. Second, SPH sheds new light on the long-term therapy with diuretics in cirrhotics. Diuretics may not only remove excess water from the body according to a symptomatic approach but it may intercept with the viscous cycle of continued water retention, SP elevation (hydrostatic component; Figure 2) and fibrosis progression. It will also be quite exciting to learn whether the liver has a more immanent role in other diseases with water retention such as cardiac insufficiency. On the diagnostic level, non-invasive LS measurement may help to monitor and optimize treatment of liver diseases especially with its direct link to SP. At the molecular level, the therapeutic desirable lowering of SP directly leads to a better understanding of hepatic mechano-signaling and the regulation of SP at the cellular level. Potential novel molecular targets or strategies could be identified that may include mechanic conditioning or pharmacotherapy acting on mechanosignaling. The mechanic role of fat has been already discussed above and, based on preliminary observation, warrants further analysis. Further studies on SP will also require the implementation of liquid physics to better understand the dynamic component of SP and its role on fibrosis progression. Finally, the role of osmotic stress, the regulation of the hydration status of the cell and the role of water channels such as aquaporins will be likewise highly interesting to study in the context of SP elevation and fibrosis progression.

ACKNOWLEDGMENTS

This hypothesis paper is dedicated to Helmut K. Seitz in memory of our longstanding friendship, the many discussions and his continued support of my work.

I would further like to thank (in alphabetical order) Pierre Bedossa, Eckart Hofmann, Carolin Lackner, Ana Elena López Echevarria; Gunda Millonig, Johannes Mueller, Felix Piecha, Vanessa Rausch, Martin Rössle, Daniel Rost, Laurent Sandrin and Helmut K. Seitz for many intensive discussions and/or for critical reading of the manuscript. I finally like to acknowledge the Dietmar Hopp foundation and the Manfred Lautenschläger foundation for their financial support over the last years.

REFERENCES

1 **WHO**. Global status report on alcohol and health. Available at: URL: http://whoint/substance_abuse/publications/global_alcohol_report/en/

2 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713 [PMID: 14698338]

3 **Mueller S**, Sandrin L. Liver stiffness: a novel parameter for the diagnosis of liver disease. *Hepat Med* 2010; **2**: 49-67 [PMID: 24367208]

4 **Cost AL**, Ringer P, Chrostek-Grashoff A, Grashoff C. How to Measure Molecular Forces in Cells: A Guide to Evaluating Genetically-Encoded FRET-Based Tension Sensors. *Cell Mol Bioeng* 2015; **8**: 96-105 [PMID: 25798203 DOI: 10.1007/s12195-014-0368-1]

5 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]

6 **Schuppan D**, Afdhal NH. Liver cirrhosis. *Lancet* 2008; **371**: 838-851 [PMID: 18328931 DOI: 10.1016/S0140-6736(08)60383-9]

7 **Bosch J**, Groszmann RJ, Shah VH. Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments. *J Hepatol* 2015; **62**: S121-S130 [PMID: 25920081 DOI: 10.1016/j.jhep.2015.01.003]

8 **Fares N**, Péron JM. [Epidemiology, natural history, and risk factors of hepatocellular carcinoma]. *Rev Prat* 2013; **63**: 216-217, 220-222 [PMID: 23513788]

9 **Lozano R**, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De Leon FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128 [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0]

10 **Berres ML**, Papen S, Pauels K, Schmitz P, Zaldivar MM, Hellerbrand C, Mueller T, Berg T, Weiskirchen R, Trautwein C, Wasmuth HE. A functional variation in CHI3L1 is associated with severity of liver fibrosis and YKL-40 serum levels in chronic hepatitis C infection. *J Hepatol* 2009; **50**: 370-376 [PMID: 19070929 DOI: 10.1016/j.jhep.2008.09.016]

11 **Seitz HK**, Mueller S. Alcoholic liver disease. In: Dancygier H, editor Clinical Hepatology: Principles and Practice of Hepatobiliary Diseases. Heidelberg, Dordrecht, London, New York: Springer, 2009: 1111-1152

12 **Benyon RC**, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. *Semin Liver Dis* 2001; **21**: 373-384 [PMID: 11586466 DOI: 10.1055/s-2001-17552]

13 **Parés A**, Caballería J. Metabolism of collagen and other extracellular proteins//Oxford textbook of clinical hepatology. Oxford University Press Oxford, 1991: 199-211

14 **Wells RG**. Tissue mechanics and fibrosis. *Biochim Biophys Acta* 2013; **1832**: 884-890 [PMID: 23434892 DOI: 10.1016/j.bbadis.2013.02.007]

15 **Olaso E**, Friedman SL. Molecular regulation of hepatic fibrogenesis. *J Hepatol* 1998; **29**: 836-847 [PMID: 9833926]

16 **Buch S**, Stickel F, Trépo E, Way M, Herrmann A, Nischalke HD, Brosch M, Rosendahl J, Berg T, Ridinger M, Rietschel M, McQuillin A, Frank J, Kiefer F, Schreiber S, Lieb W, Soyka M, Semmo N, Aigner E, Datz C, Schmelz R, Brückner S, Zeissig S, Stephan AM, Wodarz N, Devière J, Clumeck N, Sarrazin C, Lammert F, Gustot T, Deltenre P, Völzke H, Lerch MM, Mayerle J, Eyer F, Schafmayer C, Cichon S, Nöthen MM, Nothnagel M, Ellinghaus D, Huse K, Franke A, Zopf S, Hellerbrand C, Moreno C, Franchimont D, Morgan MY, Hampe J. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. *Nat Genet* 2015; **47**: 1443-1448 [PMID: 26482880 DOI: 10.1038/ng.3417]

17 **Mells GF**, Floyd JA, Morley KI, Cordell HJ, Franklin CS, Shin SY, Heneghan MA, Neuberger JM, Donaldson PT, Day DB, Ducker SJ, Muriithi AW, Wheater EF, Hammond CJ, Dawwas MF, Jones DE, Peltonen L, Alexander GJ, Sandford RN, Anderson CA. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet* 2011; **43**: 329-332 [PMID: 21399635 DOI: 10.1038/ng.789]

18 **Hillebrandt S**, Wasmuth HE, Weiskirchen R, Hellerbrand C, Keppeler H, Werth A, Schirin-Sokhan R, Wilkens G, Geier A, Lorenzen J, Köhl J, Gressner AM, Matern S, Lammert F. Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. *Nat Genet* 2005; **37**: 835-843 [PMID: 15995705 DOI: 10.1038/ng1599]

19 **Day CP**, James OF. Hepatic steatosis: innocent bystander or guilty party? *Hepatology* 1998; **27**: 1463-1466 [PMID: 9620314]

20 **Mueller S**. CAP: a novel era to better quantitate fatty liver? *J Gastrointestin Liver Dis* 2015; **24**: 11-13 [PMID: 25822428 DOI: 10.15403/jgld.2014.1121.smu]

21 **Mueller S**, Seitz HK, Rausch V. Non-invasive diagnosis of alcoholic liver disease. *World J Gastroenterol* 2014; **20**: 14626-14641 [PMID: 25356026 DOI: 10.3748/wjg.v20.i40.14626]

22 **Mueller S**. Personal observation. 2016

23 **Abraldes JG**, Pasarín M, García-Pagán JC. Animal models of

portal hypertension. *World J Gastroenterol* 2006; **12**: 6577-6584 [PMID: 17075968 DOI: 10.3748/wjg.v12.i41.6577]
24 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]
25 **Millonig G**, Friedrich S, Adolf S, Fonouni H, Golriz M, Mehrabi A, Stiefel P, Pöschl G, Büchler MW, Seitz HK, Mueller S. Liver stiffness is directly influenced by central venous pressure. *J Hepatol* 2010; **52**: 206-210 [PMID: 20022130 DOI: 10.1016/j.jhep.2009.11.018]
26 **Georges PC**, Hui JJ, Gombos Z, McCormick ME, Wang AY, Uemura M, Mick R, Janmey PA, Furth EE, Wells RG. Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G1147-G1154 [PMID: 17932231 DOI: 10.1152/ajpgi.00032.2007]
27 **Peccerella T**, Ottinger S, Seitz HK, T. L, Mueller S. Fibrosis progression in a non-inflammatory small animal model of liver congestion. *J Hepatol* 2014; **60**: 72
28 **Hézode C**, Castéra L, Roudot-Thoraval F, Bouvier-Alias M, Rosa I, Roulot D, Leroy V, Mallat A, Pawlotsky JM. Liver stiffness diminishes with antiviral response in chronic hepatitis C. *Aliment Pharmacol Ther* 2011; **34**: 656-663 [PMID: 21752038 DOI: 10.1111/j.1365-2036.2011.04765.x]
29 **Mueller S**, Millonig G, Sarovska L, Friedrich S, Reimann FM, Pritsch M, Eisele S, Stickel F, Longerich T, Schirmacher P, Seitz HK. Increased liver stiffness in alcoholic liver disease: differentiating fibrosis from steatohepatitis. *World J Gastroenterol* 2010; **16**: 966-972 [PMID: 20180235 DOI: 10.3748/wjg.v16.i8.966]
30 **Lindvig K**, Mössner BK, Pedersen C, Lillevang ST, Christensen PB. Liver stiffness and 30-day mortality in a cohort of patients admitted to hospital. *Eur J Clin Invest* 2012; **42**: 146-152 [PMID: 21793821 DOI: 10.1111/j.1365-2362.2011.02571.x]
31 **Vergniol J**, Foucher J, Terrebonne E, Bernard PH, le Bail B, Merrouche W, Couzigou P, de Ledinghen V. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. *Gastroenterology* 2011; **140**: 1970-1979, 1979.e1-3 [PMID: 21376047 DOI: 10.1053/j.gastro.2011.02.058]
32 **Rausch V**, Peccerella T, Lackner C, Yagmur E, Seitz HK, Longerich T, Mueller S. Primary liver injury and delayed resolution of liver stiffness after alcohol detoxification in heavy drinkers with the PNPLA3 variant I148M. *World J Hepatol* 2016; **8**: 1547-1556 [DOI: 10.4254/wjh.v8.i35.1547]
33 **Arena U**, Vizzutti F, Abraldes JG, Corti G, Stasi C, Moscarella S, Milani S, Lorefice E, Petrarca A, Romanelli RG, Laffi G, Bosch J, Marra F, Pinzani M. Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. *Gut* 2008; **57**: 1288-1293 [PMID: 18448567 DOI: 10.1136/gut.2008.149708]
34 **Sagir A**, Erhardt A, Schmitt M, Häussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology* 2008; **47**: 592-595 [PMID: 18098325 DOI: 10.1002/hep.22056]
35 **Millonig G**, Reimann FM, Friedrich S, Fonouni H, Mehrabi A, Büchler MW, Seitz HK, Mueller S. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. *Hepatology* 2008; **48**: 1718-1723 [PMID: 18836992 DOI: 10.1002/hep.22577]
36 **Discher DE**, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science* 2005; **310**: 1139-1143 [PMID: 16293750 DOI: 10.1126/science.1116995]
37 **Hinz B**. Tissue stiffness, latent TGF-beta1 activation, and mechanical signal transduction: implications for the pathogenesis and treatment of fibrosis. *Curr Rheumatol Rep* 2009; **11**: 120-126 [PMID: 19296884]
38 **Dragoteanu M**, Cotul SO, Pîgleşan C, Tamaş S. Liver angioscintigraphy: clinical applications. *Rom J Gastroenterol* 2004; **13**: 55-63 [PMID: 15054528]
39 **Kleber G**, Steudel N, Behrmann C, Zipprich A, Hübner G, Lotterer E, Fleig WE. Hepatic arterial flow volume and reserve in patients with cirrhosis: use of intra-arterial Doppler and adenosine infusion. *Gastroenterology* 1999; **116**: 906-914 [PMID: 10092313]
40 **Li MD**, Chen Y, Chen YX, Gao ZL, Zhu K, Yin X. [Whole-liver perfusion imaging by multi-slice spiral computed tomography based on Couinaud segments for evaluation of the blood flow state in patients with hepatitis B cirrhosis]. *Zhonghua Ganzangbing Zazhi* 2014; **22**: 817-821 [PMID: 25531377 DOI: 10.3760/cma.j.issn.1007-3418.2014.11.004]
41 **Ehling J**, Bartneck M, Wei X, Gremse F, Fech V, Möckel D, Baeck C, Hittatiya K, Eulberg D, Luedde T, Kiessling F, Trautwein C, Lammers T, Tacke F. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. *Gut* 2014; **63**: 1960-1971 [PMID: 24561613 DOI: 10.1136/gutjnl-2013-306294]
42 **Lautt WW**. Hepatic Circulation: Physiology and Pathophysiology. Colloquium Series on Integrated Systems Physiology: from Molecule to Function. *Morgan Claypool Publishers* 2009; **1**: 1-174
43 **Medina J**, Arroyo AG, Sánchez-Madrid F, Moreno-Otero R. Angiogenesis in chronic inflammatory liver disease. *Hepatology* 2004; **39**: 1185-1195 [PMID: 15122744 DOI: 10.1002/hep.20193]
44 **Rector WG**, Hoefs JC, Hossack KF, Everson GT. Hepatofugal portal flow in cirrhosis: observations on hepatic hemodynamics and the nature of the arterioportal communications. *Hepatology* 1988; **8**: 16-20 [PMID: 3338703]
45 **Mueller S**, Englert S, Seitz HK, Badea RI, Erhardt A, Bozaari B, Beaugrand M, Lupşor-Platon M. Inflammation-adapted liver stiffness values for improved fibrosis staging in patients with hepatitis C virus and alcoholic liver disease. *Liver Int* 2015; **35**: 2514-2521 [PMID: 26121926 DOI: 10.1111/liv.12904]
46 **Curtis LH**, Whellan DJ, Hammill BG, Hernandez AF, Anstrom KJ, Shea AM, Schulman KA. Incidence and prevalence of heart failure in elderly persons, 1994-2003. *Arch Intern Med* 2008; **168**: 418-424 [PMID: 18299498 DOI: 10.1001/archinternmed.2007.80]
47 **Sherlock S**. The liver in heart failure; relation of anatomical, functional, and circulatory changes. *Br Heart J* 1951; **13**: 273-293 [PMID: 14848381]
48 **Lefkowitch JH**. Scheuer's Liver Biopsy Interpretation. 8th Ed. ed: Edinburgh: Saunders Elsevier, 2010
49 **Izumi Y**, Hiramatsu N, Itose I, Inoue T, Sasagawa A, Egawa S, Nishida T, Kakiuchi Y, Toyama T, Nakanishi F, Ohkawa K, Mochizuki K, Kanto T, Tsujii M, Takehara T, Tsuji S, Kato M, Kasahara A, Hayashi N. Juvenile hepatocellular carcinoma with congestive liver cirrhosis. *J Gastroenterol* 2005; **40**: 204-208 [PMID: 15770406 DOI: 10.1007/s00535-004-1525-4]
50 **Rodríguez-Artalejo F**, Banegas Banegas JR, Guallar-Castillón P. [Epidemiology of heart failure]. *Rev Esp Cardiol* 2004; **57**: 163-170 [PMID: 14967113]
51 **Simonetto DA**, Yang HY, Yin M, de Assuncao TM, Kwon JH, Hilscher M, Pan S, Yang L, Bi Y, Beyder A, Cao S, Simari RD, Ehman R, Kamath PS, Shah VH. Chronic passive venous congestion drives hepatic fibrogenesis via sinusoidal thrombosis and mechanical forces. *Hepatology* 2015; **61**: 648-659 [PMID: 25142214 DOI: 10.1002/hep.27387]
52 **Balfour DC**, Reynolds TB, Levinson DC, Mikkelsen WP, Pattison AC. Hepatic vein pressure studies for evaluation of intrahepatic portal hypertension. *AMA Arch Surg* 1954; **68**: 442-447 [PMID: 13147661]
53 **Atkinson M**, Sherlock S. Intrasplenic pressure as index of portal venous pressure. *Lancet* 1954; **266**: 1325-1327 [PMID: 13164385]
54 **Schmidt RF**, Thews G. Physiologie des Menschen. 23. Auflage ed. Heidelberg: Springer, Verlag, 1987: 563
55 **Piecha F**, Peccerella T, Bruckner T, Seitz HK, Rausch V, Mueller S. Arterial pressure suffices to increase liver stiffness. *Am J Physiol Gastrointest Liver Physiol* 2016; **311**: G945-G953 [PMID: 27288426 DOI: 10.1152/ajpgi.00399.2015]
56 **Arena U**, Vizzutti F, Corti G, Ambu S, Stasi C, Bresci S, Moscarella S, Boddi V, Petrarca A, Laffi G, Marra F, Pinzani M. Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; **47**: 380-384 [PMID:

18095306]
57 **Dietrich C**, Yagmur E, Seitz HK, Viedt-Suelmann C, Mueller S. Cardiac cirrhosis contributes to the water retention in patients with heart failure. *J Hepatol* 2013; **58**: S242 [DOI: 10.1016/S0168-8278(13)60594-2]
58 **Moeller M**, Thonig A, Pohl S, Ripoll C, Zipprich A. Hepatic arterial vasodilation is independent of portal hypertension in early stages of cirrhosis. *PLoS One* 2015; **10**: e0121229 [PMID: 25793622 DOI: 10.1371/journal.pone.0121229]
59 **Lautt WW**. Mechanism and role of intrinsic regulation of hepatic arterial blood flow: hepatic arterial buffer response. *Am J Physiol* 1985; **249**: G549-G556 [PMID: 3904482]
60 **Arena U**, Lupsor Platon M, Stasi C, Moscarella S, Assarat A, Bedogni G, Piazzolla V, Badea R, Laffi G, Marra F, Mangia A, Pinzani M. Liver stiffness is influenced by a standardized meal in patients with chronic hepatitis C virus at different stages of fibrotic evolution. *Hepatology* 2013; **58**: 65-72 [PMID: 23447459 DOI: 10.1002/hep.26343]
61 **Ohnishi K**, Chin N, Sugita S, Saito M, Tanaka H, Terabayashi H, Saito M, Iida S, Nomura F, Okuda K. Quantitative aspects of portal-systemic and arteriovenous shunts within the liver in cirrhosis. *Gastroenterology* 1987; **93**: 129-134 [PMID: 3582900 DOI: 10.1016/0016-5085(87)90324-6]
62 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971 DOI: 10.1002/hep.21665]
63 **Pasternak BM**, Cohen H. Arteriovenous fistula and forward hypertension in the portal circulation. *Angiology* 1978; **29**: 367-373 [PMID: 655466]
64 **Harada N**, Ito T, Taura S, Tsuchiya R, Koga Y. Portal hypertension due to hepatic artery-portal vein arteriovenous fistula--a case report. *Jpn J Surg* 1975; **5**: 145-152 [PMID: 1228260]
65 **Tomasek JJ**, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol* 2002; **3**: 349-363 [PMID: 11988769 DOI: 10.1038/nrm809]
66 **Tamariz E**, Grinnell F. Modulation of fibroblast morphology and adhesion during collagen matrix remodeling. *Mol Biol Cell* 2002; **13**: 3915-3929 [PMID: 12429835 DOI: 10.1091/mbc.E02-05-0291]
67 **Mueller S**, Seitz HK, Schirmacher P, Straub BK. Nicht invasive versus invasive Beurteilung der Leberfibrose. *Gastroenterologie up2date* 2014; **10**: 51-67
68 **de Carvalho BT**, Coutinho Domingues AL, de Almeida Lopes EP, Brandão SC. Increased Hepatic Arterial Blood Flow Measured by Hepatic Perfusion Index in Hepatosplenic Schistosomiasis: New Concepts for an Old Disease. *Dig Dis Sci* 2016; **61**: 2118-2126 [PMID: 26921079 DOI: 10.1007/s10620-016-4080-y]
69 **Huang H**, Deng M, Jin H, Liu A, Dirsch O, Dahmen U. Hepatic arterial perfusion is essential for the spontaneous recovery from focal hepatic venous outflow obstruction in rats. *Am J Transplant* 2011; **11**: 2342-2352 [PMID: 21831159 DOI: 10.1111/j.1600-6143.2011.03682.x]
70 **Shahin M**, Schuppan D, Waldherr R, Risteli J, Risteli L, Savolainen ER, Oesterling C, Abdel Rahman HM, el Sahly AM, Abdel Razek SM. Serum procollagen peptides and collagen type VI for the assessment of activity and degree of hepatic fibrosis in schistosomiasis and alcoholic liver disease. *Hepatology* 1992; **15**: 637-644 [PMID: 1551641 DOI: 10.1002/hep.1840150414]
71 **Nakano M**, Worner TM, Lieber CS. Perivenular fibrosis in alcoholic liver injury: ultrastructure and histologic progression. *Gastroenterology* 1982; **83**: 777-785 [PMID: 7106508]
72 **Millonig G**, Hegedüsch S, Becker L, Seitz HK, Schuppan D, Mueller S. Hypoxia-inducible factor 1 alpha under rapid enzymatic hypoxia: cells sense decrements of oxygen but not hypoxia per se. *Free Radic Biol Med* 2009; **46**: 182-191 [PMID: 19007879]
73 **Broughan TA**, Naukam R, Tan C, Van De Wiele CJ, Refai H, Teague TK. Effects of hepatic zonal oxygen levels on hepatocyte stress responses. *J Surg Res* 2008; **145**: 150-160 [PMID: 18164035 DOI: 10.1016/j.jss.2007.04.014]
74 **Du Y**, Han R, Wen F, Ng San San S, Xia L, Wohland T, Leo HL, Yu H. Synthetic sandwich culture of 3D hepatocyte monolayer. *Biomaterials* 2008; **29**: 290-301 [PMID: 17964646 DOI: 10.1016/j.biomaterials.2007.09.016]
75 **Chen S**, Shi J, Xu X, Ding J, Zhong W, Zhang L, Xing M, Zhang L. Study of stiffness effects of poly(amidoamine)-poly(n-isopropyl acrylamide) hydrogel on wound healing. *Colloids Surf B Biointerfaces* 2016; **140**: 574-582 [PMID: 26628331 DOI: 10.1016/j.colsurfb.2015.08.041]
76 **Garcia-Tsao G**. Beta blockers in cirrhosis: The window re-opens. *J Hepatol* 2016; **64**: 532-534 [PMID: 26724557 DOI: 10.1016/j.jhep.2015.12.012]
77 **Mookerjee RP**, Pavesi M, Thomsen KL, Mehta G, Macnaughtan J, Bendtsen F, Coenraad M, Sperl J, Gines P, Moreau R, Arroyo V, Jalan R. Treatment with non-selective beta blockers is associated with reduced severity of systemic inflammation and improved survival of patients with acute-on-chronic liver failure. *J Hepatol* 2016; **64**: 574-582 [PMID: 26519600 DOI: 10.1016/j.jhep.2015.10.018]
78 **Hernández-Guerra M**, González-Méndez Y, de Ganzo ZA, Salido E, García-Pagán JC, Abrante B, Malagón AM, Bosch J, Quintero E. Role of gap junctions modulating hepatic vascular tone in cirrhosis. *Liver Int* 2014; **34**: 859-868 [PMID: 24350605 DOI: 10.1111/liv.12446]
79 **Bolognesi M**, Di Pascoli M, Verardo A, Gatta A. Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis. *World J Gastroenterol* 2014; **20**: 2555-2563 [PMID: 24627591 DOI: 10.3748/wjg.v20.i10.2555]
80 **Bataller R**, Schwabe RF, Choi YH, Yang L, Paik YH, Lindquist J, Qian T, Schoonhoven R, Hagedorn CH, Lemasters JJ, Brenner DA. NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis. *J Clin Invest* 2003; **112**: 1383-1394 [PMID: 14597764 DOI: 10.1172/JCI18212]
81 **Yokohama S**, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, Hasegawa T, Tokusashi Y, Miyokawa N, Nakamura K. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. *Hepatology* 2004; **40**: 1222-1225 [PMID: 15382153 DOI: 10.1002/hep.20420]
82 **Schrier RW**, Cadnapaphornchai MA, Ohara M. Water retention and aquaporins in heart failure, liver disease and pregnancy. *J R Soc Med* 2001; **94**: 265-269 [PMID: 11387413]
83 **Wanless IR**, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G. Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension. *Hepatology* 1995; **21**: 1238-1247 [PMID: 7737629]
84 **Cerini F**, Vilaseca M, Lafoz E, García-Irigoyen O, García-Calderó H, Tripathi DM, Avila M, Reverter JC, Bosch J, Gracia-Sancho J, García-Pagán JC. Enoxaparin reduces hepatic vascular resistance and portal pressure in cirrhotic rats. *J Hepatol* 2016; **64**: 834-842 [PMID: 26686269 DOI: 10.1016/j.jhep.2015.12.003]
85 **Nguyen-Khac E**, Saint-Leger P, Tramier B, Coevoet H, Capron D, Dupas JL. Noninvasive diagnosis of large esophageal varices by Fibroscan: strong influence of the cirrhosis etiology. *Alcohol Clin Exp Res* 2010; **34**: 1146-1153 [PMID: 20477777 DOI: 10.1111/j.1530-0277.2010.01191.x]
86 **Yamasaki M**, Ikeda K, Nakatani K, Yamamoto T, Kawai Y, Hirohashi K, Kinoshita H, Kaneda K. Phenotypical and morphological alterations to rat sinusoidal endothelial cells in arterialized livers after portal branch ligation. *Arch Histol Cytol* 1999; **62**: 401-411 [PMID: 10678569 DOI: 10.1679/aohc.62.401]
87 **Moreau R**, Lee SS, Hadengue A, Braillon A, Lebrec D. Hemodynamic effects of a clonidine-induced decrease in sympathetic tone in patients with cirrhosis. *Hepatology* 1987; **7**: 149-154 [PMID: 3542775]

88 **Hruban Z**, Russell RM, Boyer JL, Glagov S, Bagheri SA. Ultrastructural changes in livers of two patients with hypervitaminosis A. *Am J Pathol* 1974; **76**: 451-461 [PMID: 4416771]
89 **Russell RM**, Boyer JL, Bagheri SA, Hruban Z. Hepatic injury from chronic hypervitaminosis a resulting in portal hypertension and ascites. *N Engl J Med* 1974; **291**: 435-440 [PMID: 4843409 DOI: 10.1056/NEJM197408292910903]
90 **Piecha F**, Peccerella T, Seitz HK, Rausch V, Mueller S. Pharmacological vasodilatation efficiently decreases liver stiffness in rats with TAA-induced liver cirrhosis. *J Hepatol* 2016; **64**: S722 [DOI: 10.1016/S0168-8278(16)01396-9]
91 **Bedossa P**. Personal communication. 2016

P- Reviewer: Ikura Y, Niu ZS, Wang K **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Zhang FF

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.v22.i48.10502

World J Gastroenterol 2016 December 28; 22(48): 10502-10511
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Updated therapeutic outcome for patients with periampullary and pancreatic cancer related to recent translational research

Trond A Buanes

Trond A Buanes, Department of Hepato-Pancreatico-Biliary Surgery, Oslo University Hospital, N-0424 Oslo, Norway

Trond A Buanes, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, N-0424 Oslo, Norway

Author contributions: Buanes TA solely contributed to this manuscript.

Conflict-of-interest statement: The author has no conflicts of interest.

Manuscript source: Invited manuscript

Correspondence to: Trond A Buanes, MD, PhD, Professor of Surgery, Department of Hepato-Pancreatico-Biliary Surgery, Oslo University Hospital, Nydalen, N-0424 Oslo, Norway. trond.buanes@medisin.uio.no
Telephone: +47-23070958
Fax: +47-23072526

Received: August 15, 2016
Peer-review started: August 16, 2016
First decision: October 11, 2016
Revised: October 14, 2016
Accepted: November 23, 2016
Article in press: November 28, 2016
Published online: December 28, 2016

Abstract

Chemotherapy with improved effect in patients with metastatic pancreatic cancer has recently been established, launching a new era for patients with this very aggressive disease. FOLFIRINOX and gemcitabine plus nab-paclitaxel are different regimens, both capable of stabilizing the disease, thus increasing the number of patients who can reach second line and even third line of treatment. Concurrently, new windows of opportunity open for nutritional support and other therapeutic interventions, improving quality of life. Also pancreatic surgery has changed significantly during the latest years. Extended operations, including vascular/multivisceral resections are frequently performed in specialized centers, pushing borders of resectability. Potentially curative treatment including neoadjuvant and adjuvant chemotherapy is offered new patient groups. Translational research is the basis for the essential understanding of the ongoing development. Even thou biomarkers for clinical management of patients with periampullary tumors have almost been lacking, biomarker driven trials are now in progress. New insight is constantly made available for clinicians; one recent example is selection of patients for gemcitabine treatment based on the expression level of the human equilibrium nucleoside transporter 1. An example of new diagnostic tools is identification of early pancreatic cancer patients by a three-biomarker panel in urine: The proteins lymphatic vessel endothelial hyaluronan receptor 1, regenerating gene 1 alpha and translation elongation factor 1 alpha. Requirement of treatment guideline revisions is intensifying, as combined chemotherapy regimens result in unexpected advantages. The European Study Group for Pancreatic Cancer 4 trial outcome is an illustration: Addition of capecitabine in the adjuvant setting improved overall survival more than expected from the effect in advanced disease. Rapid implementation of new treatment options is mandatory when progress finally extends to patients with this serious disease.

Key words: Chemotherapy; Clinical outcome; Evidence-

based medicine; Molecular expression profiling; Pancreatic cancer; Periampullary tumor; Prognostic markers; Survival

Core tip: More effective chemotherapy and reorganized care for patients with periampullary carcinoma has opened windows of opportunity for improved surgical performance. Combined with neoadjuvant and adjuvant chemotherapy new patient groups can therefore be offered potentially curative treatment. A biomarkers can predict gemcitabine sensitivity, thus improving patient selection. A three-biomarker panel in urine can identify patients with early pancreatic adenocarcinoma. Clinical implementation of new diagnostic and therapeutic options is mandatory.

Buanes TA. Updated therapeutic outcome for patients with periampullary and pancreatic cancer related to recent translational research. *World J Gastroenterol* 2016; 22(48): 10502-10511 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10502.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10502

INTRODUCTION

The different prognosis of pancreatic ductal adenocarcinoma (PDAC) and other periampullary carcinomas generates from profound biological diversities, increasingly explained by recent translational research[1]. Most details from whole genome analysis of the mutational landscape[2] generate knowledge that cannot be directly translated into clinical applications. Nevertheless, accumulative genetic and molecular biological information constantly increase the basis for clinical studies, and together with well-designed randomized controlled trials (RCT), the scientific development is accelerated. Outcome from recent trials are summarized in this review, related to relevant translational research which is rapidly generating new windows of diagnostic and therapeutic opportunity.

Molecular expression profiling in histological subtypes

The different prognosis for periampullary carcinomas, arising from pancreatic duct cells, distal bile duct cells or the mucosa of the ampulla or duodenum is documented by numerous authors[3-6]. Intestinal or pancreaticobiliary differentiation have been shown to be prognostically more important than anatomic site of origin[7], and an integrative platform, enabling profiling of micro(mi)RNA, mRNA and proteins have recently been published[8]. Utilizing this platform, the molecular profiles of 85 periampullary adenocarcinomas, resected by pancreaticoduodenectomy (PD, or Whipple-procedure), was characterized by mRNA and miRNA expressions, comparing tumors from different anatomical sites of origin as well as different histological subtypes[9]. Six miRNA families were downregulated and four were upregulated in the pancreaticobiliary type as compared to the intestinal type. miRNA and mRNAs associated with improved survival for both histopathological subtypes were identified. The genes 3-phosphoinositide dependent protein kinase 1 (PDPK1), phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2), glucose 6-phosphatase alpha (G6PC) and miRNAs miR-127-3p and miR-377 were linked to enriched pathways and identified as prognostic markers for future clinical investigation.

In lung cancer patients a serum specific miRNA signature have been identified and validated in a study on 1115 high risk individuals for lung cancer, enrolled in a screening protocol. Overall accuracy, sensitivity and specificity were found above 70%, area under the curve (AUC) in the (receiver operating characteristics) ROC-analysis was 0.85[10]. In another report on circulating miRNA as biomarker of lung cancer, a panel of 24 miRNAs with optimal classification performance was identified[11], enabling earlier detection of patients with lung cancer. Also in breast cancer circulating cell-free miRNAs have been found to be a biomarker for diagnostic and possibly targeted therapeutic utilization[12]. A similar development is foreseeable for patients with periampullary tumors. A feasibility study on 46 patients with early stage PDAC, 29 patients with chronic pancreatitis and 26 healthy controls, investigated the discriminant ability of the combination of miR-143 and miR-30e in urine samples. Sensitivity above 80%, specificity above 90% and AUC 0.923 in the ROC analysis was recently published[13]. Also proteomics of 18 urine samples were analyzed in 192 patients with PDAC and 87 healthy controls. A three-biomarker panel was identified, able to differentiate patients with early stage pancreatic cancer from healthy controls by urine specimens[14]. The discriminant ability of the proteins lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), regenerating gene 1 alpha (REG1A) and (translation elongation factor 1 alpha) TEF1 increased further when combined with serum Ca19-9 value, resulting in AUC 0.97 in the ROC analysis. Accordingly, improved diagnosis of patients with localized *i.e.*, resectable periampullary tumors is achievable, based on recent translational research. Epigenetic downregulation of miRNA 192 expression has recently been found to promote pancreatic cancer progression[15], explaining the early metastatic behavior of PDAC. Thrombospondin-1 (TSP-1) was found to decrease preclinical, even 24 mo prior to the diagnosis of PDAC, and the combination of TSP-1 and serum Ca19-9 achieved significant diagnostic yield: AUC 0.86 in ROC analysis[16]. A circulating miRNA profile has also been found to predict disease progression in patients with metastatic pancreatic cancer, receiving second line treatment[17], illustrating that monitoring of treatment

outcome is becoming possible, based on miRNA as biomarker.

RESECTION MARGIN ASSESSMENT

A standardized protocol for PD specimen examination, defining R1 status as tumor cells within 1 mm distance from the resection margin (RM), introduced by Verbeke[18], have profoundly influenced reporting on R0/R1 status. This is illustrated by numerous reports, for example from the European Study Group for Pancreatic Cancer (ESPAC). In the ESPAC 1 study (inclusion 1994-2000), R1 status was reported in 18%[19], ESPAC 3 (inclusion 2000-2008) reported R1 in 16%[20], but in ESPAC 4 (inclusion 2009-2014) the frequency of R1 resections was 60%. Traditionally, R0 status was considered the only satisfactory outcome of PD[21], as no clinical benefit was supposed to result from R1 resection. High rates of R0 status was conceived as an important indicator of good surgical performance. However, the ESPAC data and numerous other reports leave little doubt that historical differences in R1 rates result from divergence in pathology examination, more than quality of surgery[22]. This opinion is supported by recent reports from the US[23] and France[24], underlining the importance of focused attention on standardized pathology reports in order to increase comparability of published data. In the center of Heidelberg, R0 status was not a prognostic indicator until the new standard was introduced[25].

Tumor growth is more dispersed in pancreatic head cancers than in rectal cancer, ampullary cancer and distal bile duct cancer[26]. The implication is that even R0 status according to the standardized pathology protocol, does not completely exclude the possibility of residual tumor cells in the operation field. The necessity of adjuvant chemotherapy is very well documented, and is compatible with this hypothesis.

MORPHOLOGICAL HETEROGENEITY

High levels of morphological heterogeneity is common in PDAC[27] and desmoplastic stroma is also predominant. Both characteristics are supposed to be obstacles to effective chemotherapeutic treatment. This hypothesis is supported by a report, classifying PDAC as classical, quasi mesenchymal and exocrine-like with different therapeutic outcome[28]. Genetic heterogeneity is contributing to therapeutic failure[29], and whole-genome sequencing of 100 PDACs have recently redefined the mutational landscape[2]. Further genome-wide investigation of copy number aberrations revealed significant prognostic implications. Deletion in the genes RAB12 and COLEC12 are associated with increased and amplification with decreased postoperative survival after PD[30]. Also the stromal compartment provides stimulatory signals to the cancer cells, and the interaction has therapeutic relevance[31]. Investigation of miRNA expression profiling of carcinomatous and stromal components in twenty periampullary adenocarcinomas, identified miRNA mediated interactions between carcinoma and stroma cells[32] which may be utilized as future therapeutic targets. These data has potential for clinical utilization in the near future.

PERSONALIZED MEDICINE - NEW CLINICALLY APPLICABLE BIOMARKERS

Based on microarrays from patients randomized to chemotherapy in the ESPAC 3 trial plus controls from the ESPAC 1 trial, expression of the human equilibrium nucleoside transporter 1 (hENT1) levels were determined[33]. Survival was compared between patients with high and low hENT1 expression in the gemcitabine and 5-fluorouraci (5-FU) arms. There was no difference in the 5-FU arm, whereas gemcitabine treated patients with high hENT1 expression lived median 26.2 mo *vs* 17.1 mo if hENT1 expression was low (P = 0.002). Patients with Gemcitabine sensitive tumors can thus be selected, resulting in higher response rates in future, biomarker driven trials. There has recently been an explosion of available biomarkers for PDAC which need clinical validation[34,35]. Exosomes are extracellular vesicles, containing proteins and nucleic acids, secreted by all cells, circulating in the blood. Identification of cancer cell derived exosomes has until recently not been possible, but a cell surface proteoglycan, glypican-1 (GPC1), specially enriched on cancer cell derived exosomes, was recently described, and GPC1 positive circulating exosomes ($GPC1^+$crExos) were isolated from serum in PDAC-patients[36]. Levels of $GPC1^+$crExos were also found to correlate with tumor burden in the same patient series. Exosomes have even been shown to initiate pre-metastatic niche formation in the liver[37], supporting the hypothesis proposed by Heiler *et al*[38] that cancer stem cells gain the capacity for cell to cell crosstalk from generation, loading and delivery of exosomes. Utilization of exosomes as diagnostic biomarker as well as staging instrument is probably shortly upcoming opportunities.

CHEMOTHERAPY

The development of effective oncological regimens has been slow, due to the chemoresistant character of PDAC. Table 1 shows key information from important clinical trials in first line therapy of metastatic disease, illustrating that gemcitabine became standard of care for fourteen years after Burris publication[39]. Even though S1 (tegafur, prodrug of 5-FU) could increase response rate, overall survival (OS) was no longer[40]. The addition of erlotinib to gemcitabine[41] resulted in a significant but small increase in OS. FOLFIRINOX represented a breakthrough in 2011, increasing response rate × 3, and median survival almost × 2, from 6.8 mo in the gemcitabine group to 11.1 mo[42]. In

Table 1 Important clinical trials in metastatic pancreatic ductal adenocarcinoma

Ref.	Year published	Investigated drugs	Clinical outcome	
			OS	ORR
Burris *et al*[39]	1997	Gemcitabine *vs* 5-FU	5.65 mo *vs* 4.4 mo	5.4% *vs* 0.0%
Ueno *et al*[40]	2005	S-1 (quasi mesenchymal and oteracil)	5.6 mo	21.1%
Moore *et al*[41]	2007	Gemcitabine *vs* erlotinib	5.91 mo *vs* 6.24 mo	8.0% *vs* 8.6%
Conroy *et al*[42]	2011	Gemcitabine *vs* Oxaliplatin + irinotecan + leucoverin + 5-FU (FOLFIRINOX)	6.80 mo *vs* 11.10 mo	9.4% *vs* 31.6%
Von Hoff *et al*[43]	2013	Gemcitabine *vs* gemcitabine + nab-paclitaxel	6.70 mo *vs* 8.50 mo	7.0% *vs* 23.0 %

OS: Overall survival; ORR: Overall response rate; 5-FU: 5-Fluorouraci.

Table 2 Important clinical trials evaluating adjuvant chemotherapy in pancreatic ductal adenocarcinoma

Ref.	Year published	Investigated drugs	Number of patients	Clinical outcome	
				Median survival (mo)	5-year survival (%)
Neoptolemos *et al*[19,52] (ESPAC 1)	2001 and 2004	5-FU/FA *vs* No chemotherapy	149/143	20.1/15.5	21.0/8.0
Oettle *et al*[53]	2007	Gemcitabine *vs* No chemotherapy	179/175	22.1/20.2	22.5/11.5
Neoptolemos *et al*[20] (ESPAC 3)	2010	Gemcitabine *vs* 5-FU/FA	539/551	23.6/23.0	17.5/15.9
Neoptolemos *et al*[20] (ESPAC 4)	2016	Gemcitabine *vs* Gemcitabine + Capecitabine	366/364	25.5/28.0	16.3/28.8
Uesaka *et al*[55]	2016	Gemcitabine *vs* S1	193/192	24.5/46.5	24.4/44.1

5-FU: 5-Fluorouraci; FA: Folinic acid; ESPAC: European Study Group for Pancreatic Cancer.

2013 gemcitabine plus nab-paclitaxel was also shown to stabilize metastatic disease[43]. This has opened new windows of opportunity for maintenance treatment in case of intolerable toxicity[44], second line chemotherapy in cases of progression during first line treatment[45-47] and even third line chemotherapy, even though no evidence for this is available yet. Regimen of second line chemotherapy should be chosen related to first line[48]. Nanoliposomal irinotecan with 5-FU/folinic acid (FA) increased OS to median 6.2 mo in a recently published RCT on gemcitabine refractory metastatic PDAC[49]. The American Society of Clinical Oncology (ASCO) has recently published guidelines, incorporating this new insight, for treatment of patients with locally advanced[50] and metastatic[51] PDAC, recommending practical answers to key clinical questions for each patient group.

Adjuvant chemotherapy has been evaluated in numerous RCTs, published during the last 15 years, as illustrated in Table 2. The necessity of adjuvant treatment was first documented by Neoptolemos in the ESPAC 1 trial[19,52], later verified by Oettle[53]. Medan and 5 year survival have increased during the following decennium. In a western patient population, gemcitabine plus capecitabine is now standard of care, resulting in median 28 mo and close to 30% five year survival after upfront surgery[54]. A recent report from Japan[55], suggests that even better outcome is achievable with adjuvant S1.

SURGERY

The concept that upfront surgery is the best treatment option for patients with resectable tumors, is widely accepted[1,56-58]. Nationwide centralization of PD in the Netherlands resulted in decreased postoperative in-hospital mortality from 9.8% in 2004 to 5.1% in 2009[59], and the importance of volume-outcome relationship is increasingly emphasized[60]. The reorganization of care for patients with periampullary cancer, based on multidisciplinary management, is widely supported[48,61], and it has resulted in significant change in surgical practice during recent years. Extended operations including vascular/multivisceral resections are now frequently performed, and borders of resectability are continuously being pushed[62]. The outcome value of multivisceral resections has been assessed[63], criteria for clinical evaluations are defined[64] and the basis for perfected surgical practice is continuously improving. Better handling of surgical complications is an important element of this development and a reason for reduced failure to rescue in case of serious complications[65-67]. The improvement of outcome related to patient volume continues after 40 procedures/year at one hospital[68]. Nevertheless, postoperative complications is a major problem after pancreatic surgery, precluding adjuvant chemotherapy and thus decreasing OS[69,70]. The focus on improved surgical performance is therefore increasing, generating comprehensive evaluation of new technical details[71].

Patients with borderline resectable PDAC was primarily described by Katz *et al*[72] as those with localized disease with tumor or patients characteristics precluding immediate surgery. After neoadjuvant chemotherapy, chemoradiation or both in 125 of these patients, 66 (41%) underwent pancreatic resections, 18 (27%) with vascular resection/reconstruction. Median postoperative survival was 40 mo. Subsequently, the borderline concept is defined in detail[73]. Management and treatment outcome in patients with borderline

resectable tumors is an area of intense scrutiny.

Neoadjuvant treatment, also in patients with resectable tumors, is widely accepted in the United States[74,75], whereas upfront surgery is still considered standard of care in influential European centers[76]. Clinical and preclinical data support the concept that PDAC metastases appear early in the pathogenesis, even before the tumor can be identified[77], favoring neoadjuvant chemotherapy. A meta-analysis focusing outcome in patients with resectable and unresectable tumors[78], found that resection frequencies and survival after neoadjuvant therapy in resectable patients was similar to patients undergoing upfront surgery and adjuvant chemotherapy. In patients with unresectable tumors, approximately one third became resectable after restaging. In another meta-analysis of neoadjuvant chemotherapy in patients with borderline resectable PDAC, primary outcome measures were proportion of complete or partial response, stable or progressive disease as well as percentages of exploration and resection, and these results were also similar[79]. This evidence seems to support recommendation of neoadjuvant chemotherapy in borderline resectable PDAC, as resection rates and survival can be raised to the same level as patients with resectable tumors. A report from the National Cancer Data base on patients with PDAC stage Ⅰ and Ⅱ, who underwent PD between 2006 and 2012[80], found increased rates of neoadjuvant treatment from 12.0% in 2006 to 20.2% in 2012. Patients who complete all intended neoadjuvant therapy, including surgery and adjuvant chemotherapy are by some authors supposed to experience increased OS, compared to patients undergoing upfront surgery[81]. However, evidence from well conducted RCTs is lacking, and the putative benefit of neoadjuvant treatment can be a by-product of selection bias, as patients with rapid disease progression never undergo surgery. Therefore, the real benefit or harm of neoadjuvant chemotherapy in patients with resectable PDAC still requires systematic evaluation. The International Study Group for Pancreatic Cancer recommendations is still upfront surgery for resectable and borderline resectable tumors[64,82,83].

DISTAL CHOLANGIOCARCINOMA, AMPULLARY AND DUODENAL ADENOCARCINOMA

Patients with periampullary adenocarcinomas undergo the same surgical resectional procedure as patients with PDAC, and postoperative survival is longer[84-88]. However, some data are contradictory: similar survival for patients with distal cholangiocarcinoma and PDAC was recently reported[89], as well as comparable prognosis for ampullary and extra-ampullary duodenal carcinomas[90]. Even ampullary carcinoma with the same median postoperative survival as PDAC has been described from Denmark[91]. Routine histopathology from 207 PD specimens were recently re-evaluated by two independent experienced pancreatic pathologists, and 53% of distal cholangiocarcinoma were misdiagnosed as PDAC[92]. A comprehensive assessment of tumor origin in pancreatic head cancer documented inaccurate and inconsistent distinctions between pancreatic, ampullary and distal bile duct cancer[6]. This divergence in pathology assessment may explain some of the contradictory data on prognosis in periampullary carcinomas. Ampullary carcinoma can be of pancreaticobiliary- or intestinal type, and the molecular signatures of mRNA and miRNA, linked to specific intracellular pathways, correlate to subtype above anatomical origin in periampullary tumors[9]. Histological subtype is a predictor of survival, and have also recently been found to influence response to adjuvant gemcitabine[93].

IS ADJUVANT CHEMOTHERAPY INDICATED FOR PERIAMPULLARY CARCINOMA?

A single center study from Johns Hopkins Hospital found that adjuvant chemoradiation did not improve survival[94]. The only well conducted RCT, investigating this question, is the ESPAC 3 trial, in which 297 patients with ampullary carcinoma, 96 with distal cholangiocarcinoma and 35 other carcinoma were randomized between 5-FU/FA, Gemcitabine or observation only. Median survival was 35.2 mo in the observation group *vs* 43.1 mo in the two chemotherapy groups, but the difference was not significant in the primary analysis. After multivariate analysis, adjusting for prognostic variables, statistically significant survival benefit was found after adjuvant chemotherapy[95]. The authors underline that distal cholangiocarcinoma should be analyzed separately, *i.e.*, not together with tumors with other sites of origin, as periampullary adenocarcinoma is not a separate tumor entity. As documented above[9], site of tumor origin influence expected survival and pancreaticobiliary and intestinal subtypes respond differently to adjuvant gemcitabine[93]. Accordingly, the probable survival benefit from adjuvant chemotherapy, shown in the ESPAC 3 trial, should be further investigated by evaluation of combination chemotherapies in a modified study design.

CONCLUSION

Lack of biomarkers, applicable as diagnostic tools and/or therapeutic targets has been a major hindrance for development of personalized treatment in patients with periampullary tumors. This is now rapidly changing[1,96]. A practical example is selection of patients for gemcitabine treatment guided by hENT1 expression[33]. Clinical implementation of new micromolecular markers is presently a major issue. Subsequently, treatment guidelines need revision. Surgical treatment of metastatic PDAC has been evaluated[97], resulting in median

postoperative survival 13.8 mo, estimated one year survival 58.9%. In an earlier study of 40 patients with metastases from periampullary carcinoma undergoing curative intent surgery, median survival for the pancreaticobiliary subtype was 13 mo, intestinal 23 mo[98]. Metastatic disease is therefore conceived as a palliative condition, without clinical benefit from resectional surgery[58]. However, in a recent report from Milan[99], 127 patients with metastatic PDAC were treated by the new chemotherapeutic regimens, and 11 patients with radiological and biochemical response (Ca19-9 normalization) underwent resection of the pancreatic primary tumor plus liver or lung metastases. Postoperative median survival was 39 mo *vs* 12 mo for the 116 patients without surgical resection. One year and three year survival were 100% *vs* 42% and 57% *vs* 5% respectively. The difference is obviously caused by better chemoresponse in the resected group, possibly also by additional benefit from resectional surgery. When new biomarkers enable personalized chemotherapeutic treatment and selection of patients for surgery based on improved knowledge about the malignancy potential of the tumor, cure seems achievable also for some patients with metastatic PDAC in the near future. Clarification of the role of surgery, related to chemotherapy is associated with severe methodological difficulties, as numerous patients deny inclusion in randomized trials, evaluating neoadjuvant chemotherapy. Low response rates of neoadjuvance and corresponding high risk of ending up with unresectable tumors are frequent patient concerns. These issues goes beyond the scope of this paper, but solid evidence, clarifying benefit/harm of neoadjuvant chemotherapy in pancreatic cancer will be major future scientific achievements.

A major problem for numerous patients with periampullary carcinoma is that they suffer severely during most of their residual lifetime, and palliative interventions, improving their quality of life, are important. Appropriate instruments for measuring patient reported outcome (PRO) has been lacking, but a brief, disease specific instrument has recently been developed, the PAncreatic CAncer DIsease (PACADI) score[100]. This is a brief, eight item, patient derived instrument, feasible also for patients with severe fatigue during late disease course. The future holds opportunities of well-designed interventional outcome-studies with survival and PRO as endpoints, increasing the rate of therapeutic intervention improvement.

REFERENCES

1 **Kleeff J**, Korc M, Apte M, La Vecchia C, Johnson CD, Biankin AV, Neale RE, Tempero M, Tuveson DA, Hruban RH, Neoptolemos JP. Pancreatic cancer. *Nat Rev Dis Primers* 2016; **2**: 16022 [PMID: 27158978 DOI: 10.1038/nrdp.2016.22]

2 **Waddell N**, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek K, Quinn MC, Robertson AJ, Fadlullah MZ, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, Wani S, Wilson PJ, Markham E, Cloonan N, Anderson MJ, Fink JL, Holmes O, Kazakoff SH, Leonard C, Newell F, Poudel B, Song S, Taylor D, Waddell N, Wood S, Xu Q, Wu J, Pinese M, Cowley MJ, Lee HC, Jones MD, Nagrial AM, Humphris J, Chantrill LA, Chin V, Steinmann AM, Mawson A, Humphrey ES, Colvin EK, Chou A, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Pettitt JA, Merrett ND, Toon C, Epari K, Nguyen NQ, Barbour A, Zeps N, Jamieson NB, Graham JS, Niclou SP, Bjerkvig R, Grützmann R, Aust D, Hruban RH, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Falconi M, Zamboni G, Tortora G, Tempero MA, Gill AJ, Eshleman JR, Pilarsky C, Scarpa A, Musgrove EA, Pearson JV, Biankin AV, Grimmond SM. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* 2015; **518**: 495-501 [PMID: 25719666 DOI: 10.1038/nature14169]

3 **Nordby T**, Ikdahl T, Lothe IM, Ånonsen K, Hauge T, Edwin B, Line PD, Labori KJ, Buanes T. Opportunities of improvement in the management of pancreatic and periampullary tumors. *Scand J Gastroenterol* 2013; **48**: 617-625 [PMID: 23597153 DOI: 10.3109/00365521.2013.781218]

4 **Winter JM**, Cameron JL, Lillemoe KD, Campbell KA, Chang D, Riall TS, Coleman J, Sauter PK, Canto M, Hruban RH, Schulick RD, Choti MA, Yeo CJ. Periampullary and pancreatic incidentaloma: a single institution's experience with an increasingly common diagnosis. *Ann Surg* 2006; **243**: 673-680; discussion 680-683 [PMID: 16633003 DOI: 10.1097/01.sla.0000216763. 27673.97]

5 **van Rijssen LB**, van der Geest LG, Bollen TL, Bruno MJ, van der Gaast A, Veerbeek L, Ten Kate FJ, Busch OR. National compliance to an evidence-based multidisciplinary guideline on pancreatic and periampullary carcinoma. *Pancreatology* 2015; **16**: 133-137 [PMID: 26560441 DOI: 10.1016/j.pan.2015.10.002]

6 **Verbeke CS**, Gladhaug IP. Resection margin involvement and tumour origin in pancreatic head cancer. *Br J Surg* 2012; **99**: 1036-1049 [PMID: 22517199 DOI: 10.1002/bjs.8734]

7 **Westgaard A**, Tafjord S, Farstad IN, Cvancarova M, Eide TJ, Mathisen O, Clausen OP, Gladhaug IP. Resectable adenocarcinomas in the pancreatic head: the retroperitoneal resection margin is an independent prognostic factor. *BMC Cancer* 2008; **8**: 5 [PMID: 18194510 DOI: 10.1186/1471-2407-8-5]

8 **Thomas JK**, Kim MS, Balakrishnan L, Nanjappa V, Raju R, Marimuthu A, Radhakrishnan A, Muthusamy B, Khan AA, Sakamuri S, Tankala SG, Singal M, Nair B, Sirdeshmukh R, Chatterjee A, Prasad TS, Maitra A, Gowda H, Hruban RH, Pandey A. Pancreatic Cancer Database: an integrative resource for pancreatic cancer. *Cancer Biol Ther* 2014; **15**: 963-967 [PMID: 24839966 DOI: 10.4161/cbt.29188]

9 **Sandhu V**, Bowitz Lothe IM, Labori KJ, Lingjærde OC, Buanes T, Dalsgaard AM, Skrede ML, Hamfjord J, Haaland T, Eide TJ, Børresen-Dale AL, Ikdahl T, Kure EH. Molecular signatures of mRNAs and miRNAs as prognostic biomarkers in pancreatobiliary and intestinal types of periampullary adenocarcinomas. *Mol Oncol* 2015; **9**: 758-771 [PMID: 25579086 DOI: 10.1016/j.molonc.2014.12.002]

10 **Montani F**, Marzi MJ, Dezi F, Dama E, Carletti RM, Bonizzi G, Bertolotti R, Bellomi M, Rampinelli C, Maisonneuve P, Spaggiari L, Veronesi G, Nicassio F, Di Fiore PP, Bianchi F. miR-Test: a blood test for lung cancer early detection. *J Natl Cancer Inst* 2015; **107**: djv063 [PMID: 25794889 DOI: 10.1093/jnci/djv063]

11 **Wozniak MB**, Scelo G, Muller DC, Mukeria A, Zaridze D, Brennan P. Circulating MicroRNAs as Non-Invasive Biomarkers for Early Detection of Non-Small-Cell Lung Cancer. *PLoS One* 2015; **10**: e0125026 [PMID: 25965386 DOI: 10.1371/journal.pone.0125026]

12 **Shin VY**, Siu JM, Cheuk I, Ng EK, Kwong A. Circulating cell-free miRNAs as biomarker for triple-negative breast cancer. *Br J Cancer* 2015; **112**: 1751-1759 [PMID: 25906045 DOI: 10.1038/bjc.2015.143]

13 **Debernardi S**, Massat NJ, Radon TP, Sangaralingam A, Banissi A, Ennis DP, Dowe T, Chelala C, Pereira SP, Kocher HM, Young BD, Bond-Smith G, Hutchins R, Crnogorac-Jurcevic T. Noninvasive urinary miRNA biomarkers for early detection of pancreatic adenocarcinoma. *Am J Cancer Res* 2015; **5**: 3455-3466 [PMID: 26807325]

14 **Radon TP**, Massat NJ, Jones R, Alrawashdeh W, Dumartin L,

Ennis D, Duffy SW, Kocher HM, Pereira SP, Guarner posthumous L, Murta-Nascimento C, Real FX, Malats N, Neoptolemos J, Costello E, Greenhalf W, Lemoine NR, Crnogorac-Jurcevic T. Identification of a Three-Biomarker Panel in Urine for Early Detection of Pancreatic Adenocarcinoma. *Clin Cancer Res* 2015; **21**: 3512-3521 [PMID: 26240291 DOI: 10.1158/1078-0432.ccr-14-2467]

15 **Botla SK**, Savant S, Jandaghi P, Bauer AS, Mücke O, Moskalev EA, Neoptolemos JP, Costello E, Greenhalf W, Scarpa A, Gaida MM, Büchler MW, Strobel O, Hackert T, Giese NA, Augustin HG, Hoheisel JD. Early Epigenetic Downregulation of microRNA-192 Expression Promotes Pancreatic Cancer Progression. *Cancer Res* 2016; **76**: 4149-4159 [PMID: 27216198 DOI: 10.1158/0008-5472.can-15-0390]

16 **Jenkinson C**, Elliott VL, Evans A, Oldfield L, Jenkins RE, O' Brien DP, Apostolidou S, Gentry-Maharaj A, Fourkala EO, Jacobs IJ, Menon U, Cox T, Campbell F, Pereira SP, Tuveson DA, Park BK, Greenhalf W, Sutton R, Timms JF, Neoptolemos JP, Costello E. Decreased Serum Thrombospondin-1 Levels in Pancreatic Cancer Patients Up to 24 Months Prior to Clinical Diagnosis: Association with Diabetes Mellitus. *Clin Cancer Res* 2016; **22**: 1734-1743 [PMID: 26573598 DOI: 10.1158/1078-0432.ccr-15-0879]

17 **Tian X**, Shivapurkar N, Wu Z, Hwang JJ, Pishvaian MJ, Weiner LM, Ley L, Zhou D, Zhi X, Wellstein A, Marshall JL, He AR. Circulating microRNA profile predicts disease progression in patients receiving second-line treatment of lapatinib and capecitabine for metastatic pancreatic cancer. *Oncol Lett* 2016; **11**: 1645-1650 [PMID: 26998056 DOI: 10.3892/ol.2016.4101]

18 **Verbeke CS**, Leitch D, Menon KV, McMahon MJ, Guillou PJ, Anthoney A. Redefining the R1 resection in pancreatic cancer. *Br J Surg* 2006; **93**: 1232-1237 [PMID: 16804874 DOI: 10.1002/bjs.5397]

19 **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]

20 **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]

21 **Evans DB**, Farnell MB, Lillemoe KD, Vollmer C, Strasberg SM, Schulick RD. Surgical treatment of resectable and borderline resectable pancreas cancer: expert consensus statement. *Ann Surg Oncol* 2009; **16**: 1736-1744 [PMID: 19387741 DOI: 10.1245/s10434-009-0416-6]

22 **Verbeke CS**. Resection margins in pancreatic cancer: are we entering a new era? *HPB* (Oxford) 2014; **16**: 1-2 [PMID: 24329942 DOI: 10.1111/hpb.12122]

23 **Gnerlich JL**, Luka SR, Deshpande AD, Dubray BJ, Weir JS, Carpenter DH, Brunt EM, Strasberg SM, Hawkins WG, Linehan DC. Microscopic margins and patterns of treatment failure in resected pancreatic adenocarcinoma. *Arch Surg* 2012; **147**: 753-760 [PMID: 22911074 DOI: 10.1001/archsurg.2012.1126]

24 **Delpero JR**, Bachellier P, Regenet N, Le Treut YP, Paye F, Carrere N, Sauvanet A, Autret A, Turrini O, Monges-Ranchin G, Boher JM. Pancreaticoduodenectomy for pancreatic ductal adenocarcinoma: a French multicentre prospective evaluation of resection margins in 150 evaluable specimens. *HPB* (Oxford) 2014; **16**: 20-33 [PMID: 23464850 DOI: 10.1111/hpb.12061]

25 **Hartwig W**, Hackert T, Hinz U, Gluth A, Bergmann F, Strobel O, Büchler MW, Werner J. Pancreatic cancer surgery in the new millennium: better prediction of outcome. *Ann Surg* 2011; **254**: 311-319 [PMID: 21606835 DOI: 10.1097/SLA.0b013e31821fd334]

26 **Verbeke CS**, Knapp J, Gladhaug IP. Tumour growth is more dispersed in pancreatic head cancers than in rectal cancer: implications for resection margin assessment. *Histopathology* 2011; **59**: 1111-1121 [PMID: 22175891 DOI: 10.1111/j.1365-2559.2011.04056.x]

27 **Verbeke C**. Morphological heterogeneity in ductal adenocarcinoma of the pancreas - Does it matter? *Pancreatology* 2016; **16**: 295-301 [PMID: 26924665 DOI: 10.1016/j.pan.2016.02.004]

28 **Collisson EA**, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, Cooc J, Weinkle J, Kim GE, Jakkula L, Feiler HS, Ko AH, Olshen AB, Danenberg KL, Tempero MA, Spellman PT, Hanahan D, Gray JW. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med* 2011; **17**: 500-503 [PMID: 21460848 DOI: 10.1038/nm.2344]

29 **Burrell RA**, Swanton C. Tumour heterogeneity and the evolution of polyclonal drug resistance. *Mol Oncol* 2014; **8**: 1095-1111 [PMID: 25087573 DOI: 10.1016/j.molonc.2014.06.005]

30 **Sandhu V**, Wedge DC, Bowitz Lothe IM, Labori KJ, Dentro SC, Buanes T, Skrede ML, Dalsgaard AM, Munthe E, Myklebost O, Lingjærde OC, Børresen-Dale AL, Ikdahl T, Van Loo P, Nord S, Kure EH. The Genomic Landscape of Pancreatic and Periampullary Adenocarcinoma. *Cancer Res* 2016; **76**: 5092-5102 [PMID: 27488532 DOI: 10.1158/0008-5472.can-16-0658]

31 **Whatcott C**, Han H, Posner RG, Von Hoff DD. Tumor-stromal interactions in pancreatic cancer. *Crit Rev Oncog* 2013; **18**: 135-151 [PMID: 23237556]

32 **Sandhu V**, Bowitz Lothe IM, Labori KJ, Skrede ML, Hamfjord J, Dalsgaard AM, Buanes T, Dube G, Kale MM, Sawant S, Kulkarni-Kale U, Børresen-Dale AL, Lingjærde OC, Kure EH. Differential expression of miRNAs in pancreatobiliary type of periampullary adenocarcinoma and its associated stroma. *Mol Oncol* 2016; **10**: 303-316 [PMID: 26590090 DOI: 10.1016/j.molonc.2015.10.011]

33 **Greenhalf W**, Ghaneh P, Neoptolemos JP, Palmer DH, Cox TF, Lamb RF, Garner E, Campbell F, Mackey JR, Costello E, Moore MJ, Valle JW, McDonald AC, Carter R, Tebbutt NC, Goldstein D, Shannon J, Dervenis C, Glimelius B, Deakin M, Charnley RM, Lacaine F, Scarfe AG, Middleton MR, Anthoney A, Halloran CM, Mayerle J, Oláh A, Jackson R, Rawcliffe CL, Scarpa A, Bassi C, Büchler MW. Pancreatic cancer hENT1 expression and survival from gemcitabine in patients from the ESPAC-3 trial. *J Natl Cancer Inst* 2014; **106**: djt347 [PMID: 24301456 DOI: 10.1093/jnci/djt347]

34 **Verma M**. Pancreatic cancer biomarkers and their implication in cancer diagnosis and epidemiology. *Cancers* (Basel) 2010; **2**: 1830-1837 [PMID: 24281203 DOI: 10.3390/cancers2041830]

35 **Harsha HC**, Kandasamy K, Ranganathan P, Rani S, Ramabadran S, Gollapudi S, Balakrishnan L, Dwivedi SB, Telikicherla D, Selvan LD, Goel R, Mathivanan S, Marimuthu A, Kashyap M, Vizza RF, Mayer RJ, Decaprio JA, Srivastava S, Hanash SM, Hruban RH, Pandey A. A compendium of potential biomarkers of pancreatic cancer. *PLoS Med* 2009; **6**: e1000046 [PMID: 19360088 DOI: 10.1371/journal.pmed.1000046]

36 **Melo SA**, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, LeBleu VS, Mittendorf EA, Weitz J, Rahbari N, Reissfelder C, Pilarsky C, Fraga MF, Piwnica-Worms D, Kalluri R. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* 2015; **523**: 177-182 [PMID: 26106858 DOI: 10.1038/nature14581]

37 **Costa-Silva B**, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, Becker A, Hoshino A, Mark MT, Molina H, Xiang J, Zhang T, Theilen TM, García-Santos G, Williams C, Ararso Y, Huang Y, Rodrigues G, Shen TL, Labori KJ, Lothe IM, Kure EH, Hernandez J, Doussot A, Ebbesen SH, Grandgenett PM, Hollingsworth MA, Jain M, Mallya K, Batra SK, Jarnagin WR, Schwartz RE, Matei I, Peinado H, Stanger BZ, Bromberg J, Lyden D. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol* 2015; **17**: 816-826 [PMID: 25985394 DOI: 10.1038/ncb3169]

38 **Heiler S**, Wang Z, Zöller M. Pancreatic cancer stem cell markers and exosomes - the incentive push. *World J Gastroenterol* 2016; **22**: 5971-6007 [PMID: 27468191 DOI: 10.3748/wjg.v22.i26.5971]

39 **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]

40 **Ueno H**, Okusaka T, Ikeda M, Takezako Y, Morizane C. An early phase II study of S-1 in patients with metastatic pancreatic cancer. *Oncology* 2005; **68**: 171-178 [PMID: 16006754 DOI: 10.1159/000086771]

41 **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]

42 **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]

43 **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]

44 **Reni M**, Cereda S, Milella M, Novarino A, Passardi A, Mambrini A, Di Lucca G, Aprile G, Belli C, Danova M, Bergamo F, Franceschi E, Fugazza C, Ceraulo D, Villa E. Maintenance sunitinib or observation in metastatic pancreatic adenocarcinoma: a phase II randomised trial. *Eur J Cancer* 2013; **49**: 3609-3615 [PMID: 23899530 DOI: 10.1016/j.ejca.2013.06.041]

45 **Caparello C**, Vivaldi C, Fornaro L, Musettini G, Pasquini G, Catanese S, Masi G, Lencioni M, Falcone A, Vasile E. Second-line therapy for advanced pancreatic cancer: evaluation of prognostic factors and review of current literature. *Future Oncol* 2016; **12**: 901-908 [PMID: 26883177 DOI: 10.2217/fon.16.16]

46 **Oettle H**, Riess H, Stieler JM, Heil G, Schwaner I, Seraphin J, Görner M, Mölle M, Greten TF, Lakner V, Bischoff S, Sinn M, Dörken B, Pelzer U. Second-line oxaliplatin, folinic acid, and fluorouracil versus folinic acid and fluorouracil alone for gemcitabine-refractory pancreatic cancer: outcomes from the CONKO-003 trial. *J Clin Oncol* 2014; **32**: 2423-2429 [PMID: 24982456 DOI: 10.1200/jco.2013.53.6995]

47 **Pelzer U**, Schwaner I, Stieler J, Adler M, Seraphin J, Dörken B, Riess H, Oettle H. Best supportive care (BSC) versus oxaliplatin, folinic acid and 5-fluorouracil (OFF) plus BSC in patients for second-line advanced pancreatic cancer: a phase III-study from the German CONKO-study group. *Eur J Cancer* 2011; **47**: 1676-1681 [PMID: 21565490 DOI: 10.1016/j.ejca.2011.04.011]

48 **Takaori K**, Bassi C, Biankin A, Brunner TB, Cataldo I, Campbell F, Cunningham D, Falconi M, Frampton AE, Furuse J, Giovannini M, Jackson R, Nakamura A, Nealon W, Neoptolemos JP, Real FX, Scarpa A, Sclafani F, Windsor JA, Yamaguchi K, Wolfgang C, Johnson CD. International Association of Pancreatology (IAP)/European Pancreatic Club (EPC) consensus review of guidelines for the treatment of pancreatic cancer. *Pancreatology* 2016; **16**: 14-27 [PMID: 26699808 DOI: 10.1016/j.pan.2015.10.013]

49 **Wang-Gillam A**, Li CP, Bodoky G, Dean A, Shan YS, Jameson G, Macarulla T, Lee KH, Cunningham D, Blanc JF, Hubner RA, Chiu CF, Schwartsmann G, Siveke JT, Braiteh F, Moyo V, Belanger B, Dhindsa N, Bayever E, Von Hoff DD, Chen LT. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. *Lancet* 2016; **387**: 545-557 [PMID: 26615328 DOI: 10.1016/s0140-6736(15)00986-1]

50 **Balaban EP**, Mangu PB, Khorana AA, Shah MA, Mukherjee S, Crane CH, Javle MM, Eads JR, Allen P, Ko AH, Engebretson A, Herman JM, Strickler JH, Benson AB, Urba S, Yee NS. Locally Advanced, Unresectable Pancreatic Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2016; **34**: 2654-2668 [PMID: 27247216 DOI: 10.1200/jco.2016.67.5561]

51 **Sohal DP**, Mangu PB, Khorana AA, Shah MA, Philip PA, O'Reilly EM, Uronis HE, Ramanathan RK, Crane CH, Engebretson A, Ruggiero JT, Copur MS, Lau M, Urba S, Laheru D. Metastatic Pancreatic Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2016; **34**: 2784-2796 [PMID: 27247222 DOI: 10.1200/jco.2016.67.1412]

52 **Neoptolemos JP**, Dunn JA, Stocken DD, Almond J, Link K, Beger H, Bassi C, Falconi M, Pederzoli P, Dervenis C, Fernandez-Cruz L, Lacaine F, Pap A, Spooner D, Kerr DJ, Friess H, Büchler MW. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. *Lancet* 2001; **358**: 1576-1585 [PMID: 11716884]

53 **Oettle H**, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutberlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B, Riess H. Adjuvant chemotherapy with gemcitabine *vs* observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA* 2007; **297**: 267-277 [PMID: 17227978 DOI: 10.1001/jama.297.3.267]

54 **Neoptolemos J**, Palmer D, Ghaneh P, Valle JW, Cunningham D, Wadsley J, Meyer T, Anthoney A, Glimelius B, Lind P, Falk S, Izbicki J, Middleton G, Ross P, Wasan H, McDonald A, Crosby T, Psarelli E, Hammel P, Büchler M. ESPAC-4: A multicenter, international, open label randomized controlled phase III trail og adjuvant combination chemotherapy of gemacitabine (GEM) and capecitabine (CAP), versus monotherapy gemcitamine in patients with resected pancreatic ducal ademocarcinoma. Proceedings of the ASCO Annual Meeting; 2016 Jun 6-16; Chicago, USA. Abstract No: LBA4006. Available from: URL: http://meetinglibraryascoorg/content/122648?media=vm

55 **Uesaka K**, Boku N, Fukutomi A, Okamura Y, Konishi M, Matsumoto I, Kaneoka Y, Shimizu Y, Nakamori S, Sakamoto H, Morinaga S, Kainuma O, Imai K, Sata N, Hishinuma S, Ojima H, Yamaguchi R, Hirano S, Sudo T, Ohashi Y. Adjuvant chemotherapy of S-1 versus gemcitabine for resected pancreatic cancer: a phase 3, open-label, randomised, non-inferiority trial (JASPAC 01). *Lancet* 2016; **388**: 248-257 [PMID: 27265347 DOI: 10.1016/s0140-6736(16)30583-9]

56 **Riall TS**, Lillemoe KD. Underutilization of surgical resection in patients with localized pancreatic cancer. *Ann Surg* 2007; **246**: 181-182 [PMID: 17667494 DOI: 10.1097/SLA.0b013e31811eaa2c]

57 **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]

58 **Tempero MA**, Arnoletti JP, Behrman SW, Ben-Josef E, Benson AB, Casper ES, Cohen SJ, Czito B, Ellenhorn JD, Hawkins WG, Herman J, Hoffman JP, Ko A, Komanduri S, Koong A, Ma WW, Malafa MP, Merchant NB, Mulvihill SJ, Muscarella P, Nakakura EK, Obando J, Pitman MB, Sasson AR, Tally A, Thayer SP, Whiting S, Wolff RA, Wolpin BM, Freedman-Cass DA, Shead DA. Pancreatic Adenocarcinoma, version 2.2012: featured updates to the NCCN Guidelines. *J Natl Compr Canc Netw* 2012; **10**: 703-713 [PMID: 22679115]

59 **de Wilde RF**, Besselink MG, van der Tweel I, de Hingh IH, van Eijck CH, Dejong CH, Porte RJ, Gouma DJ, Busch OR, Molenaar IQ. Impact of nationwide centralization of pancreaticoduodenectomy on hospital mortality. *Br J Surg* 2012; **99**: 404-410 [PMID: 22237731 DOI: 10.1002/bjs.8664]

60 **Finks JF**, Osborne NH, Birkmeyer JD. Trends in hospital volume and operative mortality for high-risk surgery. *N Engl J Med* 2011; **364**: 2128-2137 [PMID: 21631325 DOI: 10.1056/NEJMsa1010705]

61 **Ducreux M**, Cuhna AS, Caramella C, Hollebecque A, Burtin P, Goéré D, Seufferlein T, Haustermans K, Van Laethem JL, Conroy T, Arnold D. Cancer of the pancreas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015; **26** Suppl 5: v56-v68 [PMID: 26314780 DOI: 10.1093/

annonc/mdv295]
62 **Hackert T**, Schneider L, Büchler MW. Current State of Vascular Resections in Pancreatic Cancer Surgery. *Gastroenterol Res Pract* 2015; **2015**: 120207 [PMID: 26609306 DOI: 10.1155/2015/120207]
63 **Kulemann B**, Hoeppner J, Wittel U, Glatz T, Keck T, Wellner UF, Bronsert P, Sick O, Hopt UT, Makowiec F, Riediger H. Perioperative and long-term outcome after standard pancreatico-duodenectomy, additional portal vein and multivisceral resection for pancreatic head cancer. *J Gastrointest Surg* 2015; **19**: 438-444 [PMID: 25567663 DOI: 10.1007/s11605-014-2725-8]
64 **Hartwig W**, Vollmer CM, Fingerhut A, Yeo CJ, Neoptolemos JP, Adham M, Andrén-Sandberg A, Asbun HJ, Bassi C, Bockhorn M, Charnley R, Conlon KC, Dervenis C, Fernandez-Cruz L, Friess H, Gouma DJ, Imrie CW, Lillemoe KD, Milićević MN, Montorsi M, Shrikhande SV, Vashist YK, Izbicki JR, Büchler MW. Extended pancreatectomy in pancreatic ductal adenocarcinoma: definition and consensus of the International Study Group for Pancreatic Surgery (ISGPS). *Surgery* 2014; **156**: 1-14 [PMID: 24856668 DOI: 10.1016/j.surg.2014.02.009]
65 **Schneider EB**, Ejaz A, Spolverato G, Hirose K, Makary MA, Wolfgang CL, Ahuja N, Weiss M, Pawlik TM. Hospital volume and patient outcomes in hepato-pancreatico-biliary surgery: is assessing differences in mortality enough? *J Gastrointest Surg* 2014; **18**: 2105-2115 [PMID: 25297443 DOI: 10.1007/s11605-014-2619-9]
66 **Spolverato G**, Ejaz A, Hyder O, Kim Y, Pawlik TM. Failure to rescue as a source of variation in hospital mortality after hepatic surgery. *Br J Surg* 2014; **101**: 836-846 [PMID: 24760705 DOI: 10.1002/bjs.9492]
67 **Amini N**, Spolverato G, Kim Y, Pawlik TM. Trends in Hospital Volume and Failure to Rescue for Pancreatic Surgery. *J Gastrointest Surg* 2015; **19**: 1581-1592 [PMID: 25794484 DOI: 10.1007/s11605-015-2800-9]
68 **van der Geest LG**, van Rijssen LB, Molenaar IQ, de Hingh IH, Groot Koerkamp B, Busch OR, Lemmens VE, Besselink MG. Volume-outcome relationships in pancreatoduodenectomy for cancer. *HPB* (Oxford) 2016; **18**: 317-324 [PMID: 27037200 DOI: 10.1016/j.hpb.2016.01.515]
69 **Labori KJ**, Katz MH, Tzeng CW, Bjørnbeth BA, Cvancarova M, Edwin B, Kure EH, Eide TJ, Dueland S, Buanes T, Gladhaug IP. Impact of early disease progression and surgical complications on adjuvant chemotherapy completion rates and survival in patients undergoing the surgery first approach for resectable pancreatic ductal adenocarcinoma - A population-based cohort study. *Acta Oncol* 2016; **55**: 265-277 [PMID: 26213211 DOI: 10.3109/0284186x.2015.1068445]
70 **Tzeng CW**, Tran Cao HS, Lee JE, Pisters PW, Varadhachary GR, Wolff RA, Abbruzzese JL, Crane CH, Evans DB, Wang H, Abbott DE, Vauthey JN, Aloia TA, Fleming JB, Katz MH. Treatment sequencing for resectable pancreatic cancer: influence of early metastases and surgical complications on multimodality therapy completion and survival. *J Gastrointest Surg* 2014; **18**: 16-24; discussion 24-25 [PMID: 24241967 DOI: 10.1007/s11605-013-2412-1]
71 **Halloran CM**, Platt K, Gerard A, Polydoros F, O'Reilly DA, Gomez D, Smith A, Neoptolemos JP, Soonwalla Z, Taylor M, Blazeby JM, Ghaneh P. PANasta Trial; Cattell Warren versus Blumgart techniques of panreatico-jejunostomy following pancreato-duodenectomy: Study protocol for a randomized controlled trial. *Trials* 2016; **17**: 30 [PMID: 26772736 DOI: 10.1186/s13063-015-1144-9]
72 **Katz MH**, Pisters PW, Evans DB, Sun CC, Lee JE, Fleming JB, Vauthey JN, Abdalla EK, Crane CH, Wolff RA, Varadhachary GR, Hwang RF. Borderline resectable pancreatic cancer: the importance of this emerging stage of disease. *J Am Coll Surg* 2008; **206**: 833-846; discussion 846-848 [PMID: 18471707 DOI: 10.1016/j.jamcollsurg.2007.12.020]
73 **Malafa MP**. Defining borderline resectable pancreatic cancer: emerging consensus for an old challenge. *J Natl Compr Canc Netw* 2015; **13**: 501-504 [PMID: 25964634]
74 **Roland CL**, Yang AD, Katz MH, Chatterjee D, Wang H, Lin H, Vauthey JN, Pisters PW, Varadhachary GR, Wolff RA, Crane CH, Lee JE, Fleming JB. Neoadjuvant therapy is associated with a reduced lymph node ratio in patients with potentially resectable pancreatic cancer. *Ann Surg Oncol* 2015; **22**: 1168-1175 [PMID: 25352267 DOI: 10.1245/s10434-014-4192-6]
75 **Roland CL**, Katz MH, Tzeng CW, Lin H, Varadhachary GR, Shroff R, Javle M, Fogelman D, Wolff RA, Vauthey JN, Crane CH, Lee JE, Fleming JB. The Addition of Postoperative Chemotherapy is Associated with Improved Survival in Patients with Pancreatic Cancer Treated with Preoperative Therapy. *Ann Surg Oncol* 2015; **22** Suppl 3: S1221-S1228 [PMID: 26350371 DOI: 10.1245/s10434-015-4854-z]
76 **Werner J**, Combs SE, Springfeld C, Hartwig W, Hackert T, Büchler MW. Advanced-stage pancreatic cancer: therapy options. *Nat Rev Clin Oncol* 2013; **10**: 323-333 [PMID: 23629472 DOI: 10.1038/nrclinonc.2013.66]
77 **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]
78 **Gillen S**, Schuster T, Meyer Zum Büschenfelde C, Friess H, Kleeff J. Preoperative/neoadjuvant therapy in pancreatic cancer: a systematic review and meta-analysis of response and resection percentages. *PLoS Med* 2010; **7**: e1000267 [PMID: 20422030 DOI: 10.1371/journal.pmed.1000267]
79 **Tang K**, Lu W, Qin W, Wu Y. Neoadjuvant therapy for patients with borderline resectable pancreatic cancer: A systematic review and meta-analysis of response and resection percentages. *Pancreatology* 2016; **16**: 28-37 [PMID: 26687001 DOI: 10.1016/j.pan.2015.11.007]
80 **Lutfi W**, Talamonti MS, Kantor O, Wang CH, Liederbach E, Stocker SJ, Bentrem DJ, Roggin KK, Winchester DJ, Marsh R, Prinz RA, Baker MS. Perioperative chemotherapy is associated with a survival advantage in early stage adenocarcinoma of the pancreatic head. *Surgery* 2016; **160**: 714-724 [PMID: 27422328 DOI: 10.1016/j.surg.2016.05.029]
81 **Tsai S**, Evans DB. Therapeutic Advances in Localized Pancreatic Cancer. *JAMA Surg* 2016; **151**: 862-868 [PMID: 27276001 DOI: 10.1001/jamasurg.2016.1113]
82 **Bockhorn M**, Uzunoglu FG, Adham M, Imrie C, Milicevic M, Sandberg AA, Asbun HJ, Bassi C, Büchler M, Charnley RM, Conlon K, Cruz LF, Dervenis C, Fingerhutt A, Friess H, Gouma DJ, Hartwig W, Lillemoe KD, Montorsi M, Neoptolemos JP, Shrikhande SV, Takaori K, Traverso W, Vashist YK, Vollmer C, Yeo CJ, Izbicki JR. Borderline resectable pancreatic cancer: a consensus statement by the International Study Group of Pancreatic Surgery (ISGPS). *Surgery* 2014; **155**: 977-988 [PMID: 24856119 DOI: 10.1016/j.surg.2014.02.001]
83 **Asbun HJ**, Conlon K, Fernandez-Cruz L, Friess H, Shrikhande SV, Adham M, Bassi C, Bockhorn M, Büchler M, Charnley RM, Dervenis C, Fingerhutt A, Gouma DJ, Hartwig W, Imrie C, Izbicki JR, Lillemoe KD, Milicevic M, Montorsi M, Neoptolemos JP, Sandberg AA, Sarr M, Vollmer C, Yeo CJ, Traverso LW. When to perform a pancreatoduodenectomy in the absence of positive histology? A consensus statement by the International Study Group of Pancreatic Surgery. *Surgery* 2014; **155**: 887-892 [PMID: 24661765 DOI: 10.1016/j.surg.2013.12.032]
84 **Nordby T**, Ikdahl T, Bowitz Lothe IM, Fagerland MW, Heiberg T, Hauge T, Labori KJ, Buanes T. Improved survival and quality of life in patients undergoing R1 pancreatic resection compared to patients with locally advanced unresectable pancreatic adenocarcinoma. *Pancreatology* 2013; **13**: 180-185 [PMID: 23561977 DOI: 10.1016/j.pan.2013.01.003]
85 **Westgaard A**, Tafjord S, Farstad IN, Cvancarova M, Eide TJ, Mathisen O, Clausen OP, Gladhaug IP. Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma. *BMC Cancer* 2008; **8**: 170 [PMID: 18547417 DOI: 10.1186/1471-2407-8-170]
86 **Westgaard A**, Pomianowska E, Clausen OP, Gladhaug IP.

Intestinal-type and pancreatobiliary-type adenocarcinomas: how does ampullary carcinoma differ from other periampullary malignancies? *Ann Surg Oncol* 2013; **20**: 430-439 [PMID: 22956064 DOI: 10.1245/s10434-012-2603-0]

87 **Poultsides GA**, Huang LC, Cameron JL, Tuli R, Lan L, Hruban RH, Pawlik TM, Herman JM, Edil BH, Ahuja N, Choti MA, Wolfgang CL, Schulick RD. Duodenal adenocarcinoma: clinicopathologic analysis and implications for treatment. *Ann Surg Oncol* 2012; **19**: 1928-1935 [PMID: 22167476 DOI: 10.1245/s10434-011-2168-3]

88 **Albores-Saavedra J**, Schwartz AM, Batich K, Henson DE. Cancers of the ampulla of vater: demographics, morphology, and survival based on 5,625 cases from the SEER program. *J Surg Oncol* 2009; **100**: 598-605 [PMID: 19697352 DOI: 10.1002/jso.21374]

89 **Courtin-Tanguy L**, Rayar M, Bergeat D, Merdrignac A, Harnoy Y, Boudjema K, Meunier B, Sulpice L. The true prognosis of resected distal cholangiocarcinoma. *J Surg Oncol* 2016; **113**: 575-580 [PMID: 26776934 DOI: 10.1002/jso.24165]

90 **Onkendi EO**, Boostrom SY, Sarr MG, Farnell MB, Nagorney DM, Donohue JH, Kendrick ML, Reid-Lombardo KM, Harmsen WS, Que FG. 15-year experience with surgical treatment of duodenal carcinoma: a comparison of periampullary and extra-ampullary duodenal carcinomas. *J Gastrointest Surg* 2012; **16**: 682-691 [PMID: 22350721 DOI: 10.1007/s11605-011-1808-z]

91 **Buchbjerg T**, Fristrup C, Mortensen MB. The incidence and prognosis of true duodenal carcinomas. *Surg Oncol* 2015; **24**: 110-116 [PMID: 25936244 DOI: 10.1016/j.suronc.2015.04.004]

92 **Pomianowska E**, Grzyb K, Westgaard A, Clausen OP, Gladhaug IP. Reclassification of tumour origin in resected periampullary adenocarcinomas reveals underestimation of distal bile duct cancer. *Eur J Surg Oncol* 2012; **38**: 1043-1050 [PMID: 22883964 DOI: 10.1016/j.ejso.2012.07.113]

93 **Schiergens TS**, Reu S, Neumann J, Renz BW, Niess H, Boeck S, Heinemann V, Bruns CJ, Jauch KW, Kleespies A. Histomorphologic and molecular phenotypes predict gemcitabine response and overall survival in adenocarcinoma of the ampulla of Vater. *Surgery* 2015; **158**: 151-161 [PMID: 25819575 DOI: 10.1016/j.surg.2015.02.001]

94 **Showalter TN**, Zhan T, Anne PR, Chervoneva I, Mitchell EP, Yeo CJ, Rosato EL, Kennedy EP, Berger AC. The influence of prognostic factors and adjuvant chemoradiation on survival after pancreaticoduodenectomy for ampullary carcinoma. *J Gastrointest Surg* 2011; **15**: 1411-1416 [PMID: 21523621 DOI: 10.1007/s11605-011-1518-6]

95 **Neoptolemos JP**, Moore MJ, Cox TF, Valle JW, Palmer DH, McDonald AC, Carter R, Tebbutt NC, Dervenis C, Smith D, Glimelius B, Charnley RM, Lacaine F, Scarfe AG, Middleton MR, Anthoney A, Ghaneh P, Halloran CM, Lerch MM, Oláh A, Rawcliffe CL, Verbeke CS, Campbell F, Büchler MW. Effect of adjuvant chemotherapy with fluorouracil plus folinic acid or gemcitabine *vs* observation on survival in patients with resected periampullary adenocarcinoma: the ESPAC-3 periampullary cancer randomized trial. *JAMA* 2012; **308**: 147-156 [PMID: 22782416 DOI: 10.1001/jama.2012.7352]

96 **Bailey P**, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, Anderson MJ, Kazakoff S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quek K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Pinese M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Hair J, Grützmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH, Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson JV, Waddell N, Biankin AV, Grimmond SM. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* 2016; **531**: 47-52 [PMID: 26909576 DOI: 10.1038/nature16965]

97 **Shrikhande SV**, Kleeff J, Reiser C, Weitz J, Hinz U, Esposito I, Schmidt J, Friess H, Büchler MW. Pancreatic resection for M1 pancreatic ductal adenocarcinoma. *Ann Surg Oncol* 2007; **14**: 118-127 [PMID: 17066229 DOI: 10.1245/s10434-006-9131-8]

98 **de Jong MC**, Tsai S, Cameron JL, Wolfgang CL, Hirose K, van Vledder MG, Eckhauser F, Herman JM, Edil BH, Choti MA, Schulick RD, Pawlik TM. Safety and efficacy of curative intent surgery for peri-ampullary liver metastasis. *J Surg Oncol* 2010; **102**: 256-263 [PMID: 20740584 DOI: 10.1002/jso.21610]

99 **Crippa S**, Bittoni A, Sebastiani E, Partelli S, Zanon S, Lanese A, Andrikou K, Muffatti F, Balzano G, Reni M, Cascinu S, Falconi M. Is there a role for surgical resection in patients with pancreatic cancer with liver metastases responding to chemotherapy? *Eur J Surg Oncol* 2016; **42**: 1533-1539 [PMID: 27423449 DOI: 10.1016/j.ejso.2016.06.398]

100 **Heiberg T**, Nordby T, Kvien TK, Buanes T. Development and preliminary validation of the pancreatic cancer disease impact score. *Support Care Cancer* 2013; **21**: 1677-1684 [PMID: 23314652 DOI: 10.1007/s00520-012-1713-3]

P- Reviewer: Chen YC, Neri V, Takahashi H **S- Editor**: Yu J
L- Editor: A **E- Editor**: Liu WX

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.v22.i48.10512

World J Gastroenterol 2016 December 28; 22(48): 10512-10522
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets

Chong-Yang Zhang, Wei-Gang Yuan, Pei He, Jia-Hui Lei, Chun-Xu Wang

Chong-Yang Zhang, Jia-Hui Lei, Department of Pathogenic Biology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China

Wei-Gang Yuan, Chun-Xu Wang, Department of Anthropotomy, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China

Pei He, Department of Obstetrics and Gynecology, Wuhan NO.1 Hospital Affiliated with Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei Province, China

Author contributions: Zhang CY and Yuan WG contributed equally to this work; Zhang CY and Yuan WG wrote the paper; He P and Lei JH created the schematics; Wang CX provided technical support.

Supported by the National Natural Science Foundation of China, No. 81300251.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Chun-Xu Wang, Department of Anthropotomy, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Road, Wuhan 430030, Hubei Province, China. wangchunxu12345@126.com
Telephone: +86-133-17146583
Fax: +86-27-83692617

Received: August 25, 2016
Peer-review started: August 26, 2016
First decision: September 29, 2016
Revised: October 12, 2016
Accepted: November 15, 2016
Article in press: November 16, 2016
Published online: December 28, 2016

Abstract

Liver fibrosis is a reversible wound-healing process aimed at maintaining organ integrity, and presents as the critical pre-stage of liver cirrhosis, which will eventually progress to hepatocellular carcinoma in the absence of liver transplantation. Fibrosis generally results from chronic hepatic injury caused by various factors, mainly viral infection, schistosomiasis, and alcoholism; however, the exact pathological mechanisms are still unknown. Although numerous drugs have been shown to have antifibrotic activity *in vitro* and in animal models, none of these drugs have been shown to be efficacious in the clinic. Importantly, hepatic stellate cells (HSCs) play a key role in the initiation, progression, and regression of liver fibrosis by secreting fibrogenic factors that encourage portal fibrocytes, fibroblasts, and bone marrow-derived myofibroblasts to produce collagen and thereby propagate fibrosis. These cells are subject to intricate cross-talk with adjacent cells, resulting in scarring and subsequent liver damage. Thus, an understanding of the molecular mechanisms of liver fibrosis and their relationships with HSCs is essential for the discovery of new therapeutic targets. This comprehensive review outlines the role of HSCs in liver fibrosis and details novel strategies to suppress HSC activity, thereby providing new insights into potential treatments for liver fibrosis.

Key words: Liver cirrhosis; Fibrosis; Hepatic stellate cells; Etiology; Pathology; Treatment

Core tip: This review discusses the molecular mechanisms of liver fibrosis with respect to hepatic stellate cells (HSCs). In particular, we describe the functional significance of HSCs with respect to major events triggering fibrosis and novel therapeutic strategies to suppress the activity of activated HSCs.

Zhang CY, Yuan WG, He P, Lei JH, Wang CX. Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. *World J Gastroenterol* 2016; 22(48): 10512-10522 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10512.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10512

INTRODUCTION

Liver fibrosis is a complex fibrogenic and inflammatory process that results from chronic liver injury and represents an early step in the progression of liver cirrhosis. Cirrhosis is a major health problem worldwide, owing to the lack of effective treatment methods[1,2]. During hepatic fibrosis, continuous accumulation of extracellular matrix (ECM) extremely rich in collagen Ⅰ and Ⅲ leads to scar deposition and liver fibrosis[3,4]. When left untreated, this condition can develop into cirrhosis and subsequent portal hypertension, hepatic encephalopathy, and/or liver failure, and lead to an increased risk of hepatocellular carcinoma (HCC), which can ultimately cause organ failure and death[2,4]. Liver transplantation is currently regarded as the only treatment method for cirrhosis and is generally inadequate[3]. During chronic liver disease, ongoing liver injury results in excessive ECM deposition with limited remodeling, which inevitably leads to scarring and fibrosis[5]. In comparison, the liver can quickly re-establish its structural integrity in response to acute injury, even when a substantial portion of the organ is damaged[6].

Hepatic stellate cells (HSCs) localize to the perisinusoidal space between hepatocytes and sinusoidal endothelial cells and are the primary source of activated myofibroblasts and portal fibroblasts that drive the fibrogenic process[7]. Quiescent HSCs (qHSCs) mostly function as vitamin A reserves[8]. In response to liver injury, inflammatory mediators promote HSC activation and subsequent differentiation into myofibroblasts[9]. Activated HSCs (aHSCs) are a major source of collagen in the liver and can abundantly secrete ECM proteins, tissue inhibitors of metalloproteinases, and matrix metalloproteinases (MMPs) that elicit liver architecture remodeling[9,10]. Importantly, HSCs are responsible for as much as 80% of total fibrillar collagen Ⅰ in the fibrotic liver[8-11]; thus, aHSC depletion is critical for the resolution of fibrosis.

Based on these findings, we provide a comprehensive review summarizing the etiology and pathological characteristics of hepatic fibrosis, and detail the potential therapeutic targets for suppression of aHSC function.

ETIOLOGY AND PATHOLOGICAL CHARACTERISTICS OF HEPATIC FIBROSIS

Liver fibrosis is a complex process that results from various forms of chronic hepatic disease and is associated with excess hepatocellular death[2,12,13]. The main etiologies of liver fibrosis are schistosome and chronic viral hepatitis infection, nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and cholestatic and autoimmune liver disease[1,14-17]. Liver fibrosis, which is characterized by the excessive deposition ECM proteins[18], involves both parenchymal and nonparenchymal hepatic cells, as well as infiltrating immune cells[3,19]. Furthermore, different organs, such as the adipose tissue, bile duct, intestine, and muscle, can also affect the development of liver fibrosis. Moreover, several essential signaling pathways have important roles in fibrosis. The complex interactions among these signaling pathways, diverse cells, and different organs contribute to the progression of liver fibrosis[20]. Upon fibrogenic initiation, qHSCs differentiate into aHSCs, upon which they lose the intracellular lipid droplets and acquire a myofibroblastic phenotype characterized by marked upregulation of a-smooth muscle actin (a-SMA, ACTA2), desmin (DES), and type Ⅰ collagen (COL1A1)[8-10]. The sustained buildup of collagens distorts the liver parenchyma and vascular architecture, resulting in impaired liver function, scar deposition, and liver fibrosis[1,2,12,14,17]. The initiation, progression, and resolution of liver fibrosis involving HSCs are present in Figure 1.

Viral and schistosome infection

Viral infections such as those caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) induce hepatic inflammation and thereby contribute to the cyclical process of inflammation, necrosis, and regeneration[21]. Within this inflammatory microenvironment, continuous infiltration of immune cells and secreted inflammatory cytokines leads to liver injury, triggering a progressive cascade of hepatic lobule reconstruction that promotes liver fibrosis and cirrhosis[22].

Schistosomiasis is a major chronic disease that occurs in humans living in endemic regions, owing to substantial pathologic liver fibrosis caused by from an accumulation of parasitic eggs[23]. Ongoing antigenic stimulation from the trapped ova results in immune cell recruitment to the sites of infection, leading to the formation of periovular granulomas and eventual fibrosis[24]. Liver fibrosis often begins 6 wk after infection, when the Th2 immune response predominates

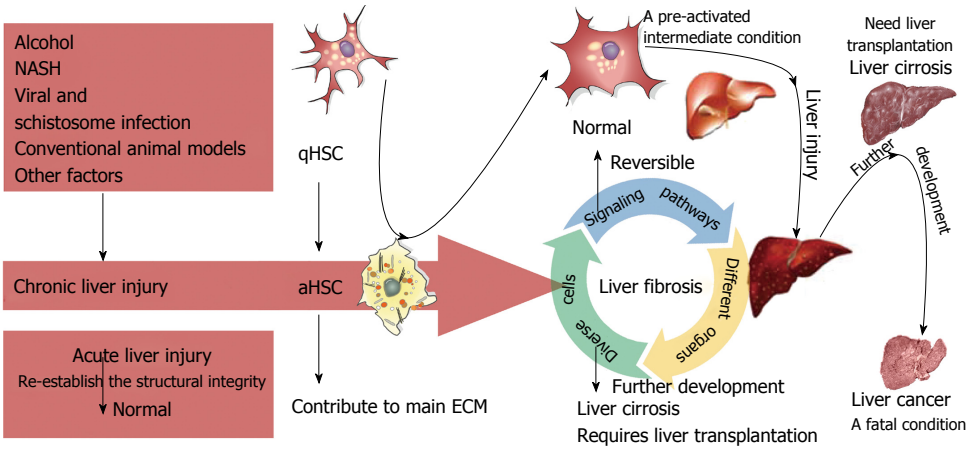

Figure 1 Initiation, progression, and resolution of liver fibrosis involving hepatic stellate cells. Upon various types of chronic injury - including that caused by alcohol, viral and schistosome infection, nonalcoholic steatohepatitis (NASH), and other factors - hepatic stellate cells (HSCs) transdifferentiate from quiescent HSCs to activated HSCs, the latter of which secrete abundant extracellular proteins that contribute to liver fibrosis. Liver fibrosis is thought to be a reversible condition owing to the elimination of causative agents and different strategies of limiting HSC activation; however, they cannot totally return to a quiescent status of the naive HSCs. Instead, they exhibit a pre-activated intermediate condition with an increased sensitivity to injury. Thus, preventing recurrent chronic liver injury is of great importance in patients undergoing treatment for liver fibrosis. Untreated or relapsed fibrosis progresses to liver cirrhosis, which often requires hepatic transplantation.

and subsequently subsides at 12 wk postinfection. The Th17 response has also been associated with severe hepatic inflammation; however, the function of B cells in schistosome-induced pathology remains controversial. Because immune cell-derived chemokines play a vital role in schistosome-induced pathology[25,26], one method to hamper disease progression could be by modulating chemokine production to limit hepatic eosinophil recruitment[27]. Importantly, although praziquantel therapy effectively kills adult *Schistosoma*, it has diminutive effects on liver fibrogenesis or portal hypertension[28,29]; thus, new strategies to treat schistosomiasis are urgently needed.

Alcohol

Excessive alcohol abuse causes steatohepatitis that can progress to ALD. Most patients are generally asymptomatic, and ALD is easily reversible when patients abstain from alcohol consumption. Otherwise, they will develop into liver fibrosis. Acetaldehyde is regarded as a major intermediate in alcohol-induced fibrogenesis[30,31], and recent studies have delineated the mechanisms through which transforming growth factor (TGF)-β/small mother against decapentaplegic (SMAD) signaling is enhanced by acetaldehyde[32]. Additionally, acetaldehyde-induced fibrogenesis is also thought to involve members of the basic transcription element binding protein[33,34], CAAT/enhanced-binding protein[35,36], and acetaldehyde-responsive element[37]. Cytochrome P450 2E1 (CYP2E1) protein is a member of the microsomal ethanol oxidizing system responsible for ethanol metabolism and is crucial for alcohol-induced fibrogenesis[38]. This mechanism is readily observed in hepatocyte and HSC cocultures with enhanced collagen I protein synthesis resulting from CYP2E1-dependent reactive oxygen species generation[39]. Correspondingly, ethanol-mediated lipid peroxidation is effectively blocked in *CYP2E1*$^{-/-}$ mice[40], whereas oxidative stress and hepatic fibrogenesis is elevated in transgenic mice with CYP2E1 overexpression[41]. Moreover, the calcium regulatory protein osteopontin (OPN) has demonstrated protective effects in early alcohol-induced liver injury by binding lipopolysaccharide and blocking tumor necrosis factor-alpha (TNF-α) function in the liver[42]. OPN is also positively correlated with fibrosis in patients with ALD[43].

Nonalcoholic steatohepatitis

Nonalcoholic steatohepatitis (NASH) is a relatively common chronic liver disease with histological characteristics similar to that of ALD[44]. NASH presents as balloon-like hepatocellular injury with or without hepatic fibrosis in liver biopsies[45] and is the intermediate between NAFLD and cirrhosis[46]. NASH occurs when sustained oxidative stress prevents the proliferation of mature liver cells, resulting in excess necrosis and an overgrowth of liver progenitor cells (oval cells)[47]. In addition, the inflammatory response to cellular necrosis induces the progressive release of platelet-derived growth factor, TGF-β, TNF-α, and other inflammatory factors, such as interleukin (IL)-1, by resident immune cells[48]. These inflammatory signals result in the activation and proliferation of HSCs and induce differentiation of HSCs into myofibroblasts, further driving ECM synthesis and ultimately liver fibrosis[49].

Animal models of liver fibrogenesis

Liver fibrosis takes years to develop in most patients and results from an interplay of several risk factors, including HBV and HCV infection, alcohol abuse, and metabolic syndromes attributed to obesity, insulin resistance, and diabetes[50]. Accordingly, animal models used to study the pathophysiology of liver fibrosis,

cirrhosis, and HCC should mimic the general disease patterns found in human counterparts.

Currently, *in vivo* models of liver fibrosis can be divided into five categories based on etiology: chemical, dietary, surgical, genetically modified, and infection[51]. The chemicals commonly used to cause hepatic lesions and induce liver fibrosis include ethanol, carbon tetrachloride (CCl_4)[52], thioacetamide[53], dimethylnitrosamine[54], and diethylnitrosamine[55]. A number of specific diets, such as the methionine- and choline-deficient diet[56], high-fat diet[57], and choline-deficient L-amino acid-defined diet[58], can be used to induce progression of NAFLD to hepatic fibrosis in experimental animals. Moreover, common bile duct ligation (BDL) can also lead to cholestatic injury and periportal biliary fibrosis[59]. In the past decade, multidrug resistance-associated protein 2-deficient (*Mdr2*$^{-/-}$) mice[60] and *Alms1*$^{foz/foz}$ fat Aussie mice[61] have been used to study the functional relevance of specific signaling pathways in the formation of liver fibrosis and identify novel drug targets. Finally, infections with HBV[62] and *Schistosoma* parasites[63] are also popular models of liver fibrosis.

NOVEL THERAPEUTIC TARGETS IN LIVER FIBROSIS

Liver fibrosis was once deemed irreversible; however, early liver fibrosis is now managed by clinical treatment, and overwhelming evidence suggests that advanced fibrosis may likely be reversible once the injurious stimulus is removed[64]. Since aHSCs are the primary mediators of liver pathology in this process, several molecules required for HSC activation are considered potential therapeutic targets[9,64,65]. The following section details recent novel targets identified for the treatment of liver fibrosis through suppression of HSC activation.

Key molecules in liver fibrosis

Mitra and colleagues reported that IL-30 attenuates hepatic fibrosis by inducing natural killer group 2D (NKG2D)/ribonucleic acid export 1 crosstalk between aHSCs and natural killer T (NKT) cells and is therefore an ideal therapy for liver fibrosis. Mechanistically, IL-30 treatment promotes surface NKG2D expression on liver NKT cells to subsequently enhance their cytotoxic activity towards aHSCs, thereby inhibiting liver fibrosis[66]. Another molecule, hydrogen peroxide-inducible clone-5 (Hic-5) is a TGF-β1-inducible focal adhesion protein that facilitates cell proliferation and ECM expansion in various organs[67]. Previous studies have shown that Hic-5 contributes to vascular restoration and restructuring[67,68]; however, a recent study revealed that Hic-5 expression also plays a critical role in attenuating fibrosis by enhancing TGF-β-induced Smad2 phosphorylation via the downregulation of Smad7 in both human and mouse aHSCs[69]. Taken together, these data indicate that Hic-5 is a novel therapeutic target and a potential marker of activated HSCs. Additionally, acyl-coenzyme A: cholesterol acyltransferase (ACAT) is comprised of two isoenzymes-ACAT1 and ACAT2-and functions as a catalyst to convert free cholesterol (FC) to cholesteryl esters[70]. FC accumulation has been shown to regulate HSC activation and the development of liver fibrosis by promoting Toll-like receptor 4 signal transduction. Because ACAT1 plays an essential role in regulating FC accumulation in HSCs[71], studies have focused on developing new ACAT1-directed therapeutic interventions for the treatment of liver fibrosis. The roles of IL-30, Hic-5, and ACAT1 in liver fibrosis are presented in Figure 2.

Regulatory CD4$^+$ T cells

Regulatory T (Treg) cells function to modulate HCV-dependent liver fibrosis by regulating the interaction between NK cells and aHSCs[72,73]. Specifically, Treg cells act in a cell-contact-dependent manner to reduce NK cell activity against HSCs and downregulate vital NKT-activating ligands on HSCs by secreting soluble IL-8 and/or TGF-β1[73]. This mechanism may also be present in fibrosis, resulting from other etiologies; however, further studies are needed to confirm this hypothesis.

Macrophages

Macrophages, which can be classified as M1 (classically activated) macrophages and M2 (alternatively activated) macrophages, play dual roles in the progression and resolution of liver fibrosis. Typically, M1 macrophages produce inflammatory cytokines, whereas M2 macrophages regulate inflammatory responses and tissue repair. The imbalance of M1 and M2 macrophages mediates the progression and resolution of liver fibrosis[74]. During the early stages of liver injury, bone marrow-derived monocytes are extensively recruited to the liver and then differentiate into inflammatory macrophages (mostly M1 macrophages) to produce pro-inflammatory and profibrotic cytokines, thereby promoting inflammatory responses and HSC activation. Afterwards, recruited macrophages switch their phenotypic (mostly M2 macrophages) to secrete MMPs, the main enzymes degrading ECM, to facilitate fibrosis resolution[20,75,76].

Role of signal transduction in the progression of liver fibrosis

Several intracellular signaling pathways are involved in the pathophysiology of liver fibrosis. In this section, we detail the functional significance of three key signaling axes in this process: Gas6/Axl, TGF-β/Smad, and target of Wnt signaling pathway (Figure 3).

Gas6/Axl pathway: The TAM (Tyro3, Axl, Mer) receptor ligand Gas6 is a vitamin K-dependent protein with an extremely high affinity for the Axl receptor.

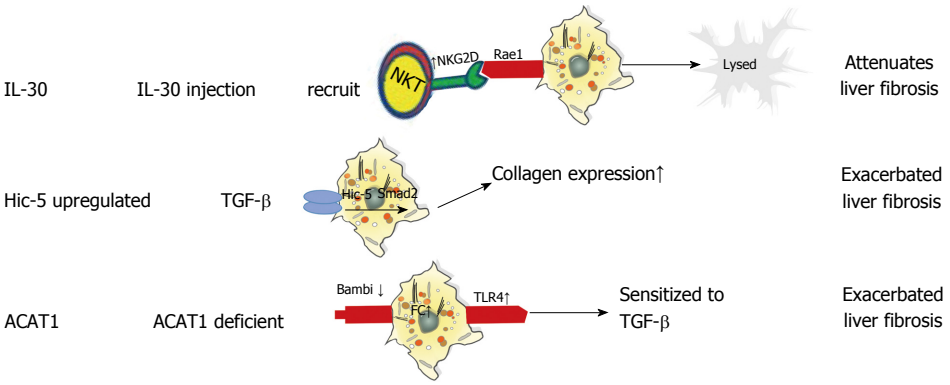

Figure 2 Roles of interleukin-30, hydrogen peroxide inducible clone 5, and cholesterol acyltransferase 1 in liver fibrosis.

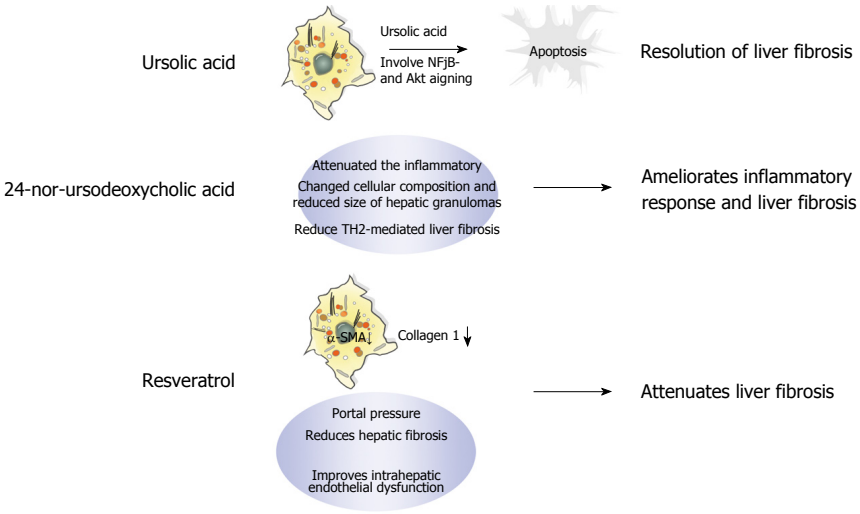

Figure 3 Roles of the Wnt, TGF-β/Smad, and Gas6/Axl signaling pathways in the progression of liver fibrosis.

Gas6 is primarily expressed by Kupffer cells, whereas Axl is found in both macrophages and qHSCs in the normal liver[77,78]. Study has demonstrated that CCl_4-induced liver fibrosis elicits Gas6/Axl pathway activation to promote HSC activation. Notably, Axl knockout disrupts this pathway, thereby attenuating hepatic fibrosis[78]. Clinical trials have also shown increased Gas6 and Axl serum levels in patients with HCV infection and ALD[78]. As such, targeting Axl may be a potential method to remediate liver fibrosis.

TGF-β/Smad signaling: TGF-β regulates ECM metabolism and tissue fibrosis through the overproduction of type Ⅰ collagen in both mice and humans. Recent studies have demonstrated that TGF-β/Smad signaling plays a crucial role in the progression of hepatic fibrosis caused by parasitic infection, including *Schistosoma*, *Clonorchis sinensis*, and *Echinococcus multilocularis*, as well as other etiological factors[79,80]. More specifically, TGF-β1 ligation to TGF-β type Ⅰ (TGFβRI) and type Ⅱ receptors induces Smad2/3 phosphorylation and its subsequent interaction with Smad4. The Smad2/3/4 complex can then translocate to the nucleus and induce the expression of profibrotic genes, namely collagen type Ⅰ. Strikingly, Smad7 can block TGF-β signaling through various means[80-82], such as binding TGFβRI to inhibit the interaction-dependent activation of Smad2, collaborating with other effectors to induce TGFβRI degradation, and regulating the Wnt/β-catenin pathway to influence TGF-β-induced apoptosis[83]. Similarly, targeting of Smad7 enhances TGF-β pathway activation[84].

Wnt pathway: Several studies have demonstrated that aberrant Wnt/β-catenin signaling affects the progression of fibrotic disorders. Wnt comprises an evolutionarily conserved family of excreted lipid-modified glycoproteins that can be classified into at least three signaling pathways: Necdin-Wnt, noncanonical (β-catenin-independent), and canonical (β-catenin-dependent). In the Necdin-Wnt pathway, HSC activation and differentiation require the down-regulation of peroxisome proliferator-activated receptor γ (PPARγ). Necdin is a melanoma antigen family protein preferentially expressed in aHSCs that promotes myogenic and neuronal differentiation while suppressing adipogenesis. Notably, Necdin silencing restores PPARγ-mediated Wnt pathway inhibition to

effectively reverse HSC activation[85,86]. In the canonical pathway, Wnt ligation to cell surface receptors elicits downstream signaling that stabilizes β-catenin, which can then translocate into the nucleus, bind T cell factor/lymphoid enhancer-binding factor (TCF/LEF) promoter, and induce gene expression to exert biological effects[87,88]. Alternatively, noncanonical Wnt signaling occurs *via* the β-catenin-independent planar cell polarity and noncanonical Wnt/Ca^{2+} pathways. Thus, a collective understanding of Wnt signaling mechanisms may provide novel insights into the pathophysiology of liver fibrosis. A recent study also showed that DKK2 (a Wnt antagonist and target of the Wnt pathway) connects Sept4 (a subunit of the septin cytoskeleton expressed in qHSCs) and the activation of HSCs, thereby mediating the progression of liver fibrosis. The expression of DKK2 is high in primary cultured HSCs. However, DKK2 expression is reduced when Sept is not expressed in a mice model of CCL4-induced fibrosis. The high expression of DKK2 in qHSCs inhibits Wnts and thereby affects downstream β-catenin signaling. This results in suppression of the Wnt signaling pathway, leading to increased expression of Sept4 and preventing HSC activation[87].

HAb18G/CD147: HAb18G/CD147 is induced by TGF-β1 stimulation and is highly expressed on sinusoidal aHSCs, where it colocalizes with a-SMA. Transient transfection of CD147 in LX-2 cells results in increased expression of mRNAs encoding α-SMA, TIMP-1, α1(I) collagen, and TGF-β1. In contrast, MMP-13 and MMP-2 levels are markedly reduced, suggesting that HAb18G/CD147 promotes HSC activation. Consistent with this, HAb18G/CD147-targeting antibodies block HSC activation, thereby inhibiting liver fibrogenesis[89]. These data support the potential role for HAb18G/CD147 in liver fibrosis; however, further studies are needed to confirm these findings.

microRNAs and HSCs in liver fibrosis

Recently, microRNAs (miRNAs) have also been found to play multifaceted roles in hepatic fibrosis, including those in HSC activation and proliferation and production of ECM proteins[3,11]. Previous studies have indicated that human and murine miRNAs participate in liver fibrosis. For example, *miR-199a*, antisense *miR-199a**, *miR-200a*, and *miR-200b* are dramatically upregulated in a mouse model of liver fibrosis[90]. Conversely, the *miR-29* family is downregulated in aHSCs when compared with that in qHSCs, both *in vivo* and *in vitro*[91].

miR-133a is specifically downregulated in HSCs during fibrogenesis, but is overexpressed in primary murine HSC, resulting in attenuation of collagen expression[91]. Similarly, CCL4-induced *miR-122* expression is markedly lower in aHSCs and fibrotic liver tissue. Cell experiments have also shown that *miR-133a* overexpression inhibits both LX2 and primary murine HSC proliferation and prevents the progression of liver fibrosis[92-94]. Furthermore, both *miR-15b* and *miR-16* facilitate qHSC apoptosis by targeting Bcl-2 and the caspase signaling cascade[95].

Promising therapies for liver fibrosis

Although several antifibrotic drug candidates have recently been evaluated, these drugs have failed to show increased therapeutic efficacy over those drugs currently used in the clinic, *e.g.,* ursolic acid (UA), 24-nor-ursodeoxycholic acid (norUDCA), and resveratrol. UA is a pentacyclic triterpenoid compound with a wide spectrum of pharmacological activities found in various edible fruits and medicinal plants. Studies have demonstrated that UA induces apoptotic culture-activated HSC death due to inhibition of nuclear factor kappa B and AKT in HSCs, but not in isolated qHSCs *in vitro*. In addition, UA alleviates liver fibrosis induced by both BDL and chronic thioacetamide administration *in vivo*. As shown in Figure 4, the mechanism of UA-induced apoptosis may be attributed to its suppression of cell survival pathways and the activation of downstream caspases via the mitochondrial permeability transition[96].

The bile acid derivative norUDCA is a promising new treatment option for liver fibrosis that significantly reduces liver fibrosis in chronically infected *Schistosoma mansoni* mice by limiting T-cell proliferation and IL-13 and IL-4 serum levels (Figure 4). Moreover, norUDCA has anti-inflammatory properties demonstrated by the low expression of MHC class Ⅱ on dendritic cells and macrophages after norUDCA treatment[28].

Finally, the natural polyphenol flavonoid resveratrol has a broad range of beneficial biological functions, including anti-inflammatory[97] and antioxidant[98] properties[99]. In addition, resveratrol is believed to ameliorate obesity-related complications by mimicking caloric restriction[100] through activation of key metabolic regulators, including NAD^+-dependent deacetylase (SIRT1)[101], AMP-activated protein kinase[102], and nuclear factor erythroid-2 related factor 2[103]. Furthermore, oxidative damage and inflammation are closely related to the HSC activation process. For example, SIRT1 activation inhibits the expression of muscle-related genes, such as *MyoD*[104]. Moreover, studies have demonstrated the beneficial effects of resveratrol in different models of liver steatosis[105-108]. Superoxide dismutase activity is necessary for the reduction of oxygen free radicals and protects against lipid peroxidation, thereby inhibiting HSC activation and limiting the progression of liver fibrosis[109]. The mechanisms through which resveratrol alleviates fibrosis are shown in Figure 4. Although resveratrol has been shown to have beneficial biological functions in the antifibrotic response, its efficacy in NAFLD is insignificant; indeed, a meta-analysis conducted by Zhang *et al*[110] indicated that resveratrol can only improve LDL and total cholesterol levels in patients with NAFLD.

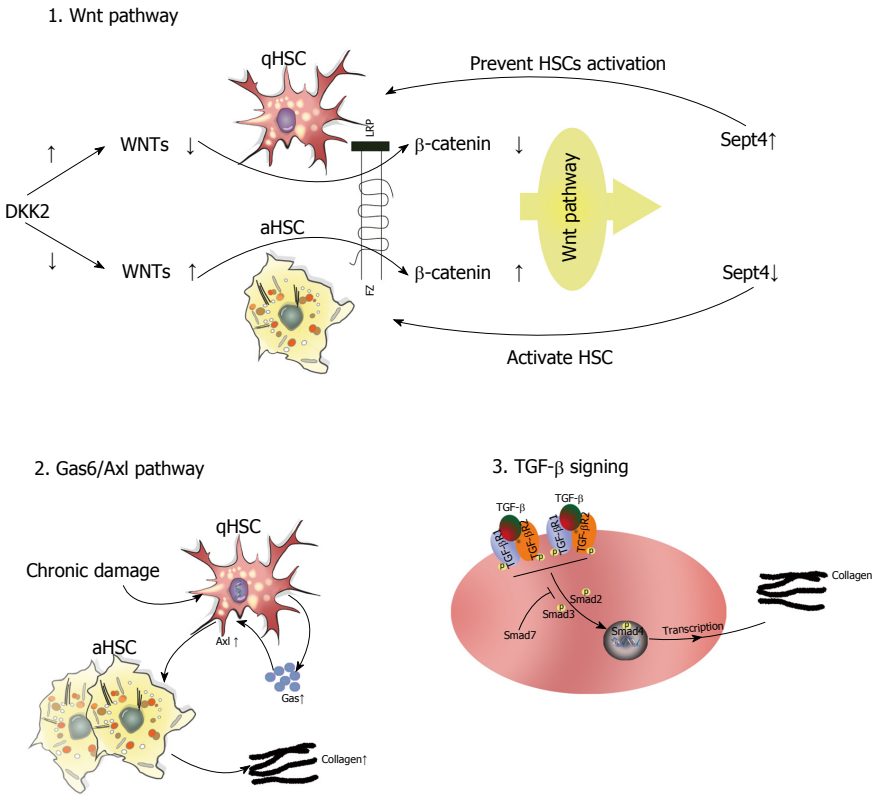

Figure 4 Mechanism of action of three potential therapeutic drugs-ursolic acid, 24-nor-ursodeoxycholic acid, and resveratrol-for treating fibrosis.

CONCLUSION

In this review, we outlined some major etiological and pathological characteristics of hepatic fibrosis and described several promising approaches for liver fibrosis therapy. We strongly believe that liver fibrosis will be cured through the combined application of these therapeutics; however, further studies are necessary to support this hypothesis.

REFERENCES

1 **Weiskirchen R**, Tacke F. Liver Fibrosis: From Pathogenesis to Novel Therapies. *Dig Dis* 2016; **34**: 410-422 [PMID: 27170396 DOI: 10.1159/000444556]
2 **Pinzani M**. Pathophysiology of Liver Fibrosis. *Dig Dis* 2015; **33**: 492-497 [PMID: 26159264 DOI: 10.1159/000374096]
3 **van Dijk F**, Olinga P, Poelstra K, Beljaars L. Targeted Therapies in Liver Fibrosis: Combining the Best Parts of Platelet-Derived Growth Factor BB and Interferon Gamma. *Front Med* (Lausanne) 2015; **2**: 72 [PMID: 26501061 DOI: 10.3389/fmed.2015.00072]
4 **Tacke F**, Trautwein C. Mechanisms of liver fibrosis resolution. *J Hepatol* 2015; **63**: 1038-1039 [PMID: 26232376 DOI: 10.1016/j.jhep.2015.03.039]
5 **Schuppan D**. Liver fibrosis: Common mechanisms and antifibrotic therapies. *Clin Res Hepatol Gastroenterol* 2015; **39** Suppl 1: S51-S59 [PMID: 26189980 DOI: 10.1016/j.clinre.2015.05.005]
6 **Nicolas CT**, Wang Y, Nyberg SL. Cell therapy in chronic liver disease. *Curr Opin Gastroenterol* 2016; **32**: 189-194 [PMID: 26950359 DOI: 10.1097/MOG.0000000000000262]
7 **Josan S**, Billingsley K, Orduna J, Park JM, Luong R, Yu L, Hurd R, Pfefferbaum A, Spielman D, Mayer D. Assessing inflammatory liver injury in an acute CCl4 model using dynamic 3D metabolic imaging of hyperpolarized [1-(13)C]pyruvate. *NMR Biomed* 2015; **28**: 1671-1677 [PMID: 26474216 DOI: 10.1002/nbm.3431]
8 **Lepreux S**, Desmoulière A. Human liver myofibroblasts during development and diseases with a focus on portal (myo)fibroblasts. *Front Physiol* 2015; **6**: 173 [PMID: 26157391 DOI: 10.3389/fphys.2015.00173]
9 **Li D**, He L, Guo H, Chen H, Shan H. Targeting activated hepatic stellate cells (aHSCs) for liver fibrosis imaging. *EJNMMI Res* 2015; **5**: 71 [PMID: 26650603 DOI: 10.1186/s13550-015-0151-x]
10 **Puche JE**, Saiman Y, Friedman SL. Hepatic stellate cells and liver fibrosis. *Compr Physiol* 2013; **3**: 1473-1492 [PMID: 24265236 DOI: 10.1002/cphy.c120035]
11 **Rao HY**, Wei L, Li J, Zhang LF, Chen HY, Zhu LM, Liu F, Sun Y, Wang H. Liver fibrosis and hepatic stellate cells improvement of chronic hepatitis C patients by interferon-beta-1a with or without sustained viral response. *Hepatogastroenterology* 2009; **56**: 328-334 [PMID: 19579592]
12 **Kitano M**, Bloomston PM. Hepatic Stellate Cells and microRNAs in Pathogenesis of Liver Fibrosis. *J Clin Med* 2016; **5**: pii. E38 [PMID: 26999230 DOI: 10.3390/jcm5030038]
13 **Elpek GÖ**. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. *World J Gastroenterol* 2014; **20**: 7260-7276 [PMID: 24966597 DOI: 10.3748/wjg.v20.i23.7260]
14 **Poelstra K**. Liver fibrosis in 2015: Crucial steps towards an effective treatment. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 67-68 [PMID: 26758787 DOI: 10.1038/nrgastro.2015.224]
15 **Seki E**, Brenner DA. Recent advancement of molecular mechanisms of liver fibrosis. *J Hepatobiliary Pancreat Sci* 2015; **22**: 512-518 [PMID: 25869468 DOI: 10.1002/jhbp.245]
16 **Schmid P**, Bregenzer A, Huber M, Rauch A, Jochum W, Müllhaupt B, Vernazza P, Opravil M, Weber R. Progression of Liver Fibrosis in HIV/HCV Co-Infection: A Comparison between Non-Invasive Assessment Methods and Liver Biopsy. *PLoS One* 2015; **10**: e0138838 [PMID: 26418061 DOI: 10.1371/journal.pone.0138838]
17 **Sacchi P**, Cima S, Corbella M, Comolli G, Chiesa A, Baldanti F, Klersy C, Novati S, Mulatto P, Mariconti M, Bazzocchi C, Puoti M, Pagani L, Filice G, Bruno R. Liver fibrosis, microbial translocation

and immune activation markers in HIV and HCV infections and in HIV/HCV co-infection. *Dig Liver Dis* 2015; **47**: 218-225 [PMID: 25544657 DOI: 10.1016/j.dld.2014.11.012]
18 **Baiocchini A**, Montaldo C, Conigliaro A, Grimaldi A, Correani V, Mura F, Ciccosanti F, Rotiroti N, Brenna A, Montalbano M, D' Offizi G, Capobianchi MR, Alessandro R, Piacentini M, Schininà ME, Maras B, Del Nonno F, Tripodi M, Mancone C. Extracellular Matrix Molecular Remodeling in Human Liver Fibrosis Evolution. *PLoS One* 2016; **11**: e0151736 [PMID: 26998606 DOI: 10.1371/journal.pone.0151736]
19 **Toosi AE**. Liver Fibrosis: Causes and Methods of Assessment, A Review. *Rom J Intern Med* 2015; **53**: 304-314 [PMID: 26939206]
20 **Sun M**, Kisseleva T. Reversibility of liver fibrosis. *Clin Res Hepatol Gastroenterol* 2015; **39** Suppl 1: S60-S63 [PMID: 26206574 DOI: 10.1016/j.clinre.2015.06.015]
21 **Nagao Y**, Kawahigashi Y, Sata M. Association of Periodontal Diseases and Liver Fibrosis in Patients With HCV and/or HBV infection. *Hepat Mon* 2014; **14**: e23264 [PMID: 25737729 DOI: 10.5812/hepatmon.23264]
22 **Lin H**, Ha NB, Ahmed A, Ayoub W, Daugherty TJ, Lutchman GA, Garcia G, Nguyen MH. Both HCV and HBV are major causes of liver cancer in Southeast Asians. *J Immigr Minor Health* 2013; **15**: 1023-1029 [PMID: 23864445 DOI: 10.1007/s10903-013-9871-z]
23 **Zoni AC**, Catalá L, Ault SK. Schistosomiasis Prevalence and Intensity of Infection in Latin America and the Caribbean Countries, 1942-2014: A Systematic Review in the Context of a Regional Elimination Goal. *PLoS Negl Trop Dis* 2016; **10**: e0004493 [PMID: 27007193 DOI: 10.1371/journal.pntd.0004493]
24 Immunity to schistosomiasis. *Lancet* 1987; **1**: 1015-1016 [PMID: 2883351]
25 **al-Fawaz IM**, al-Rasheed SA, al-Majed SA, Ashour M. Schistosomiasis associated with a mediastinal mass: case report and review of the literature. *Ann Trop Paediatr* 1990; **10**: 293-297 [PMID: 1703747]
26 Immunopathology of schistosomiasis. *Lancet* 1987; **2**: 194-195 [PMID: 2885645 DOI: 10.1016/S0140-6736(87)90771-9]
27 **Chuah C**, Jones MK, Burke ML, McManus DP, Gobert GN. Cellular and chemokine-mediated regulation in schistosome-induced hepatic pathology. *Trends Parasitol* 2014; **30**: 141-150 [PMID: 24433721 DOI: 10.1016/j.pt.2013.12.009]
28 **Sombetzki M**, Fuchs CD, Fickert P, Österreicher CH, Mueller M, Claudel T, Loebermann M, Engelmann R, Langner C, Sahin E, Schwinge D, Guenther ND, Schramm C, Mueller-Hilke B, Reisinger EC, Trauner M. 24-nor-ursodeoxycholic acid ameliorates inflammatory response and liver fibrosis in a murine model of hepatic schistosomiasis. *J Hepatol* 2015; **62**: 871-878 [PMID: 25463533 DOI: 10.1016/j.jhep.2014.11.020]
29 **Praticò L**, Mariani B, Brunetti E, Maserati R, Bruno A, Novati S, Chichino G. Failure of repeated treatment with praziquantel and arthemeter in four patients with acute schistosomiasis. *J Travel Med* 2014; **21**: 133-136 [PMID: 24455977 DOI: 10.1111/jtm.12098]
30 **Moshage H**, Casini A, Lieber CS. Acetaldehyde selectively stimulates collagen production in cultured rat liver fat-storing cells but not in hepatocytes. *Hepatology* 1990; **12**: 511-518 [PMID: 2169453]
31 **Casini A**, Cunningham M, Rojkind M, Lieber CS. Acetaldehyde increases procollagen type I and fibronectin gene transcription in cultured rat fat-storing cells through a protein synthesis-dependent mechanism. *Hepatology* 1991; **13**: 758-765 [PMID: 2010171]
32 **Svegliati-Baroni G**, Inagaki Y, Rincon-Sanchez AR, Else C, Saccomanno S, Benedetti A, Ramirez F, Rojkind M. Early response of alpha2(I) collagen to acetaldehyde in human hepatic stellate cells is TGF-beta independent. *Hepatology* 2005; **42**: 343-352 [PMID: 16025520 DOI: 10.1002/hep.20798]
33 **Anania FA**, Potter JJ, Rennie-Tankersley L, Mezey E. Effects of acetaldehyde on nuclear protein binding to the nuclear factor I consensus sequence in the alpha 2(I) collagen promoter. *Hepatology* 1995; **21**: 1640-1648 [PMID: 7768510]
34 **Attard FA**, Wang L, Potter JJ, Rennie-Tankersley L, Mezey E. CCAAT/enhancer binding protein beta mediates the activation of the murine alpha1(I) collagen promoter by acetaldehyde. *Arch Biochem Biophys* 2000; **378**: 57-64 [PMID: 10871045 DOI: 10.1006/abbi.2000.1803]
35 **García-Trevijano ER**, Iraburu MJ, Fontana L, Domínguez-Rosales JA, Auster A, Covarrubias-Pinedo A, Rojkind M. Transforming growth factor beta1 induces the expression of alpha1(I) procollagen mRNA by a hydrogen peroxide-C/EBPbeta-dependent mechanism in rat hepatic stellate cells. *Hepatology* 1999; **29**: 960-970 [PMID: 10051504 DOI: 10.1002/hep.510290346]
36 **Greenwel P**, Domínguez-Rosales JA, Mavi G, Rivas-Estilla AM, Rojkind M. Hydrogen peroxide: a link between acetaldehyde-elicited alpha1(I) collagen gene up-regulation and oxidative stress in mouse hepatic stellate cells. *Hepatology* 2000; **31**: 109-116 [PMID: 10613735 DOI: 10.1002/hep.510310118]
37 **Svegliati-Baroni G**, Ridolfi F, Di Sario A, Saccomanno S, Bendia E, Benedetti A, Greenwel P. Intracellular signaling pathways involved in acetaldehyde-induced collagen and fibronectin gene expression in human hepatic stellate cells. *Hepatology* 2001; **33**: 1130-1140 [PMID: 11343241 DOI: 10.1053/jhep.2001.23788]
38 **Lu Y**, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. *Free Radic Biol Med* 2008; **44**: 723-738 [PMID: 18078827 DOI: 10.1016/j.freeradbiomed.2007.11.004]
39 **Bell LN**, Temm CJ, Saxena R, Vuppalanchi R, Schauer P, Rabinovitz M, Krasinskas A, Chalasani N, Mattar SG. Bariatric surgery-induced weight loss reduces hepatic lipid peroxidation levels and affects hepatic cytochrome P-450 protein content. *Ann Surg* 2010; **251**: 1041-1048 [PMID: 20485142 DOI: 10.1097/SLA.0b013e3181dbb572]
40 **Butura A**, Nilsson K, Morgan K, Morgan TR, French SW, Johansson I, Schuppe-Koistinen I, Ingelman-Sundberg M. The impact of CYP2E1 on the development of alcoholic liver disease as studied in a transgenic mouse model. *J Hepatol* 2009; **50**: 572-583 [PMID: 19157621 DOI: 10.1016/j.jhep.2008.10.020]
41 **Patouraux S**, Bonnafous S, Voican CS, Anty R, Saint-Paul MC, Rosenthal-Allieri MA, Agostini H, Njike M, Barri-Ova N, Naveau S, Le Marchand-Brustel Y, Veillon P, Calès P, Perlemuter G, Tran A, Gual P. The osteopontin level in liver, adipose tissue and serum is correlated with fibrosis in patients with alcoholic liver disease. *PLoS One* 2012; **7**: e35612 [PMID: 22530059 DOI: 10.1371/journal.pone.0035612]
42 **Nagoshi S**. Osteopontin: Versatile modulator of liver diseases. *Hepatol Res* 2014; **44**: 22-30 [PMID: 23701387 DOI: 10.1111/hepr.12166]
43 **Morales-Ibanez O**, Domínguez M, Ki SH, Marcos M, Chaves JF, Nguyen-Khac E, Houchi H, Affò S, Sancho-Bru P, Altamirano J, Michelena J, García-Pagán JC, Abraldes JG, Arroyo V, Caballería J, Laso FJ, Gao B, Bataller R. Human and experimental evidence supporting a role for osteopontin in alcoholic hepatitis. *Hepatology* 2013; **58**: 1742-1756 [PMID: 23729174 DOI: 10.1002/hep.26521]
44 **Teli MR**, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. *Hepatology* 1995; **22**: 1714-1719 [PMID: 7489979]
45 **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]
46 **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]
47 **Feldstein AE**, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, Burgart LJ, Gores GJ. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. *Hepatology* 2004; **40**: 185-194 [PMID: 15239102 DOI: 10.1002/hep.20283]
48 **Gustot T**, Lemmers A, Moreno C, Nagy N, Quertinmont E, Nicaise C, Franchimont D, Louis H, Devière J, Le Moine O. Differential liver sensitization to toll-like receptor pathways in mice with alcoholic fatty liver. *Hepatology* 2006; **43**: 989-1000

[PMID: 16628628 DOI: 10.1002/hep.21138]
49 **Fujii H**, Kawada N. Inflammation and fibrogenesis in steatohepatitis. *J Gastroenterol* 2012; **47**: 215-225 [PMID: 22310735 DOI: 10.1007/s00535-012-0527-x]
50 **Blachier M**, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; **58**: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]
51 **Crespo Yanguas S**, Cogliati B, Willebrords J, Maes M, Colle I, van den Bossche B, de Oliveira CP, Andraus W, Alves VA, Leclercq I, Vinken M. Experimental models of liver fibrosis. *Arch Toxicol* 2016; **90**: 1025-1048 [PMID: 26047667 DOI: 10.1007/s00204-015-1543-4]
52 **Khan RA**, Khan MR, Sahreen S, Ahmed M, Shah NA. Carbon tetrachloride-induced lipid peroxidation and hyperglycemia in rat: a novel study. *Toxicol Ind Health* 2015; **31**: 546-553 [PMID: 23416881 DOI: 10.1177/0748233713475503]
53 **Wallace MC**, Hamesch K, Lunova M, Kim Y, Weiskirchen R, Strnad P, Friedman SL. Standard operating procedures in experimental liver research: thioacetamide model in mice and rats. *Lab Anim* 2015; **49**: 21-29 [PMID: 25835735 DOI: 10.1177/0023677215573040]
54 **Oh SW**, Kim DH, Ha JR, Kim DY. Anti-fibrotic effects of a methylenedioxybenzene compound, CW209292 on dimethylnitrosamine-induced hepatic fibrosis in rats. *Biol Pharm Bull* 2009; **32**: 1364-1370 [PMID: 19652375]
55 **Jin N**, Deng J, Chadashvili T, Zhang Y, Guo Y, Zhang Z, Yang GY, Omary RA, Larson AC. Carbogen gas-challenge BOLD MR imaging in a rat model of diethylnitrosamine-induced liver fibrosis. *Radiology* 2010; **254**: 129-137 [PMID: 20032147 DOI: 10.1148/radiol.09090410]
56 **Jha P**, Knopf A, Koefeler H, Mueller M, Lackner C, Hoefler G, Claudel T, Trauner M. Role of adipose tissue in methionine-choline-deficient model of non-alcoholic steatohepatitis (NASH). *Biochim Biophys Acta* 2014; **1842**: 959-970 [PMID: 24594481 DOI: 10.1016/j.bbadis.2014.02.012]
57 **Ito M**, Suzuki J, Tsujioka S, Sasaki M, Gomori A, Shirakura T, Hirose H, Ito M, Ishihara A, Iwaasa H, Kanatani A. Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to high-fat diet. *Hepatol Res* 2007; **37**: 50-57 [PMID: 17300698 DOI: 10.1111/j.1872-034X.2007.00008.x]
58 **Denda A**, Kitayama W, Kishida H, Murata N, Tsutsumi M, Tsujiuchi T, Nakae D, Konishi Y. Development of hepatocellular adenomas and carcinomas associated with fibrosis in C57BL/6J male mice given a choline-deficient, L-amino acid-defined diet. *Jpn J Cancer Res* 2002; **93**: 125-132 [PMID: 11856475]
59 **Park KC**, Park JH, Jeon JY, Kim SY, Kim JM, Lim CY, Lee TH, Kim HK, Lee HG, Kim SM, Kwon HJ, Suh JS, Kim SW, Choi SH. A new histone deacetylase inhibitor improves liver fibrosis in BDL rats through suppression of hepatic stellate cells. *Br J Pharmacol* 2014; **171**: 4820-4830 [PMID: 24467283 DOI: 10.1111/bph.12590]
60 **Popov Y**, Patsenker E, Fickert P, Trauner M, Schuppan D. Mdr2 (Abcb4)-/- mice spontaneously develop severe biliary fibrosis via massive dysregulation of pro- and antifibrogenic genes. *J Hepatol* 2005; **43**: 1045-1054 [PMID: 16223543 DOI: 10.1016/j.jhep.2005.06.025]
61 **Arsov T**, Larter CZ, Nolan CJ, Petrovsky N, Goodnow CC, Teoh NC, Yeh MM, Farrell GC. Adaptive failure to high-fat diet characterizes steatohepatitis in Alms1 mutant mice. *Biochem Biophys Res Commun* 2006; **342**: 1152-1159 [PMID: 16516152 DOI: 10.1016/j.bbrc.2006.02.032]
62 **Sitia G**, Aiolfi R, Di Lucia P, Mainetti M, Fiocchi A, Mingozzi F, Esposito A, Ruggeri ZM, Chisari FV, Iannacone M, Guidotti LG. Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. *Proc Natl Acad Sci USA* 2012; **109**: E2165-E2172 [PMID: 22753481 DOI: 10.1073/pnas.1209182109]
63 **Zhang Y**, Huang D, Gao W, Yan J, Zhou W, Hou X, Liu M, Ren C, Wang S, Shen J. Lack of IL-17 signaling decreases liver fibrosis in murine schistosomiasis japonica. *Int Immunol* 2015; **27**: 317-325 [PMID: 25840437 DOI: 10.1093/intimm/dxv017]
64 **Trautwein C**, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: Concept to treatment. *J Hepatol* 2015; **62**: S15-S24 [PMID: 25920084 DOI: 10.1016/j.jhep.2015.02.039]
65 **Mallat A**, Lotersztajn S. Reversion of hepatic stellate cell to a quiescent phenotype: From myth to reality? *J Hepatol* 2013; **59**: 383-386 [PMID: 23567083 DOI: 10.1016/j.jhep.2013.03.031]
66 **Mitra A**, Satelli A, Yan J, Xueqing X, Gagea M, Hunter CA, Mishra L, Li S. IL-30 (IL27p28) attenuates liver fibrosis through inducing NKG2D-rae1 interaction between NKT and activated hepatic stellate cells in mice. *Hepatology* 2014; **60**: 2027-2039 [PMID: 25351459 DOI: 10.1002/hep.27392]
67 **Kim-Kaneyama JR**, Lei XF, Arita S, Miyauchi A, Miyazaki T, Miyazaki A. Hydrogen peroxide-inducible clone 5 (Hic-5) as a potential therapeutic target for vascular and other disorders. *J Atheroscler Thromb* 2012; **19**: 601-607 [PMID: 22472216]
68 **Wu JR**, Hu CT, You RI, Pan SM, Cheng CC, Lee MC, Wu CC, Chang YJ, Lin SC, Chen CS, Lin TY, Wu WS. Hydrogen peroxide inducible clone-5 mediates reactive oxygen species signaling for hepatocellular carcinoma progression. *Oncotarget* 2015; **6**: 32526-32544 [PMID: 26416447 DOI: 10.18632/oncotarget.5322]
69 **Lei XF**, Fu W, Kim-Kaneyama JR, Omoto T, Miyazaki T, Li B, Miyazaki A. Hic-5 deficiency attenuates the activation of hepatic stellate cells and liver fibrosis through upregulation of Smad7 in mice. *J Hepatol* 2016; **64**: 110-117 [PMID: 26334580 DOI: 10.1016/j.jhep.2015.08.026]
70 **Billheimer JT**, Cromley DA, Kempner ES. The functional size of acyl-coenzyme A (CoA): cholesterol acyltransferase and acyl-CoA hydrolase as determined by radiation inactivation. *J Biol Chem* 1990; **265**: 8632-8635 [PMID: 1971274]
71 **Tomita K**, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, Usui S, Furuhashi H, Kimura A, Nishiyama K, Maejima T, Okada Y, Kurihara C, Shimamura K, Ebinuma H, Saito H, Yokoyama H, Watanabe C, Komoto S, Nagao S, Sugiyama K, Aosasa S, Hatsuse K, Yamamoto J, Hibi T, Miura S, Hokari R, Kanai T. Acyl-CoA: cholesterol acyltransferase 1 mediates liver fibrosis by regulating free cholesterol accumulation in hepatic stellate cells. *J Hepatol* 2014; **61**: 98-106 [PMID: 24657401 DOI: 10.1016/j.jhep.2014.03.018]
72 **Xue Y**, Michalopoulos G. Tregs: A Therapeutic Target for the Treatment of Portal Fibrosis? *Dig Dis Sci* 2015; **60**: 1878-1880 [PMID: 25972149 DOI: 10.1007/s10620-015-3687-8]
73 **Glässner A**, Eisenhardt M, Kokordelis P, Krämer B, Wolter F, Nischalke HD, Boesecke C, Sauerbruch T, Rockstroh JK, Spengler U, Nattermann J. Impaired $CD4^+$ T cell stimulation of NK cell anti-fibrotic activity may contribute to accelerated liver fibrosis progression in HIV/HCV patients. *J Hepatol* 2013; **59**: 427-433 [PMID: 23665286 DOI: 10.1016/j.jhep.2013.04.029]
74 **Pradere JP**, Kluwe J, De Minicis S, Jiao JJ, Gwak GY, Dapito DH, Jang MK, Guenther ND, Mederacke I, Friedman R, Dragomir AC, Aloman C, Schwabe RF. Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice. *Hepatology* 2013; **58**: 1461-1473 [PMID: 23553591 DOI: 10.1002/hep.26429]
75 **Barnes MA**, McMullen MR, Roychowdhury S, Madhun NZ, Niese K, Olman MA, Stavitsky AB, Bucala R, Nagy LE. Macrophage migration inhibitory factor is required for recruitment of scar-associated macrophages during liver fibrosis. *J Leukoc Biol* 2015; **97**: 161-169 [PMID: 25398607 DOI: 10.1189/jlb.3A0614-280R]
76 **Ehling J**, Bartneck M, Wei X, Gremse F, Fech V, Möckel D, Baeck C, Hittatiya K, Eulberg D, Luedde T, Kiessling F, Trautwein C, Lammers T, Tacke F. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. *Gut* 2014; **63**: 1960-1971 [PMID: 24561613 DOI: 10.1136/gutjnl-2013-306294]
77 **Tsai WB**, Long Y, Kuo MT. Gas6/Axl in arginine-starvation therapy. *Oncoscience* 2015; **2**: 659-660 [PMID: 26425646 DOI: 10.18632/oncoscience.218]
78 **Bárcena C**, Stefanovic M, Tutusaus A, Joannas L, Menéndez A, García-Ruiz C, Sancho-Bru P, Marí M, Caballeria J, Rothlin CV, Fernández-Checa JC, de Frutos PG, Morales A. Gas6/Axl pathway

is activated in chronic liver disease and its targeting reduces fibrosis via hepatic stellate cell inactivation. *J Hepatol* 2015; **63**: 670-678 [PMID: 25908269 DOI: 10.1016/j.jhep.2015.04.013]
79 **Yan C**, Wang L, Li B, Zhang BB, Zhang B, Wang YH, Li XY, Chen JX, Tang RX, Zheng KY. The expression dynamics of transforming growth factor-β/Smad signaling in the liver fibrosis experimentally caused by Clonorchis sinensis. *Parasit Vectors* 2015; **8**: 70 [PMID: 25649869 DOI: 10.1186/s13071-015-0675-y]
80 **Calabrese F**, Valente M, Giacometti C, Pettenazzo E, Benvegnu L, Alberti A, Gatta A, Pontisso P. Parenchymal transforming growth factor beta-1: its type II receptor and Smad signaling pathway correlate with inflammation and fibrosis in chronic liver disease of viral etiology. *J Gastroenterol Hepatol* 2003; **18**: 1302-1308 [PMID: 14535988]
81 **Qu Y**, Zong L, Xu M, Dong Y, Lu L. Effects of 18α-glycyrrhizin on TGF-β1/Smad signaling pathway in rats with carbon tetrachloride-induced liver fibrosis. *Int J Clin Exp Pathol* 2015; **8**: 1292-1301 [PMID: 25973013]
82 **Lee JH**, Jang EJ, Seo HL, Ku SK, Lee JR, Shin SS, Park SD, Kim SC, Kim YW. Sauchinone attenuates liver fibrosis and hepatic stellate cell activation through TGF-β/Smad signaling pathway. *Chem Biol Interact* 2014; **224**: 58-67 [PMID: 25451574 DOI: 10.1016/j.cbi.2014.10.005]
83 **Luo L**, Li N, Lv N, Huang D. SMAD7: a timer of tumor progression targeting TGF-β signaling. *Tumour Biol* 2014; **35**: 8379-8385 [PMID: 24935472 DOI: 10.1007/s13277-014-2203-7]
84 **Wang ZH**, Zhang QS, Duan YL, Zhang JL, Li GF, Zheng DL. TGF-β induced miR-132 enhances the activation of TGF-β signaling through inhibiting SMAD7 expression in glioma cells. *Biochem Biophys Res Commun* 2015; **463**: 187-192 [PMID: 25983322 DOI: 10.1016/j.bbrc.2015.05.001]
85 **Yu F**, Lu Z, Huang K, Wang X, Xu Z, Chen B, Dong P, Zheng J. MicroRNA-17-5p-activated Wnt/β-catenin pathway contributes to the progression of liver fibrosis. *Oncotarget* 2016; **7**: 81-93 [PMID: 26637809 DOI: 10.18632/oncotarget.6447]
86 **Ma ZG**, Lv XD, Zhan LL, Chen L, Zou QY, Xiang JQ, Qin JL, Zhang WW, Zeng ZJ, Jin H, Jiang HX, Lv XP. Human urokinase-type plasminogen activator gene-modified bone marrow-derived mesenchymal stem cells attenuate liver fibrosis in rats by down-regulating the Wnt signaling pathway. *World J Gastroenterol* 2016; **22**: 2092-2103 [PMID: 26877613 DOI: 10.3748/wjg.v22.i6.2092]
87 **Miao CG**, Yang YY, He X, Huang C, Huang Y, Zhang L, Lv XW, Jin Y, Li J. Wnt signaling in liver fibrosis: progress, challenges and potential directions. *Biochimie* 2013; **95**: 2326-2335 [PMID: 24036368 DOI: 10.1016/j.biochi.2013.09.003]
88 **Li W**, Zhu C, Li Y, Wu Q, Gao R. Mest attenuates CCl4-induced liver fibrosis in rats by inhibiting the Wnt/β-catenin signaling pathway. *Gut Liver* 2014; **8**: 282-291 [PMID: 24827625 DOI: 10.5009/gnl.2014.8.3.282]
89 **Zhang DW**, Zhao YX, Wei D, Li YL, Zhang Y, Wu J, Xu J, Chen C, Tang H, Zhang W, Gong L, Han Y, Chen ZN, Bian H. HAb18G/CD147 promotes activation of hepatic stellate cells and is a target for antibody therapy of liver fibrosis. *J Hepatol* 2012; **57**: 1283-1291 [PMID: 22878468 DOI: 10.1016/j.jhep.2012.07.042]
90 **Murakami Y**, Toyoda H, Tanaka M, Kuroda M, Harada Y, Matsuda F, Tajima A, Kosaka N, Ochiya T, Shimotohno K. The progression of liver fibrosis is related with overexpression of the miR-199 and 200 families. *PLoS One* 2011; **6**: e16081 [PMID: 21283674 DOI: 10.1371/journal.pone.0016081]
91 **He Y**, Huang C, Lin X, Li J. MicroRNA-29 family, a crucial therapeutic target for fibrosis diseases. *Biochimie* 2013; **95**: 1355-1359 [PMID: 23542596 DOI: 10.1016/j.biochi.2013.03.010]
92 **Roderburg C**, Luedde M, Vargas Cardenas D, Vucur M, Mollnow T, Zimmermann HW, Koch A, Hellerbrand C, Weiskirchen R, Frey N, Tacke F, Trautwein C, Luedde T. miR-133a mediates TGF-β-dependent derepression of collagen synthesis in hepatic stellate cells during liver fibrosis. *J Hepatol* 2013; **58**: 736-742 [PMID: 23183523 DOI: 10.1016/j.jhep.2012.11.022]
93 **Halász T**, Horváth G, Pár G, Werling K, Kiss A, Schaff Z, Lendvai G. miR-122 negatively correlates with liver fibrosis as detected by histology and FibroScan. *World J Gastroenterol* 2015; **21**: 7814-7823 [PMID: 26167081 DOI: 10.3748/wjg.v21.i25.7814]
94 **Shen WJ**, Dong R, Chen G, Zheng S. microRNA-222 modulates liver fibrosis in a murine model of biliary atresia. *Biochem Biophys Res Commun* 2014; **446**: 155-159 [PMID: 24569080 DOI: 10.1016/j.bbrc.2014.02.065]
95 **Guo CJ**, Pan Q, Li DG, Sun H, Liu BW. miR-15b and miR-16 are implicated in activation of the rat hepatic stellate cell: An essential role for apoptosis. *J Hepatol* 2009; **50**: 766-778 [PMID: 19232449 DOI: 10.1016/j.jhep.2008.11.025]
96 **Wang X**, Ikejima K, Kon K, Arai K, Aoyama T, Okumura K, Abe W, Sato N, Watanabe S. Ursolic acid ameliorates hepatic fibrosis in the rat by specific induction of apoptosis in hepatic stellate cells. *J Hepatol* 2011; **55**: 379-387 [PMID: 21168456 DOI: 10.1016/j.jhep.2010.10.040]
97 **Das S**, Das DK. Anti-inflammatory responses of resveratrol. *Inflamm Allergy Drug Targets* 2007; **6**: 168-173 [PMID: 17897053]
98 **Schmatz R**, Perreira LB, Stefanello N, Mazzanti C, Spanevello R, Gutierres J, Bagatini M, Martins CC, Abdalla FH, Daci da Silva Serres J, Zanini D, Vieira JM, Cardoso AM, Schetinger MR, Morsch VM. Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid dehydratase in liver and kidney of streptozotocin-induced diabetic rats. *Biochimie* 2012; **94**: 374-383 [PMID: 21864646 DOI: 10.1016/j.biochi.2011.08.005]
99 **Di Pascoli M**, Diví M, Rodríguez-Vilarrupla A, Rosado E, Gracia-Sancho J, Vilaseca M, Bosch J, García-Pagán JC. Resveratrol improves intrahepatic endothelial dysfunction and reduces hepatic fibrosis and portal pressure in cirrhotic rats. *J Hepatol* 2013; **58**: 904-910 [PMID: 23262250 DOI: 10.1016/j.jhep.2012.12.012]
100 **Howitz KT**, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature* 2003; **425**: 191-196 [PMID: 12939617 DOI: 10.1038/nature01960]
101 **Lagouge M**, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 2006; **127**: 1109-1122 [PMID: 17112576 DOI: 10.1016/j.cell.2006.11.013]
102 **Um JH**, Park SJ, Kang H, Yang S, Foretz M, McBurney MW, Kim MK, Viollet B, Chung JH. AMP-activated protein kinase-deficient mice are resistant to the metabolic effects of resveratrol. *Diabetes* 2010; **59**: 554-563 [PMID: 19934007 DOI: 10.2337/db09-0482]
103 **Haskó G**, Pacher P. Endothelial Nrf2 activation: a new target for resveratrol? *Am J Physiol Heart Circ Physiol* 2010; **299**: H10-H12 [PMID: 20472762 DOI: 10.1152/ajpheart.00436.2010]
104 **Amat R**, Planavila A, Chen SL, Iglesias R, Giralt M, Villarroya F. SIRT1 controls the transcription of the peroxisome proliferator-activated receptor-gamma Co-activator-1alpha (PGC-1alpha) gene in skeletal muscle through the PGC-1alpha autoregulatory loop and interaction with MyoD. *J Biol Chem* 2009; **284**: 21872-21880 [PMID: 19553684 DOI: 10.1074/jbc.M109.022749]
105 **Baur JA**, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006; **444**: 337-342 [PMID: 17086191 DOI: 10.1038/nature05354]
106 **Ahn J**, Cho I, Kim S, Kwon D, Ha T. Dietary resveratrol alters lipid metabolism-related gene expression of mice on an atherogenic diet. *J Hepatol* 2008; **49**: 1019-1028 [PMID: 18930334 DOI: 10.1016/j.jhep.2008.08.012]
107 **Ajmo JM**, Liang X, Rogers CQ, Pennock B, You M. Resveratrol alleviates alcoholic fatty liver in mice. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G833-G842 [PMID: 18755807 DOI: 10.1152/ajpgi.90358.2008]
108 **Cho SJ**, Jung UJ, Choi MS. Differential effects of low-dose

resveratrol on adiposity and hepatic steatosis in diet-induced obese mice. *Br J Nutr* 2012; **108**: 2166-2175 [PMID: 22414733 DOI: 10.1017/S0007114512000347]

109 **Tian XP**, Yin YY, Li X. Effects and mechanisms of Acremoniumterricola milleretal mycelium on liver fibrosis induced by carbon tetrachloride in rats. *Am J Chin Med* 2011; **39**: 537-550 [PMID: 21598420 DOI: 10.1142/S0192415X11009019]

110 **Zhang C**, Yuan W, Fang J, Wang W, He P, Lei J, Wang C. Efficacy of Resveratrol Supplementation against Non-Alcoholic Fatty Liver Disease: A Meta-Analysis of Placebo-Controlled Clinical Trials. *PLoS One* 2016; **11**: e0161792 [PMID: 27560482 DOI: 10.1371/journal.pone.0161792]

P- Reviewer: Chow WK **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Zhang FF

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.v22.i48.10523

World J Gastroenterol 2016 December 28; 22(48): 10523-10531
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Gastric adenocarcinoma of the fundic gland (chief cell-predominant type): A review of endoscopic and clinicopathological features

Masaki Miyazawa, Mitsuru Matsuda, Masaaki Yano, Yasumasa Hara, Fumitaka Arihara, Yosuke Horita, Koichiro Matsuda, Akito Sakai, Yatsugi Noda

Masaki Miyazawa, Mitsuru Matsuda, Masaaki Yano, Yasumasa Hara, Fumitaka Arihara, Yosuke Horita, Koichiro Matsuda, Akito Sakai, Yatsugi Noda, Department of Internal Medicine, Toyama Prefectural Central Hospital, Toyama 930-8550, Japan

Author contributions: Miyazawa M designed the study and wrote the manuscript; Matsuda M, Yano M, Hara Y, Arihara F, Horita Y, Matsuda K, Sakai A and Noda Y were involved in editing the manuscript.

Conflict-of-interest statement: Authors declare no Conflict of Interests for this article.

Manuscript source: Invited manuscript

Correspondence to: Masaki Miyazawa, MD, Department of Internal Medicine, Toyama Prefectural Central Hospital, 2-2-78 Nishi-Nagae, Toyama, Toyama 930-8550, Japan. miyacchi_1985@yahoo.co.jp
Telephone: +81-76-4241531
Fax: +81-76-4220667

Received: July 27, 2016
Peer-review started: August 6, 2016
First decision: October 10, 2016
Revised: October 27, 2016
Accepted: November 23, 2016
Article in press: November 28, 2016
Published online: December 28, 2016

Abstract

Gastric adenocarcinoma of the fundic gland (chief cell-predominant type, GA-FG-CCP) is a rare variant of well-differentiated adenocarcinoma, and has been proposed to be a novel disease entity. GA-FG-CCP originates from the gastric mucosa of the fundic gland region without chronic gastritis or intestinal metaplasia. The majority of GA-FG-CCPs exhibit either a submucosal tumor-like superficial elevated shape or a flat shape on macroscopic examination. Narrow-band imaging with endoscopic magnification may reveal a regular or an irregular microvascular pattern, depending on the degree of tumor exposure to the mucosal surface. Pathological analysis of GA-FG-CCPs is characterized by a high frequency of submucosal invasion, rare occurrences of lymphatic and venous invasion, and low-grade malignancy. Detection of diffuse positivity for pepsinogen-I by immunohistochemistry is specific for GA-FG-CCP. Careful endoscopic examination and detailed pathological evaluation are essential for early and accurate diagnosis of GA-FG-CCP. Nearly all GA-FG-CCPs are treated by endoscopic resection due to their small tumor size and low risk of recurrence or metastasis.

Key words: Narrow-band imaging; Pepsinogen-I; Fundic gland; Gastric adenocarcinoma; Chief cell

Core tip: Gastric adenocarcinoma of the fundic gland (chief cell-predominant type, GA-FG-CCP) was recently proposed as a novel disease entity. The endoscopic and clinicopathological characteristics of GA-FG-CCP are distinct from those of gastric adenocarcinomas with an intestinal phenotype that originate from the mucosa

with chronic gastritis or intestinal metaplasia. Careful endoscopic examination and detailed pathological evaluation are essential for early and accurate diagnosis of GA-FG-CCP.

Miyazawa M, Matsuda M, Yano M, Hara Y, Arihara F, Horita Y, Matsuda K, Sakai A, Noda Y. Gastric adenocarcinoma of the fundic gland (chief cell-predominant type): A review of endoscopic and clinicopathological features. *World J Gastroenterol* 2016; 22(48): 10523-10531 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10523.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10523

INTRODUCTION

It had been thought that differentiated adenocarcinomas which originate from the intestinal metaplasia involving *Helicobactor pylori* (*H. pylori*) infection have an intestinal phenotype, whereas undifferentiated adenocarcinomas which originate from the gastric mucosa without the process of intestinal metaplasia have a gastric phenotype[1]. However, progress in immunohistochemistry has revealed that some differentiated adenocarcinomas have a gastric phenotype[2].

In 2006, Yao *et al*[3] described extremely well-differentiated gastric adenocarcinoma with a gastric phenotype similar to gastric foveolar epithelium, mucous neck cells and pyloric glands. Thereafter, the first case of gastric adenocarcinoma with differentiation into chief cells within the fundic gland by Tsukamoto *et al*[4]. Furthermore, 10 cases of gastric adenocarcinoma with differentiation into chief cells were documented by Ueyama *et al*[5] who proposed a novel disease concept: gastric adenocarcinoma of the fundic gland (chief cell-predominant type, GA-FG-CCP) based on the characteristic morphology and immunohistochemistry. Because GA-FG-CCPs are thought to originate from gastric mucosa of the fundic gland region without chronic gastritis or intestinal metaplasia, it is expected that the proportion of gastric adenocarcinomas diagnosed as GA-FG-CCP will increase in parallel with the declining frequency of *H. pylori* infection.

GA-FG-CCP is defined as a neoplastic lesion, which is composed of cells that resemble the fundic gland cells and is positive for pepsinogen-I: a marker of chief cells on immunohistochemistry[5]. For early and precise diagnosis, it is vital to undertake careful endoscopic examination and detailed pathological evaluation using immunohistochemistry. In particular, endoscopists should be able to reliably detect a suspicious lesion that may be a GA-FG-CCP during screening. As the concept of GA-FG-CCP as an individual disease entity has become better established, information concerning the pathological features of GA-FG-CCP has occasionally been published. Moreover, several detailed endoscopic investigations of GA-FG-CCP have recently become available. Here, the unique endoscopic and clinicopathological features of GA-FG-CCP will be discussed.

CLINICAL CHARACTERISTICS

GA-FG-CCP is a very rare variant of gastric adenocarcinoma. A review of studies on GA-FG-CCP published in English language indicates that 46 cases have been reported to date[4-16]. Almost all patients were Japanese, but a study by Singhi *et al*[9] in 2012 comprised 10 non-Asian patients, including Hispanics, Caucasians and African-Americans. The clinical characteristics of the 46 previously reported cases are presented in Table 1. According to Ueyama *et al*[12], GA-FG-CCP was detected in only 10 of 14080 patients who underwent esophagogastroduodenoscopy (EGD). Park *et al*[8] identified GA-FG-CCP in only 3 of over 6000 Korean patients with gastric adenocarcinomas resected by endoscopy or surgery. During the 3 years after 2010 when the concept of GA-FG-CCP was first proposed, Miyazawa *et al*[13] reported that GA-FG-CCP accounted for 0.98% of early gastric adenocarcinomas treated by endoscopic submucosal dissection (ESD). Among Japanese cases of GA-FG-CCP, the male-to-female ratio is approximately 1.4, which is lower than that of the estimated morbidity from all gastric adenocarcinomas in 2011 in Japan (2.1)[17]. The average age of Japanese patients is 67.7 years, with a range of 42 to 82 years. Early cases of GA-FG-CCP generally have no or mild subjective symptoms, although Singhi *et al*[9] found that all 10 cases had clinical symptoms of gastroesophageal reflux, suggesting that gastric acid secretion was maintained due to a lack of mucosal atrophy. To the best of the authors' knowledge, none of the previous cases of GA-FG-CCP had evidence of *H. pylori* infection.

ENDOSCOPIC FINDINGS

Endoscopic findings from patients with GA-FG-CCP are shown in Figure 1. The endoscopic characteristics of 46 previously reported cases are summarized in Table 2. GA-FG-CCP was located in the upper third of the stomach in 87% of cases. The average tumor diameter was 7.5 mm, and approximately 80% of all tumors were less than 10 mm in diameter at the time of diagnosis. Macroscopically, about three-quarters of GA-FG-CCPs were recognized by an elevated shape, especially a submucosal tumor (SMT)-like shape, while the other one-quarter had a flat or depressed shape. GA-FG-CCP with an SMT-like elevated shape has a poorly demarcated border and softness[12]. Miyazawa *et al*[13] suggested that a possible reason for the macroscopic similarity to SMT is that GA-FG-CCP originates from deep layers of the gastric mucosa. GA-FG-CCP may be likely to grow vertically into the submucosa and develop laterally toward the surrounding tissue. If GA-FG-CCP grew in a straight direction towards the mucosal surface, it would be recognized as a superficial unsmooth tumor. Ueyama *et al*[12] speculated that surface mucosal epithelial cells are maintained because

Table 1 Clinical characteristics of previously reported cases

Author (yr)	Number of patients	Sex (M:F)	Age (yr, average)	Race or nationality	Clinical symptoms	Therapeutic method	Survival time (mo, average)	Outcome
Tsukamoto *et al*[4] (2007)	1	F	82	Japanese	No symptom	EMR	ND	ND
Ueyama *et al*[5] (2010)	10	6:4	65.5 (42-79)	Japanese: 10	ND	EMR: 2 ESD: 5 Operation: 3	37.1 (10-70)	Alive, NED: 10
Fukatsu *et al*[6] (2011)	1	M	56	Japanese	No symptom	EAM	12	Alive, NED
Terada *et al*[7] (2011)	1	M	78	Japanese	Abdominal pain (colon cancer)	No therapy (only biopsy)	3	Died (colon cancer)
Park *et al*[8] (2012)	3	3:0	65.3 (47-76)	Korean: 3	ND	ESD: 1 Operation: 1 Operation after ESD: 1	24.3 (11-32)	Alive, NED: 3
Singhi *et al*[9] (2012)	10	4:6	64.2 (44-79)	Hispanic: 4 Caucasian: 2 African American: 2 Chinese: 1 Unknown: 1	GERD: 10	polypectomy: 10	15.4 (6-39)	Alive, NED: 8 Alive, persistence: 1 ND: 1
Chen *et al*[10] (2012)	1	M	79	Caucasian	GERD Esophageal stricture	EMR	2	Alive, NED
Abe *et al*[11] (2013)	1	F	71	Japanese	No symptom	EMR	12	Alive, NED
Ueyama *et al*[12] (2014)	10	6:4	66.5 (55-78)	Japanese: 10	ND	EMR: 2 ESD: 8	13.2 (1-19)	Alive, NED: 10
Miyazawa *et al*[13] (2015)	5	3:2	72.2 (67-78)	Japanese: 5	No symptom: 5	ESD: 4 Operation after ESD: 1	19.4 (10-28)	Alive, NED: 5
Parikh *et al*[14] (2015)	1	M	66	Caucasian	Heartburn (GERD)	EMR	ND	Alive, NED
Kato *et al*[15] (2015)	1	M	80s	Japanese	ND	CLEAN-NET	3	Alive, NED
Fujii *et al*[16] (2015)	1	F	64	Japanese	ND	ESD	ND	ND

CLEAN-NET: Combination of laparoscopic and endoscopic approaches to neoplasia with non-exposure technique; EAM: Endoscopic aspiration mucosectomy; EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection; F: Female; M: Male; GERD: Gastroesophageal reflux disease; NED: No evidence of disease; ND: Not described.

tumors barely destroy the surrounding tissue. GA-FG-CCP with a flat or depressed shape, which accounts for about 20% of all cases, appears to be more difficult to recognize than GA-FG-CCP with an SMT-like elevated shape. With respect to coloration, GA-FG-CCP is often covered with normal-colored or faded (*i.e.*, whitish or yellowish) mucosa. Fujii *et al*[16] suggested that the faded appearance is caused by atrophy in the foveolar epithelium above cancer tubules, but not in the surrounding mucosa. Another characteristic of GA-FG-CCP is vasodilation on the tumor surface, which is attributed to the displacement of surface vessels by tumor tissue followed by congestion[13]. Branched vessels on the tumor surface were also thought to be present in the deep region of the mucosal layer[12]. Narrow-band imaging (NBI) may clarify the presence of vasodilatation and branched vessels on the tumor surface[12,13,16]. However, it has not been possible to pathologically confirm the above explanations for fine vessels.

There are few reports on magnifying endoscopy for GA-FG-CCP[13,16]. NBI with magnification is assessed using the worldwide standard diagnostic system, the VS (vessel plus surface) classification, as proposed by Yao *et al*[18]. The VS classification system incorporates three indices: (1) the demarcation line (DL), which is the border between the tumor and the surrounding mucosa; (2) the microsurface pattern (MSP), which is microstructural patterns on the tumor surface such as marginal crypt epithelium; and (3) the microvascular pattern (MVP), which is the pattern of vascular architecture on the tumor surface such as subepithelial capillary. Miyazawa *et al*[13] demonstrated that NBI with magnification enabled detection of the absence of MSPs, indicating a lack of microstructure, and irregular MVPs, indicating non-uniformity and heterogeneity of microvascular structures, depending on the degree of tumor exposure to the mucosal surface. In contrast, NBI with magnification indicated that some GA-FG-CCPs had normal MSP and MVP. The presence of the DL, and irregular MVP and MSP, which are specific features of early gastric adenocarcinoma, may not be found in GA-FG-CCP[16]. Because GA-FG-CCP is located in the deep mucosal layer and is not exposed on the surface, detection of abnormal inner microstructures is prevented by the thick mucosa[13]. Consequently, evaluation by magnifying endoscopy may be used to assist, but not to make an accurate diagnosis of GA-FG-CCP[16].

Despite the use of endoscopy, a definite diagnosis is provided by histopathology. If GA-FG-CCP is suspected by an endoscopist, a pathologist should perform immu-

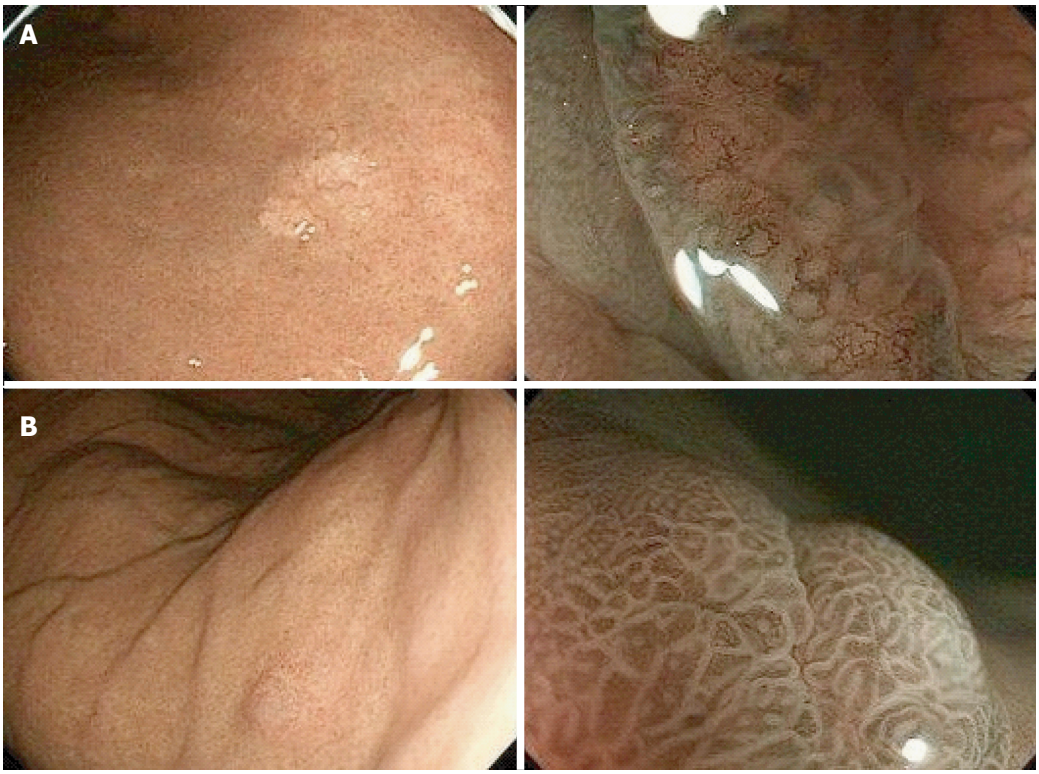

Figure 1 Endoscopic findings from representative GA-FG-CCP cases. A: White light endoscopy revealed a submucosal tumor-like elevated tumor with a whitish mucosal surface and dilatation of microvessels. The surrounding mucosa had no atrophic changes (left). Narrow-band imaging with magnification showed an absent microsurface pattern and irregular microvascular pattern on a small portion of the tumor (right); B: White light endoscopy revealed a submucosal tumor-like elevated tumor with normal-colored mucosal surface. The surrounding mucosa had no atrophic changes (left). Narrow-band imaging with magnification showed a regular microsurface pattern and microvascular pattern on the entire tumor surface (right).

Table 2 Endoscopic characteristics of previously reported cases

Author (yr)	Number of patients	Location (U:M:L)	Size (mm, average)	Macroscopic shape	Color tone	Vessel findings	NBI with magnification	
							MSP	MVP
Tsukamoto *et al*[4] (2007)	1	U	16	Elevated	Normal	Vasodilation	ND	ND
Ueyama *et al*[5] (2010)	10	10:0:0	8.6 (4-20)	Elevated: 5 Depressed: 5	ND	ND	ND	ND
Fukatsu *et al*[6] (2011)	1	U	5	Elevated	Yellowish	ND	ND	ND
Terada *et al*[7] (2011)	1	U	20	Elevated	Reddish	Vasodilation	ND	ND
Park *et al*[8] (2012)	3	1:1:1	2.6 (1.2-3.6)	Elevated and depressed: 3	ND	ND	ND	ND
Singhi *et al*[9] (2012)	10	10:0:0	4.3 (2-8)	Elevated: 10	ND	ND	ND	ND
Chen *et al*[10] (2012)	1	U	12	Elevated	Whitish	Vasodilation	ND	ND
Abe *et al*[11] (2013)	1	U	ND	Elevated	Yellowish	ND	ND	ND
Ueyama *et al*[12] (2014)	10	6:4:0	9.3 (3-31)	Elevated: 6 Depressed: 3	Whitish: 8 Reddish: 2	Vasodilation: 5 Normal: 5	ND	ND
Miyazawa *et al*[13] (2015)	5	5:0:0	7.8 (5-13)	Elevated: 4 Flat: 1	Whitish: 3 Normal: 2	Vasodilation: 5	Regular: 3 Absent: 2	Regular: 3 Irregular: 2
Parikh *et al*[14] (2015)	1	U	7	Elevated	Reddish	Vasodilation	ND	ND
Kato *et al*[15] (2015)	1	U	15	Elevated	Whitish	Vasodilation	Regular	Regular
Fujii *et al*[16] (2015)	1	U	< 10	Depressed	Whitish	Vasodilation	Absent	Irregular

U: Upper third; M: Middle third; L: Lower third; NBI: Narrow-band imaging; MSP: Microsurface pattern; MVP: Microvascular pattern; ND: Not described.

nohistochemical staining to confirm the diagnosis[13]. The biopsy may be needed for a lesion suggesting a submucosal or carcinoid tumor but resembling GA-FG-CCP in order to distinguish GA-FG-CCP from these tumors. It is crucial to obtain an adequate amount of tissue because the tumorous tissue of GA-FG-CCP is usually located in the deep mucosal layer.

HISTOPATHOLOGICAL FINDINGS

Histopathological findings from GA-FG-CCP cases are depicted in Figures 2 and 3. The histopathological characteristics of the 45 previously reported cases are shown in Table 3. GA-FG-CCP is a well-differentiated tubular adenocarcinoma composed of a variety of

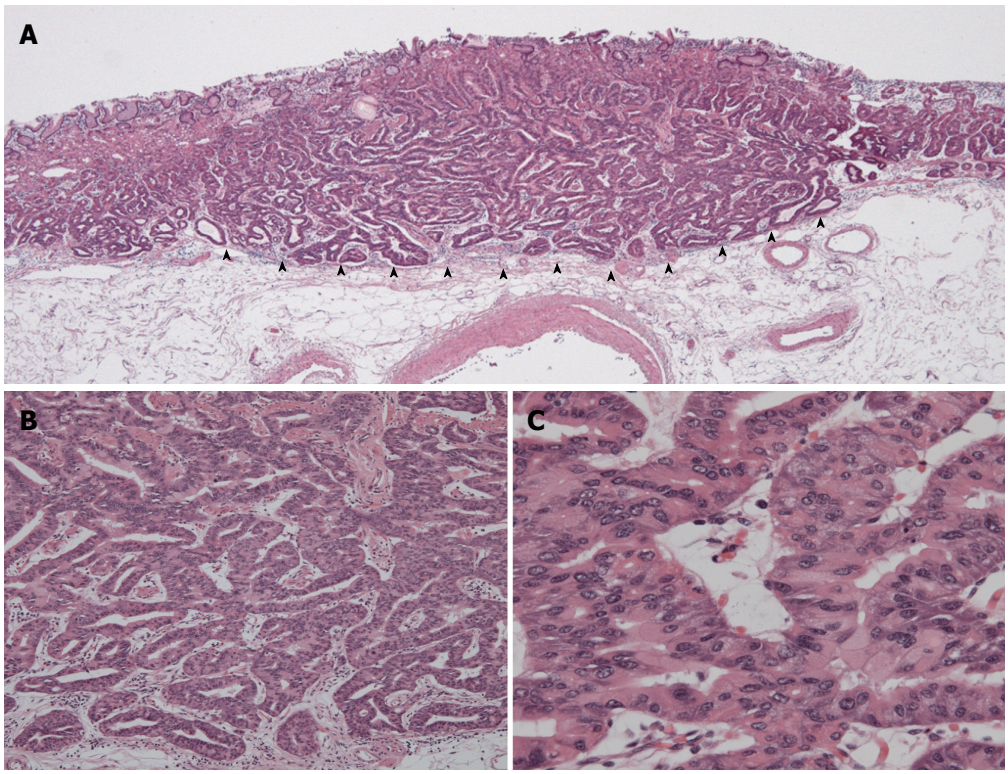

Figure 2 Histopathological findings from a representative GA-FG-CCP case. A: In low-power view, the tumor arose from the deep layer of the lamina propria mucosa and invaded the submucosal layer (arrowhead). Most of the surface was covered with non-atypical foveolar epithelium; B and C: In high-power view, the tumor was composed of well-differentiated columnar cells mimicking the fundic gland cells with mild nuclear atypia.

mildly atypical columnar cells that mimic the fundic glands[5]. The tumor originates from chief cells, which lies at the bottom of the fundic glands. A surface of tumor is primarily covered by non-atypical foveolar epithelium. Although the majority of tumors exhibit submucosal invasion, observations of lymphatic or venous invasion are rare[5,12,13]. With regard to biological behavior, GA-FG-CCPs are expected to be low-grade malignancies in view of their mild atypia. Due to the lack of recurrence or progression, Singhi *et al*[9] suggested that the term "GA-FG-CCP" is excessive, and the lesions should be considered as benign. As an alternative, the authors preferred the term "oxyntic gland polyp/adenoma" for GA-FG-CCP lesions. Although in some cases endoscopy revealed chronic atrophic gastritis or intestinal metaplasia, tumors in most cases were surrounded by the gastric mucosa without pathological evidence of mucosal changes.

Immunohistochemical analysis using the following biomarkers is important for the diagnosis of GA-FG-CCP: MUC5AC for foveolar cells; MUC6 for mucous neck cells or pyloric gland cells; MUC2 for goblet cells; CD10 for intestinal brush border cells; and pepsinogen-I for chief cells. Mucin phenotypes are assessed by the expression of gastric-type markers such as MUC5AC and MUC6, and intestinal-type markers such as MUC2 and CD10[19]. GA-FG-CCP is categorized as a purely gastric phenotype because MUC6 is strongly expressed in GA-FG-CCP which is composed of cells that mimic the fundic gland cells. In contrast, MUC5AC-postive cells, which differentiate into foveolar epithelium, are rarely detected in GA-FG-CCP[5,9,12,13]. Ueyama *et al*[5] speculated that MUC5AC is only expressed in advanced GA-FG-CCP lesion with a large diameter and massive submucosal invasion, suggesting that cell differentiation changes from the fundic gland type to the foveolar type during disease progression. All of the reported GA-FG-CCPs were negative for MUC2 staining, whereas a few cases displayed CD10 positivity. CD10 expression has been suggested to occur in GA-FG-CCP with a flat or depressed shape, which have an intestinal phenotype. A Flat or depressed lesion is thought to have different clinicopathological characteristics from GA-FG-CCP with an SMT-like elevated shape[12]. Immunohistochemical analysis for pepsinogen-I, the most specific marker of differentiation into chief cells, is indispensable for the diagnosis of GA-FG-CCP.

Regardless of its ability for submucosal invasion, GA-FG-CCP is generally considered to have a low potential for malignancy because none of the reported lesions displayed overexpression of p53 protein or a high labeling index of Ki-67[5,9,12,13].

DIFFERENTIAL DIAGNOSIS

GA-FG-CCP with an SMT-like elevated shape should be distinguished from true SMT. In particular, the possibility of diagnosis as a neuroendocrine tumor including

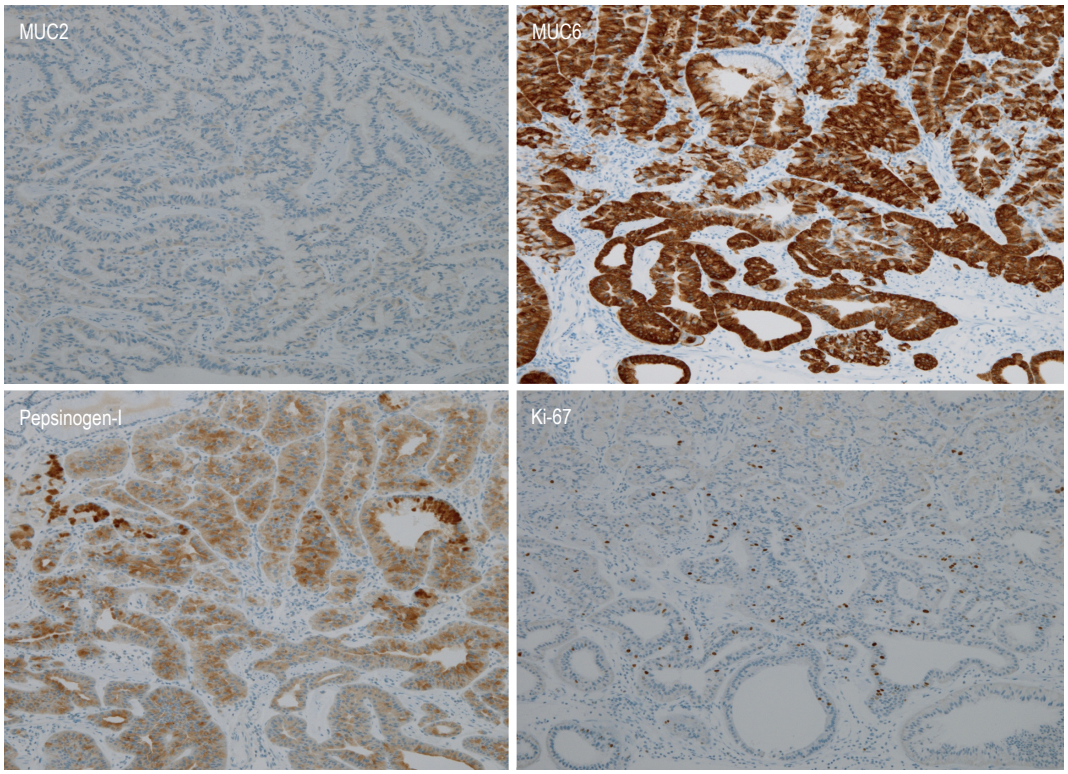

Figure 3 Immunohistochemical analysis of a representative GA-FG-CCP case. The tumor had diffuse positivity for MUC6 and pepsinogen-I, but was negative for MUC2 staining. The Ki-67 labeling index was very low.

neuroendocrine carcinoma and mixed adenoneuroendocrine carcinoma, has been emphasized. However, there are some discriminating features between the two types of lesions. Neuroendocrine tumor has a yellow color, with few vessels on the tumor surface and a solid appearance, whereas GA-FG-CCP with an SMT-like elevated shape has a faded whitish color, with vasodilation or some branched vessels on the tumor surface and a soft appearance[12]. Histological features of GA-FG-CCP resemble those of neuroendocrine tumor: both tumors are composed small round tumor cells and originate from the deep layer. Although it is a little difficult to distinguish the two tumors by conventional hematoxylin and eosin staining, immunohhistochemical examination is useful for it. Ueyama *et al*[5] said these two types of lesions can be easily distinguished by immunohistochemical staining using chromogranin A. Synaptophysin and neural cell adhesion molecule are expressed in neuroendocrine tumor, but it should be noted that these markers may also be positive in GA-FG-CCP[6,20]. Moreover, pepsinogen-I positivity is the most specific finding to characterize GA-FG-CCP.

Because fundic gland polyp (FGP) resembles normal fundic gland cells and has mild atypia, GA-FG-CCP can be misdiagnosed as FGP[5,9]. Müller-Höcker *et al*[21] and Matsukawa *et al*[22] have described FGPs with chief cell hyperplasia. These lesions, which are not consistent with usual adenocarcinoma, display mild structural and nuclear atypia and a low Ki-67 labeling index, and may be GA-FG-CCP. Histological investigations by Jalving *et al*[23] and Stolte *et al*[24] demonstrated that dysplasia in FGP tends to involve the foveolar epithelium (mucosal surface), not the fundic gland (deep mucosal layer). In a report by Garrean *et al*[25], a patient with familial adenomatous polyposis had gastric adenocarcinomas originating from FGP, but the adenocarcinomas were thought to originate from the foveolar epithelium, not the fundic gland. These lesions associated with FGP, which have a relatively clear margin from the surrounding mucosa, do not appear to have an SMT-like elevated shape on endoscopic examination.

Gastritis cystica profunda is a distinct diagnosis, characterized by dilated glands within the submucosal layer and surrounded by a lamina propria with a normal appearance[5]. On endoscopic ultrasonography, low echoic lesions are detected in the submucosal layer, which is useful to discriminate from GA-FG-CCP with an SMT-like elevated shape[26]. Histological features that help to exclude the possibility of malignancy include the absence of cytologic atypia, lack of desmoplasia, and the presence of a surrounding lamina propria.

Early gastric cancer, normal gastric mucosa with focal atrophy, and mucosa-associated lymphoid tissue lymphoma may resemble GA-FG-CCP with a flat or depressed shape[12]. Histopathological examination is necessary for accurate diagnosis because it is difficult to distinguish these lesions from GA-FG-CCP using endoscopy.

Table 3 Histopathological characteristics of previously reported cases

Author (year)	Number of patients	Background mucosa	Depth (M:SM) (μm, average)	Lymphatic invasion (+:-)	Venous invasion (+:-)	MUC2 (+:-)	MUC5AC (+:-)	MUC6 (+:-)	CD10 (+:-)	Overexpression of p53 (+:-)	Ki-67 LI (%, average)
Tsukamoto *et al*[4] (2007)	1	ND	M	(-)	(-)	ND	ND	(+)	ND	ND	7.9
Ueyama *et al*[5] (2010)	10	Normal: 7 Metaplasia: 1 ND: 2	1:9 844 (150 to 4000)	0:10	0:10	0:10	1:9	10:0	0:10	0:10	3.6
Fukatsu *et al*[6] (2011)	1	ND	SM, 100	(-)	(-)	ND	(-)	(+)	ND	ND	ND
Park *et al*[8] (2012)	3	Metaplasia: 2 Gastritis: 1	1:2	0:3	0:3	0:3	3:0	3:0	0:3	ND	ND
Singhi *et al*[9] (2012)	10	Normal: 7 Gastritis: 3	10:0	0:10	0:10	0:3	0:10	10:0	ND	0:9 ND: 1	2.6 (0.2-10)
Chen *et al*[10] (2012)	1	Normal	SM (Details unknown)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	3.8
Abe *et al*[11] (2013)	1	Normal	SM (Details unknown)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	1.9
Ueyama *et al*[12] (2014)	10	Normal: 9 Gastritis: 1	5:5 360 (100 to 1200)	1:9	0:10	0:10	4:6	10:0	2:8	0:10	8.6 (1-20)
Miyazawa *et al*[13] (2015)	5	Normal: 4 Gastritis: 1	0:5 620 (80 to 1230)	1:4	0:5	0:5	0:5	5:0	ND	0:5	Very low: 5 (Details unknown)
Parikh *et al*[14] (2015)	1	ND	SM (Details unknown)	(-)	(-)	ND	ND	ND	ND	ND	ND
Kato *et al*[15] (2015)	1	Normal	SM, 300	(-)	(-)	ND	ND	(+)	ND	ND	ND
Fujii *et al*[16] (2015)	1	Normal	SM, 300	(-)	(-)	ND	(-)	(+)	ND	ND	ND

LI: Labeling index; M: Mucosal; SM: Submucosal; ND: Not described.

TREATMENT AND PROGNOSIS

Recently, the majority of GA-FG-CCPs have been treated endoscopically. GA-FG-CCP is often considered to be an indication for endoscopic resection because of its small tumor size and benign biological behavior. However, according to the 2010 version of Japanese gastric cancer treatment guidelines (version 3), endoscopic resection of lesions with submucosal invasion deeper than 500 μm is deemed to be inadequate with respect to curative criteria[27]. Applying this guideline to reported GA-FG-CCPs, several cases did not meet curative criteria based on the depth of massive submucosal invasion. Considering the high frequency of submucosal invasion, Kato *et al*[15] suggested the efficacy of the combination of laparoscopic and endoscopic approaches to neoplasia with non-exposure technique (CLEAN-NET), a form of non-exposure laparoscopy and endoscopy cooperative surgery (LECS). CLEAN-NET may represent a therapeutic option for GA-FG-CCP because it facilitates easy resection of tumors located in the upper third of stomach, whereas ESD is technically challenging. CLEAN-NET also prevents excess wall defects and dissemination of cancer cells into the peritoneal cavity. In contrast to submucosal invasion, GA-FG-CCP rarely exhibits lymphatic and venous invasion. None of the reported cases had recurrence or metastasis, except for a case with local residual recurrence. However, due to the low number of reported GA-FG-CCP cases, the rate of lymph node metastasis and long-term survival in patients with GA-FG-CCP showing massive submucosal invasion remains unclear. Taking into account that GA-FG-CCP is a low-grade malignancy and relatively common among the elderly, a future controversy will be whether an additional therapeutic approach that is suitable for general gastric adenocarcinoma should be administered to GA-FG-CCP after resection inadequate for current curative criteria[13]. GA-FG-CCP is considered to have a favorable prognosis; however, long-term follow-up investigations are necessary. Comparative surveys and prognostic analyses of cases that undergo additional therapeutic approaches or observation without treatment after resection inadequate for current curative criteria will be required.

CONCLUSION

Although GA-FG-CCP is rare, it is expected to account for an increasing proportion of gastric adenocarcinomas. In addition to reports from Japan, data on non-Japanese cases have been recently published. GA-FG-CCP tends to have the following features: (1) it originates commonly from gastric mucosa of the fundic gland region without chronic gastritis or intestinal metaplasia; (2) it is likely to be recognized as a lesion with an SMT-like elevated shape, covered by normal-colored or faded-whitish mucosa, and vasodilatation or branched vessels on the tumor surface; (3) invasion of the submucosal layer, despite only mild histological atypia and rare lymphatic or venous invasion; (4) expression of immunohistochemical markers such as

MUC6 and pepsinogen-I; and (5) a low recurrence risk and favorable prognosis.

It is necessary for endoscopists to pay careful attention to the existence of GA-FG-CCP during routine examinations. Even in the absence of atrophic mucosa due to *H. pylori* infection, if a lesion with the above endoscopic characteristics is recognized, detailed examinations should be performed for suspected GA-FG-CCP. While a consensus on conventional white light endoscopy for diagnosis of GA-FG-CCP has recently been formed, few reports are available on advanced diagnostic endoscopy techniques such as NBI with magnification for GA-FG-CCP. Therefore, it is required to accumulate further endoscopic and histopathological data to identify the morphological characteristics of GA-FG-CCP. Furthermore, the biological characteristics of GA-FG-CCP, including its natural disease course, the rate of recurrence, and survival after resection inadequate for current curative criteria, as well as the genetic aberrations that trigger carcinogenesis remain to be elucidated.

REFERENCES

1 **Nakamura K**, Sugano H, Takagi K. Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. *Gan* 1968; **59**: 251-258 [PMID: 5726267]

2 **Kabashima A**, Yao T, Sugimachi K, Tsuneyoshi M. Gastric or intestinal phenotypic expression in the carcinomas and background mucosa of multiple early gastric carcinomas. *Histopathology* 2000; **37**: 513-522 [PMID: 11122433]

3 **Yao T**, Utsunomiya T, Oya M, Nishiyama K, Tsuneyoshi M. Extremely well-differentiated adenocarcinoma of the stomach: clinicopathological and immunohistochemical features. *World J Gastroenterol* 2006; **12**: 2510-2516 [PMID: 16688795 DOI: 10.3748/wjg.v12.i16.2510]

4 **Tsukamoto T**, Yokoi T, Maruta S, Kitamura M, Yamamoto T, Ban H, Tatematsu M. Gastric adenocarcinoma with chief cell differentiation. *Pathol Int* 2007; **57**: 517-522 [PMID: 17610477 DOI: 10.1111/j.1440-1827.2007.02134.x]

5 **Ueyama H**, Yao T, Nakashima Y, Hirakawa K, Oshiro Y, Hirahashi M, Iwashita A, Watanabe S. Gastric adenocarcinoma of fundic gland type (chief cell predominant type): proposal for a new entity of gastric adenocarcinoma. *Am J Surg Pathol* 2010; **34**: 609-619 [PMID: 20410811 DOI: 10.1097/PAS.0b013e3181d94d53]

6 **Fukatsu H**, Miyoshi H, Ishiki K, Tamura M, Yao T. Gastric adenocarcinoma of fundic gland type (chief cell predominant type) treated with endoscopic aspiration mucosectomy. *Dig Endosc* 2011; **23**: 244-246 [PMID: 21699569 DOI: 10.1111/j.1443-1661.2011.01125.x]

7 **Terada T**. Well differentiated adenocarcinoma of the stomach composed of chief cell-like cells and parietal cells (Gastric adenocarcinoma of fundic gland type). *Int J Clin Exp Pathol* 2011; **4**: 797-798 [PMID: 22135729]

8 **Park ES**, Kim YE, Park CK, Yao T, Kushima R, Kim KM. Gastric adenocarcinoma of fundic gland type: report of three cases. *Korean J Pathol* 2012; **46**: 287-291 [PMID: 23110017 DOI: 10.4132/KoreanJPathol.2012.46.3.287]

9 **Singhi AD**, Lazenby AJ, Montgomery EA. Gastric adenocarcinoma with chief cell differentiation: a proposal for reclassification as oxyntic gland polyp/adenoma. *Am J Surg Pathol* 2012; **36**: 1030-1035 [PMID: 22472957 DOI: 10.1097/PAS.0b013e31825033e7]

10 **Chen WC**, Rodriguez-Waitkus PM, Barroso A, Balsaver A, McKechnie JC. A rare case of gastric fundic gland adenocarcinoma (chief cell predominant type). *J Gastrointest Cancer* 2012; **43**: S262-S265

11 **Abe T**, Nagai T, Fukunaga J, Okawara H, Nakashima H, Syutou M, Kajimoto N, Wake R, Oyama T, Yao T. Long-term follow-up of gastric adenocarcinoma with chief cell differentiation using upper gastrointestinal tract endoscopy. *Intern Med* 2013; **52**: 1585-1588 [PMID: 23857090]

12 **Ueyama H**, Matsumoto K, Nagahara A, Hayashi T, Yao T, Watanabe S. Gastric adenocarcinoma of the fundic gland type (chief cell predominant type). *Endoscopy* 2014; **46**: 153-157 [PMID: 24338239 DOI: 10.1055/s-0033-1359042]

13 **Miyazawa M**, Matsuda M, Yano M, Hara Y, Arihara F, Horita Y, Matsuda K, Sakai A, Noda Y. Gastric adenocarcinoma of fundic gland type: Five cases treated with endoscopic resection. *World J Gastroenterol* 2015; **21**: 8208-8214 [PMID: 26185396 DOI: 10.3748/wjg.v21.i26.8208]

14 **Parikh ND**, Gibson J, Aslanian H. Gastric Fundic Gland Adenocarcinoma With Chief Cell Differentiation. *Clin Gastroenterol Hepatol* 2015; **13**: A17-A18 [PMID: 26215839 DOI: 10.1016/j.cgh.2015.07.023]

15 **Kato M**, Uraoka T, Isobe Y, Abe K, Hirata T, Takada Y, Wada M, Takatori Y, Takabayashi K, Fujiyama Y, Sekiya K, Kawaguchi Y, Sukeda A, Shiraishi J. A case of gastric adenocarcinoma of fundic gland type resected by combination of laparoscopic and endoscopic approaches to neoplasia with non-exposure technique (CLEAN-NET). *Clin J Gastroenterol* 2015; **8**: 393-399 [PMID: 26615600 DOI: 10.1007/s12328-015-0619-2]

16 **Fujii M**, Uedo N, Ishihara R, Aoi K, Matsuura N, Ito T, Yamashina T, Hanaoka N, Takeuchi Y, Higashino K, Iishi H, Tomita Y, Egashira Y. Endoscopic features of early stage gastric adenocarcinoma of fundic gland type (chief cell predominant type): a case report. *Case Rep Clin Pathol* 2015; **2**: 17 [DOI: 10.5430/crcp.v2n1p17]

17 **Matsuda A**, Matsuda T, Shibata A, Katanoda K, Sobue T, Nishimoto H. Cancer incidence and incidence rates in Japan in 2008: a study of 25 population-based cancer registries for the Monitoring of Cancer Incidence in Japan (MCIJ) project. *Jpn J Clin Oncol* 2014; **44**: 388-396 [PMID: 24503029 DOI: 10.1093/jjco/hyu003]

18 **Yao K**, Anagnostopoulos GK, Ragunath K. Magnifying endoscopy for diagnosing and delineating early gastric cancer. *Endoscopy* 2009; **41**: 462-467 [PMID: 19418401 DOI: 10.1055/s-0029-1214594]

19 **Tsukashita S**, Kushima R, Bamba M, Sugihara H, Hattori T. MUC gene expression and histogenesis of adenocarcinoma of the stomach. *Int J Cancer* 2001; **94**: 166-170 [PMID: 11668493]

20 **Yao T**, Ueyama H, Kushima R, Nakashima Y, Hirakawa K, Ohshiro Y, Hirano H, Satake T, Shimizu S, Yamane T, Hirahashi M, Iwashita A, Watanabe S. New type of gastric carcinoma-Adenocarcinoma of the fundic gland type: its clinicopathological features and tumor development. *Stomach Intest* 2010; **45**: 1203-1211

21 **Müller-Höcker J**, Rellecke P. Chief cell proliferation of the gastric mucosa mimicking early gastric cancer: an unusual variant of fundic gland polyp. *Virchows Arch* 2003; **442**: 496-500 [PMID: 12698365]

22 **Matsukawa A**, Kurano R, Takemoto T, Kagayama M, Ito T. Chief cell hyperplasia with structural and nuclear atypia: a variant of fundic gland polyp. *Pathol Res Pract* 2005; **200**: 817-821 [PMID: 15792126]

23 **Jalving M**, Koornstra JJ, Boersma-van Ek W, de Jong S, Karrenbeld A, Hollema H, de Vries EG, Kleibeuker JH. Dysplasia in fundic gland polyps is associated with nuclear beta-catenin expression and relatively high cell turnover rates. *Scand J Gastroenterol* 2003; **38**: 916-922 [PMID: 14531526]

24 **Stolte M**, Vieth M, Ebert MP. High-grade dysplasia in sporadic fundic gland polyps: clinically relevant or not? *Eur J Gastroenterol Hepatol* 2003; **15**: 1153-1156 [PMID: 14560146]

25 **Garrean S**, Hering J, Saied A, Jani J, Espat NJ. Gastric adenocarcinoma arising from fundic gland polyps in a patient with familial adenomatous polyposis syndrome. *Am Surg* 2008; **74**: 79-83

[PMID: 18274437]
26 **Machicado J**, Shroff J, Quesada A, Jelinek K, Spinn MP, Scott LD, Thosani N. Gastritis cystica profunda: Endoscopic ultrasound findings and review of the literature. *Endosc Ultrasound* 2014; **3**: 131-134 [PMID: 24955343 DOI: 10.4103/2303-9027.131041]
27 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer* 2011; **14**: 113-123 [PMID: 21573742 DOI: 10.1007/s10120-011-0042-4]

P- Reviewer: Garcia-Olmo D, Hori K **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Liu WX

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.v22.i48.10532

World J Gastroenterol 2016 December 28; 22(48): 10532-10544
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Effects of *Saccharomyces cerevisiae* or *boulardii* yeasts on acute stress induced intestinal dysmotility

Christine West, Andrew M Stanisz, Annette Wong, Wolfgang A Kunze

Christine West, Andrew M Stanisz, Annette Wong, Wolfgang A Kunze, McMaster Brain-Body Institute, St. Joseph's Healthcare, Hamilton, ON L8N 4A6, Canada

Wolfgang A Kunze, Department of Biology, McMaster University, Hamilton, ON L8S 4L8, Canada

Wolfgang A Kunze, Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON L8S 4L8, Canada

Author contributions: West C, Stanisz AM and Wong A acquired and analyzed the data; Stanisz AM performed pilot experiments; West C and Kunze WA drafted the manuscript; Kunze WA designed the research.

Supported by the Natural Sciences and Engineering Research Council of Canada Discovery Grant awarded (to Kunze W), No. 2014-05517.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Animal Research Ethics Board of McMaster University (AUP# 12-05-17).

Conflict-of-interest statement: There are no conflicts of interest.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Christine West, MSc Candidate, BSc, McMaster Brain-Body Institute, St. Joseph's Healthcare, Hamilton, 50 Charlton Street East, ON L8N 4A6, Canada. westcl2@mcmaster.ca
Telephone: +1-905-5221155
Fax: +1-905-5406593

Received: July 20, 2016
Peer-review started: July 21, 2016
First decision: August 22, 2016
Revised: September 15, 2016
Accepted: October 10, 2016
Article in press: October 10, 2016
Published online: December 28, 2016

Abstract

AIM

To investigate the capacity of *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Saccharomyces boulardii* (*S. boulardii*) yeasts to reverse or to treat acute stress-related intestinal dysmotility.

METHODS

Adult Swiss Webster mice were stressed for 1 h in a wire-mesh restraint to induce symptoms of intestinal dysmotility and were subsequently killed by cervical dislocation. Jejunal and colon tissue were excised and placed within a tissue perfusion bath in which *S. cerevisiae*, *S. boulardii*, or their supernatants were administered into the lumen. Video recordings of contractility and gut diameter changes were converted to spatiotemporal maps and the velocity, frequency, and amplitude of propagating contractile clusters (PCC) were measured. Motility pre- and post-treatment was compared between stressed animals and unstressed controls.

RESULTS

S. boulardii and *S. cerevisiae* helped to mediate the effects of stress on the small and large intestine. Restraint stress reduced jejunal transit velocity (mm/s)

from 2.635 ± 0.316 to 1.644 ± 0.238, $P < 0.001$ and jejunal transit frequency (Hz) from 0.032 ± 0.008 to 0.016 ± 0.005, $P < 0.001$. Restraint stress increased colonic transit velocity (mm/s) from 0.864 ± 0.183 to 1.432 ± 0.329, $P < 0.001$ and frequency to a lesser degree. Luminal application of *S. boulardii* helped to restore jejunal and colonic velocity towards the unstressed controls; 1.833 ± 0.688 to 2.627 ± 0.664, $P < 0.001$ and 1.516 ± 0.263 to 1.036 ± 0.21, $P < 0.001$, respectively. *S. cerevisiae* also had therapeutic effects on the stressed gut, but was most apparent in the jejunum. *S. cerevisiae* increased PCC velocity in the stressed jejunum from 1.763 ± 0.397 to 2.017 ± 0.48, $P = 0.0031$ and PCC frequency from 0.016 ± 0.009 to 0.027 ± 0.007, $P < 0.001$. *S. cerevisiae* decreased colon PCC velocity from 1.647 ± 0.187 to 1.038 ± 0.222, $P < 0.001$. Addition of *S. boulardii* or *S. cerevisiae* supernatants also helped to restore motility to unstressed values in similar capacity.

CONCLUSION

There is a potential therapeutic role for *S. cerevisiae* and *S. boulardii* yeasts and their supernatants in the treatment of acute stress-related gut dysmotility.

Key words: Intestine; *Saccharomyces cerevisiae*; *Saccharomyces boulardii*; Restraint stress; Motility

Core tip: The use of *Saccharomyces cerevisiae* and *Saccharomyces boulardii* yeasts as therapeutic agents were tested for their ability to reverse the intestinal discomfort caused by acute stress. Most studies investigate the role of microbes in the prevention of stress, however the yeasts showed promising acute therapeutic effects for the treatment of stress. Additionally, the residual supernatant (Snt) after centrifugation of the yeasts was able to recapitulate much of the effect of the microbes themselves. *Saccharomyces* yeasts or Snt may be potential probiotic therapies in the treatment of acute stress-related intestinal dysmotility.

West C, Stanisz AM, Wong A, Kunze WA. Effects of *Saccharomyces cerevisiae* or *boulardii* yeasts on acute stress induced intestinal dysmotility. *World J Gastroenterol* 2016; 22(48): 10532-10544 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10532.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10532

INTRODUCTION

Most studies on beneficial ingested microbes including probiotics have focused primarily on bacteria. However, beneficial roles have been ascribed to certain yeasts, such as the sugar-fermenting *Saccharomyces*[1]. *Saccharomyces boulardii* (*S. boulardii*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) are two closely related strains used either as a probiotic or in the preparation of food and wine. The two strains have been closely examined; revealing that although they are nearly identical at a molecular level, *S. boulardii* shows more physiological resistance to heat and acid stressors[2]. It should also be noted that *S. boulardii* does not produce ascospores or use galactose, while *S. cerevisiae* does[3,4].

S. boulardii has been systematically studied for its beneficial and probiotic effects[5], but *S. cerevisiae* has limited research supporting a probiotic role[1]. *S. boulardii* has been used to help in the prevention of antibiotic or *Clostridium difficile* induced diarrhea, and there is evidence that it may be useful in attenuating acute gastroenteritis and traveller's diarrhea[5,6]. Another study showed that treatment with *S. boulardii* helped to shorten the duration of acute diarrhea in children and to normalize the frequency and consistency of stool[7]. *S. boulardii* may also help in the treatment of bowel inflammation and infection by reversing mucosal injury[8].

S. cerevisiae food and wine strains have a long history in the food and wine industry[9] and are generally considered safe for consumption[1]. Suggestive for a potential beneficial role for *S. cerevisiae* are reports indicating that this strain may provoke immune stimulation in mice infected with *Staphylococcus aureus*[10]. Supplementation with a *S. cerevisiae* I-3856 strain may improve symptoms in constipation-predominant irritable bowel syndrome patients[11]. There is also evidence that a *S. cerevisiae* UFMG 905 strain can bind to bacteria and modulate inflammation pathways in a murine model of *Salmonella enterica serovar Typhimurium* infection[12]. In summary, there appears to be published data supporting roles for *S. boulardii* or *S. cerevisiae* as beneficial or probiotic microbes. The evidence seems to be stronger for *S. boulardii* than *S. cerevisiae*, perhaps because the latter has been less frequently investigated in this regard.

We used an acute *ex vivo*, before and after, motility recording paradigm similar to that used previously to test for the effects of JB-1™ on stress-induced dysmotility[13]. *Saccharomyces* or *Saccharomyces* supernatant (Snt) were added to the Krebs buffer perfusing the lumen of isolated, previously stressed or unstressed, mouse intestinal segments. Treatment effects were interpreted by comparing propagating contractile clusters (PCC) of control with treatment recording periods. The *ex vivo* design allowed us to localize any effects to the intestine, thus avoiding multisystem homeostatic feedback between the gut and its extrinsic nervous system. This design also allowed us to separate treatment effects from confounding preventative actions, as would have been the case if the yeasts had been fed to the animal.

The preventative effect of *Saccharomyces* strains in relation to diarrhea suggests a possible action on

disordered gut motility. It has not been experimentally tested whether *S. boulardii* or *S. cerevisiae* are able to treat (effectively reverse) stress-related dysmotility in an experimental model. We have recently shown that restraint stress induces colonic propulsive hypermotility, while disorganizing and reducing motility in the small intestine[13]. The effects of stress on motility could be reversed *ex vivo* by introducing a bacterial probiotic (*Lactobacillus rhamnosus* JB-1™) into the lumen[13]. The example of JB-1™ in treating stress-induced dysmotility provides a way to compare putative beneficial actions of the *Saccharomyces* strains with a probiotic bacterium whose effects on motility and the enteric nervous system have been previously studied[14-20].

MATERIALS AND METHODS

Animals

We used 6-8-wk-old adult male Swiss Webster mice (20-30 g) from Charles River Laboratories (Wilmington, MA, United States). All procedures following acute restraint were *ex vivo*[20] and their conduct were approved by the Animal Research Ethics Board of McMaster University (AUP 12-05-17).

Gut motility

The following experiments and data analysis were performed as described as in West *et al*[13]. Mice were placed in a wire mesh restraint device for 1 h or kept in their cage for 1 h; after which they were killed by cervical dislocation. A 4 cm long segment of jejunum or colon was excised and placed into a tissue bath perfusion chamber[20]. The oral and anal ends were cannulated with silicone tubing and the oral end was attached to a stopcock manifold, allowing inflow with oxygenated Krebs buffer or buffer to which yeast or Snt had been added. Krebs buffer was of the following composition (mmol/L): 118 NaCl, 4.8 KCl, 25 $NaHCO_3$, 1.0 NaH_2PO_4, 1.2 $MgSO_4$, 11.1 glucose, and 2.5 $CaCl_2$ bubbled with carbogen gas (95% O_2 and 5% CO_2). PCCs were evoked by filling the lumen with Krebs buffer using a pressure differential of 2 hPa (cmH_2O) for the inflow and 3 hPa for outflow for jejunum, and 2-3 hPa for colon inflow with the outflow raised 1 cm above the inflow.

Contractions of the gut were video-recorded on a JVC camcorder placed 10 cm above the tissue bath. Conversion of videos to spatiotemporal diameter maps (Dmaps) were performed using Image J software, as described in Wu *et al*[20]. Dmaps are a form of heat map in which the oral to anal propagation of the intestine runs down the vertical axis and time runs across the horizontal axis. The intestine's diameter is colour coded using red to represent contraction, and yellow to green to represent varying degrees of relaxation (Figure 1). PCCs are identified in Dmaps as described in West *et al*[13]. The PCCs appear as broad bands[20] that propagate in the oral to anal direction. They are believed to require ENS activity because they are abolished by the Na channel blocker tetrodotoxin[18-23]. From these Dmaps, velocity can be measured as the slope of the bands (distance/time), frequency from the intervals between the bands, and amplitude as the difference between gut diameters.

Luminal stimuli

Lyophilized *S. boulardii* CNCM I-1079 or *S. cerevisiae* LYCC 6029 were obtained from Lallemand Health Solutions (Montreal, QC, CA). The microbes (starter counts) were diluted in 50 mL Krebs buffer and incubated for 45 min at 37 ℃. In pilot experiments, 5×10^7, 5×10^8 and 5×10^9 starter counts of *S. boulardii* reduced PCC velocity in unstressed colon segments by 5%, 25% and 27%. We thus used 5×10^8-lyophilized microbes for dilution in all other experiments as this starter count produced near maximal effect in the pilots. In some experiments we used *S. boulardii* or *S. cerevisiae* Snt after completion of the incubation period. The microbes were diluted and incubated as previously described above. The Snt was separated from the post-incubation yeast microbe solution by centrifugation at 1400 g. using a Beckman Model TJ-6 centrifuge for 30 min. After sediment removal, the mixture was passed through a 0.2 μm pore-size filter and the filtered Snt was applied to the lumen of gut segment to be tested.

Statistical analysis

Effects of restraint stress on motility were measured in unpaired experiments by comparing velocity, frequency and peak amplitude for PCCs. The same parameters were compared before and after application of *S. boulardii*, *S. cerevisiae*, or their Snt. Control recordings were made in stressed or unstressed mice perfused with Krebs for a maximum of 20 min. Treatment recordings were made during and after addition of one of the stimuli (yeast or Snt) to the same segment for additional 20 min duration. Descriptive statistics were given as mean ± SD (N, where N denotes the number of mice used). In the results, treatment effect sizes are presented as % mean differences and the probability of superiority (PS) based on distribution of difference scores and standard deviations is presented in brackets[24]. Differences were also presented in the tables using unpaired or paired *t* tests under the null hypothesis of no difference.

RESULTS

Effects of stress on PCCs

Stress had different effects on the jejunum *vs* the colon. Stress decreased propulsive motility and decreased the regularity of PCCs in jejunum, but increased motility in colon (Figure 1). Stress decreased jejunal PCC velocity

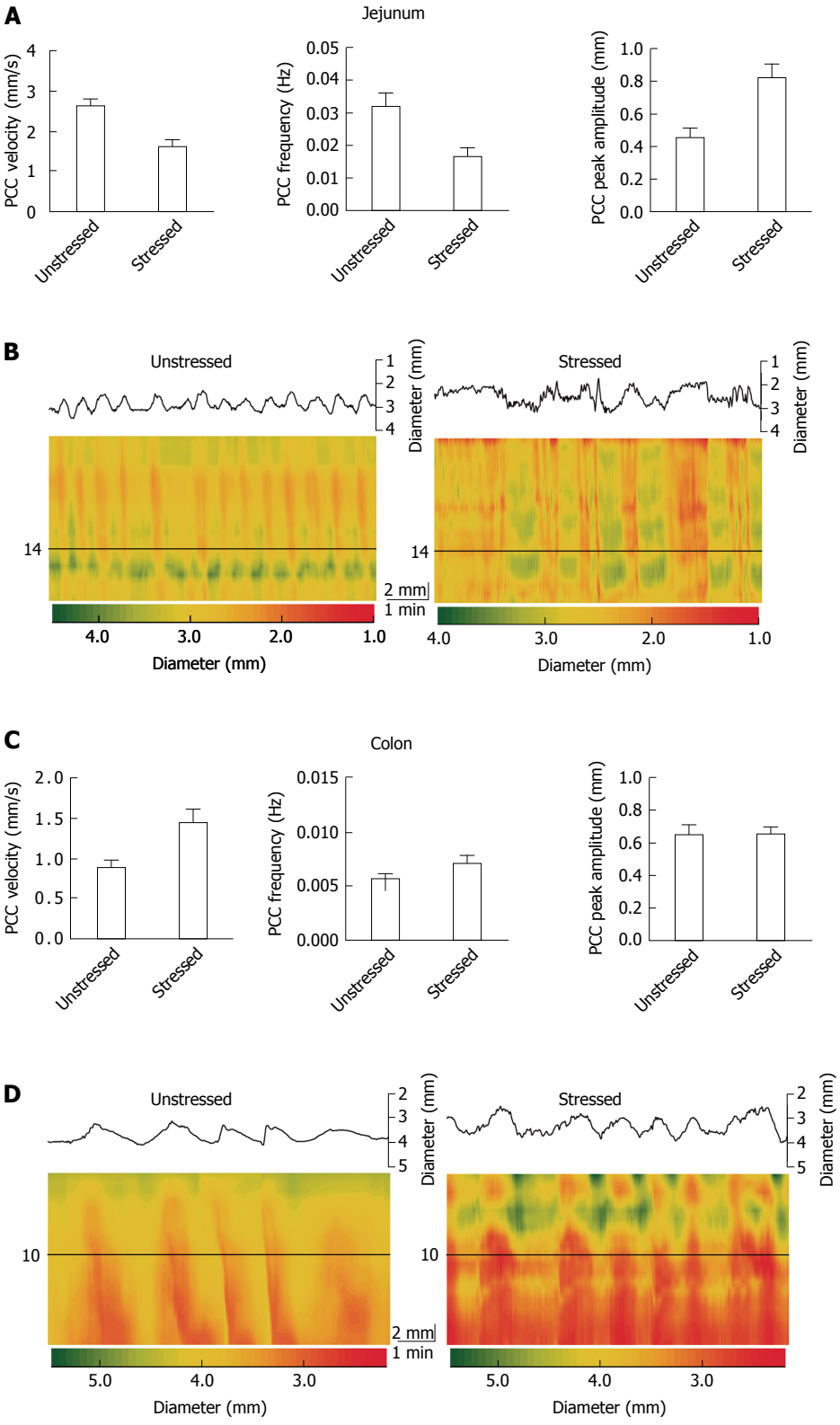

Figure 1 Effects of acute restraint stress on gut contractility, *ex vivo*. A and C: Plot PCC velocity, frequency, and peak amplitude in the jejunum and colon respectively. The bar graphs show the mean difference between the control and treatment parameters. Stress decreased PCC velocity ($P < 0.001$) and frequency ($P < 0.001$) and increased peak amplitude in the jejunum ($P < 0.001$). Stress increased PCC velocity ($P < 0.001$) and frequency ($P = 0.008$), but had virtually no effect on peak amplitude in the colon ($P = 0.902$); B and D: Spatiotemporal diameter maps demonstrating contractility of the gut over time. The colour scale of the heat maps denote gut diameter starting from red for the smallest diameter (contraction) to green for the largest diameter (relaxation). The contractions run orally to anally across the vertical axis, and across time along the horizontal axis. Above the heat map is a plot of diameter *vs* time or a single time-point along the vertical (oral-anal) axis, which is denoted by the horizontal line. The total vertical length of the map is 20 mm and time is 480 s for the jejunum and 560 s for the colon. The scale bar measures 1 min (60 s) horizontally and 2 mm vertically. All subsequent spatiotemporal (Dmaps) followed the same parameters.

Table 1 Effects of stress on gut propagating contractile clusters

Parameter	Tissue	Unstressed	Stressed	*P* value (*t* test)
Velocity (mm/s)	Jejunum	2.635 ± 0.316 (17)	1.644 ± 0.238 (17)	< 0.001
	Colon	0.864 ± 0.183 (17)	1.432 ± 0.329 (17)	< 0.001
Frequency (Hz)	Jejunum	0.032 ± 0.008 (17)	0.016 ± 0.005 (17)	< 0.001
	Colon	0.005 ± 0.001 (17)	0.007 ± 0.001 (17)	0.008
Peak amplitude (mm)	Jejunum	0.437 ± 0.020 (17)	0.790 ± 0.015 (17)	< 0.001
	Colon	0.640 ± 0.118 (17)	0.645 ± 0.101 (17)	0.902

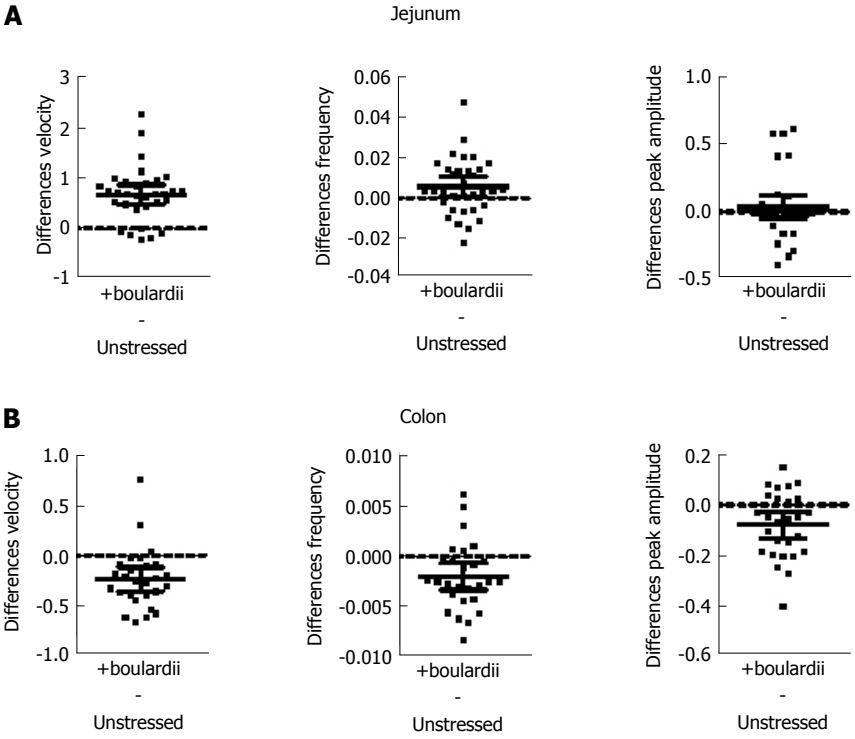

Figure 2 *Saccharomyces boulardii* increased motility across most parameters in the unstressed jejunum and decreased motility across parameters in the unstressed colon, *ex vivo*. Mean differences in parameters are plotted (unpaired) and standard deviations of the mean are indicated. A: *Saccharomyces boulardii* (*S. boulardii*) increased PCC velocity and frequency, but had little to no effect on peak amplitude, in the unstressed jejunum; B: *S. boulardii* decreased PCC velocity, frequency, and peak amplitude in the unstressed colon.

by 38% (PS = 99%, $P < 0.001$), but increased it by 66% (PS = 98%, $P < 0.001$) for colon (Table 1 and Figure 1). Frequency decreased by 50% (PS = 99%, $P < 0.001$) for jejunum, but increased by 27% (PS = 93%, $P = 0.008$) for colon. Peak amplitude increased by 81% (PS = 99%, $P < 0.001$) for jejunum and by 1% (PS = 51%, $P = 0.902$) for colon.

S. boulardii unstressed gut

S. boulardii increased PCC velocity in unstressed jejunum by 26% (PS = 94%, $P < 0.001$), but decreased velocity by 27% (PS = 89%, $P < 0.001$) for colon. Frequency was increased by 19% (PS = 74%, $P = 0.030$) for jejunum, but decreased by 22% (PS = 85%, $P = 0.005$) for colon. Peak amplitude increased by 6% (PS = 60%, $P = 0.290$) for jejunum, but decreased by 13% (PS = 85%, $P = 0.005$) for colon (Table 2). Figure 2 also shows paired mean differences (Unstressed + *S. boulardii* Unstressed) with 95%CIs. When the confidence intervals did not straddle the "difference = 0" line, the value of no paired difference was excluded for these intervals with 95% confidence[25]; when it did straddle the "difference = 0" line the value of no paired difference was included within the 95%CIs.

S. boulardii stressed gut

S. boulardii counter the effects of stress in both jejunal and colon segments, with the exception of peak amplitude (Figure 3 and Table 3). *S. boulardii* restored the regularity and frequency of contractions in both tissues, as shown in Figure 3. There is a marked increase in frequency of bands in the jejunal Dmap and a decrease in the colon Dmap. Addition of *S. boulardii* increased velocity by 43% (PS = 93%, $P < 0.001$) for stressed jejunum and decreased velocity by 32% (PS = 96%, $P < 0.001$) for colon. PCC frequency was increased by 69% (PS = 85%, $P = 0.005$) for jejunum but decreased by 29% (PS = 90%, $P = 0.001$) for colon. Peak amplitude changed by +1% (PS =

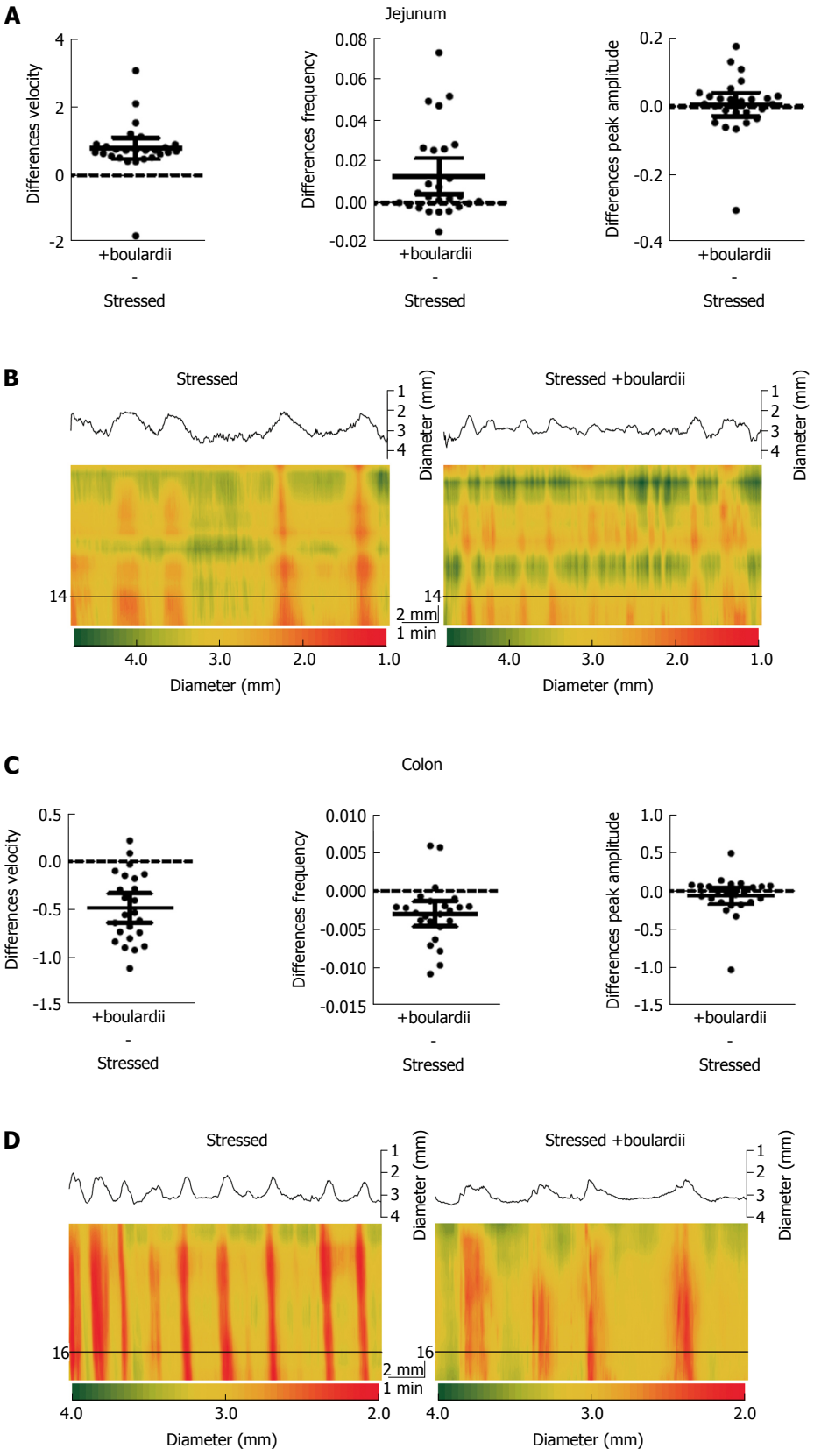

Figure 3 ***Saccharomyces boulardii*** **reduced the effects of acute stress on the small and large intestine.** A and C: Mean paired differences, using 95%CIs across parameters for jejunum and colon [(Stressed + *S. boulardii*) - stressed]. *Saccharomyces boulardii* (*S. boulardii*) increased jejunal velocity and frequency after stress. *S. boulardii* decreased colonic velocity and frequency after stress. *S. boulardii* had little to no effect on peak amplitude in the stressed jejunum and colon. Mean differences straddled 0 for peak amplitude in both jejunum and colon; B and D: Dmaps comparing stressed jejunum and colon before and after addition of *S. boulardii*.

Table 2 Effects of *Saccharomyces boulardii* on propagating contractile clusters in unstressed gut

Parameter	Tissue	Unstressed	+ boulardii	*P* value (*t* test)
Velocity (mm/s)	Jejunum	2.556 ± 0.299 (32)	3.229 ± 0.620 (32)	< 0.001
	Colon	0.865 ± 0.148 (27)	0.633 ± 0.225 (27)	< 0.001
Frequency (Hz)	Jejunum	0.030 ± 0.018 (33)	0.035 ± 0.025 (33)	0.030
	Colon	0.009 ± 0.004 (27)	0.007 ± 0.003 (27)	0.005
Peak amplitude (mm)	Jejunum	0.587 ± 0.241 (33)	0.623 ± 0.291 (33)	0.290
	Colon	0.621 ± 0.190 (27)	0.543 ± 0.179 (27)	0.005

Table 3 Effects of *Saccharomyces boulardii* on propagating contractile clusters in stressed gut

Parameter	Tissue	Stressed	+ boulardii	*P* value (*t* test)
Velocity (mm/s)	Jejunum	1.833 ± 0.688 (26)	2.627 ± 0.664 (26)	< 0.001
	Colon	1.516 ± 0.263 (24)	1.036 ± 0.210 (24)	< 0.001
Frequency (Hz)	Jejunum	0.019 ± 0.013 (26)	0.032 ± 0.024 (26)	0.005
	Colon	0.010 ± 0.003 (24)	0.007 ± 0.003 (24)	0.001
Peak amplitude (mm)	Jejunum	0.692 ± 0.215 (26)	0.698 ± 0.212 (26)	0.705
	Colon	0.719 ± 0.249 (24)	0.657 ± 0.152 (24)	0.259

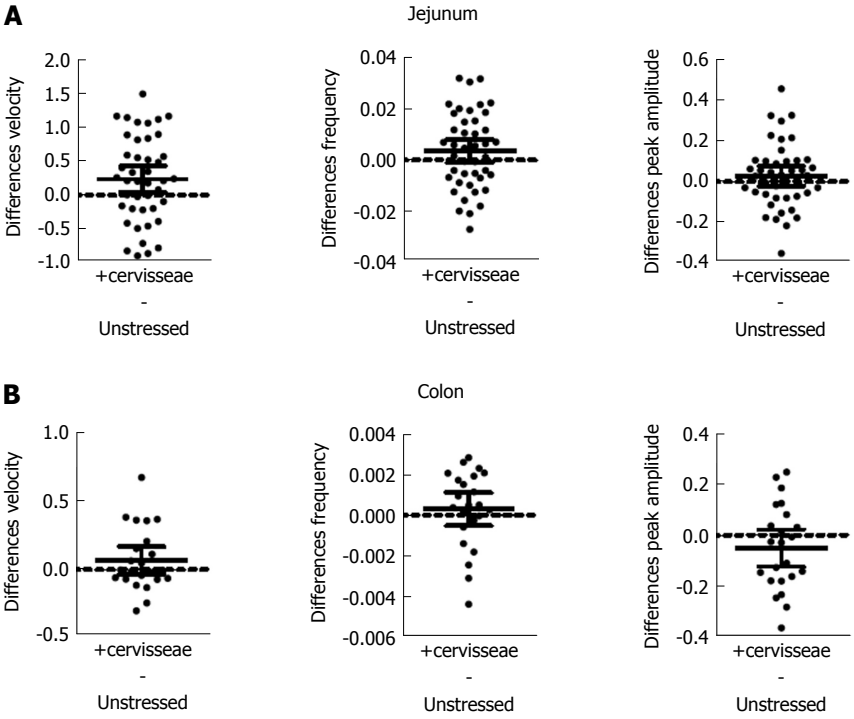

Figure 4 *Saccharomyces cerevisiae* slightly increased parameters in both the unstressed small and large intestine (excluding colon peak amplitude). A: 95%CIs for paired mean differences were near 0 for jejunal peak amplitude. Jejunal PCC velocity and frequency slightly increased; B: *Saccharomyces cerevisiae* (*S. cerevisiae*) had less potent effects in the colon, slightly increasing PCC velocity and frequency, but decreasing peak amplitude. PCC: Propagating contractile clusters.

56%, *P* = 0.705) for jejunum and decreased by 9% (PS = 68%, *P* = 0.259) for colon. Mean paired differences (Stressed + *S. boulardii* Stressed) are given in Figure 3B and D with their 95%CIs. Washout with Krebs buffer for 20 min during the control period did not moderate the effects of *S. boulardii* on the stressed segments.

S. cerevisiae unstressed gut

S. cerevisiae had the most noticeable effect on the jejunum (Figure 4 and Table 4). Velocity increased 9% (PS = 71%, *P* = 0.017) for jejunum and 9% (PS = 73%, *P* = 0.161) for colon. Frequency increased by 13% (PS = 64%, *P* = 0.003) for jejunum and by 6% (PS = 63%, *P* = 0.462) for colon, while peak amplitude increased by 7% (PS = 61%, *P* = 0.242) for jejunum, but decreased by 9% (PS = 73%, *P* = 0.164) for colon.

S. cerevisiae stressed gut

S. cerevisiae reduced most of the effects of stress, except for those of colon PCC frequency and amplitude

Table 4 Effects of *Saccharomyces cerevisiae* on propagating contractile clusters in unstressed gut

Parameter	Tissue	Unstressed	+ cerevisiae	*P* value (*t* test)
Velocity (mm/s)	Jejunum	2.779 ± 0.499 (43)	3.017 ± 0.457 (43)	0.017
	Colon	0.788 ± 0.212 (23)	0.858 ± 0.257 (23)	0.161
Frequency (Hz)	Jejunum	0.027 ± 0.012 (43)	0.031 ± 0.008 (43)	0.003
	Colon	0.0051 ± 0.001 (23)	0.0054 ± 0.001 (23)	0.462
Peak amplitude (mm)	Jejunum	0.409 ± 0.111 (43)	0.438 ± 0.098 (43)	0.242
	Colon	0.559 ± 0.176 (23)	0.508 ± 0.15 (23)	0.164

Table 5 Effects of *Saccharomyces cerevisiae* on propagating contractile clusters in stressed gut

Parameter	Tissue	Stressed	+ cerevisiae	*P* value (*t* test)
Velocity (mm/s)	Jejunum	1.763 ± 0.397 (23)	2.017 ± 0.480 (23)	0.0031
	Colon	1.647 ± 0.187 (23)	1.038 ± 0.222 (23)	< 0.001
Frequency (Hz)	Jejunum	0.016 ± 0.009 (23)	0.027 ± 0.007 (23)	< 0.001
	Colon	0.008 ± 0.002 (23)	0.008 ± 0.003 (23)	0.994
Peak amplitude (mm)	Jejunum	0.761 ± 0.316 (23)	0.559 ± 0.148 (23)	0.013
	Colon	0.640 ± 0.101 (23)	0.597 ± 0.103 (23)	0.190

Table 6 Effects of *Saccharomyces boulardii* supernatant on propagating contractile clusters in stressed gut

Parameter	Tissue	Stressed	+ boulardii Snt	*P* value (*t* test)
Velocity (mm/s)	Jejunum	1.97 ± 0.39 (6)	2.585 ± 0.468 (6)	< 0.001
	Colon	1.588 ± 0.194 (6)	1.11 ± 0.383 (6)	0.038
Frequency (Hz)	Jejunum	0.020 ± 0.014 (6)	0.044 ± 0.027 (6)	0.067
	Colon	0.010 ± 0.001 (6)	0.007 ± 0.003 (6)	0.024
Peak amplitude (mm)	Jejunum	0.75 ± 0.285 (6)	0.737 ± 0.187 (6)	0.93
	Colon	0.65 ± 0.188 (6)	0.701 ± 0.09 (6)	0.59

(Figure 5 and Table 5). Velocity was increased by 14% (PS = 89%, $P = 0.0031$) for jejunum and decreased by 37% (PS = 98%, $P < 0.001$) for colon. Frequency was increased by 74% (PS = 95%, $P < 0.001$) for jejunum, but there was only a 0.1% (PS = 50%, $P = 0.994$) change for colon. Peak amplitude decreased by 27% (PS = 86%, $P = 0.013$) for jejunum and 7% (PS = 72%, $P = 0.190$) for colon. The Dmaps in Figure 5B and D show the regulation of jejunal motility by *S. cerevisiae*, with less potent effects on the colon. However the degree in which the contractions change in diameter appear to be lessened in the colon after addition of *S. cerevisiae*, and is apparent by the reduced frequency of red, strong contractions.

S. boulardii Snt stressed gut

S. boulardii Snt decreased the effects of stress on PCC parameters, except for peak amplitude (Figure 6 and Table 6). Velocity increased by 31% (PS = 99.9%, $P < 0.001$) for jejunum, but decreased by 30% (PS = 99.9%, $P = 0.038$) for colon. Frequency increased by 114% (PS = 99.9%, $P = 0.067$) for jejunum and decreased by 37% (PS = 99%, $P = 0.024$) for colon. However, peak amplitude only decreased by 2% (PS = 56%, $P = 0.930$) for jejunum and increased 8% (PS = 74%, $P = 0.590$) for colon.

S. cerevisiae Snt stressed gut

S. cerevisiae Snt reduced the effects of stress for colon velocity, jejunal frequency and peak amplitude for jejunum and colon (Figure 7 and Table 7). Effects on other parameters were minor. Velocity decreased by 7% (PS = 56%, $P = 0.970$) for jejunum but decreased by 38% (PS = 99%, $P = 0.002$) for colon. Frequency increased by 64% (PS = 83%, $P = 0.003$) for jejunum and decreased by 2% (PS = 60%, $P = 0.830$); also, peak amplitude decreased by 35% (PS = 99%, $P = 0.0016$) for jejunum while it decreased by 31% (PS = 99%, $P = 0.100$) for colon.

DISCUSSION

We have used an *ex vivo* intestinal segment perfusion setup to show that *S. boulardii* or *S. cerevisiae* reverse much of the jejunal or colonic dysmotility induced by restraint stress. There is sometimes a potential for normal commensal, or otherwise beneficial, yeasts to adopt a pathological role in immune-compromised individuals. It is therefore of interest that the Snt from either *Saccharomyces* strains recapitulate much of the treatment effects of the live yeasts. Because the yeasts were applied intraluminally in *ex vivo* intestinal segments the treatment effect must have occurred

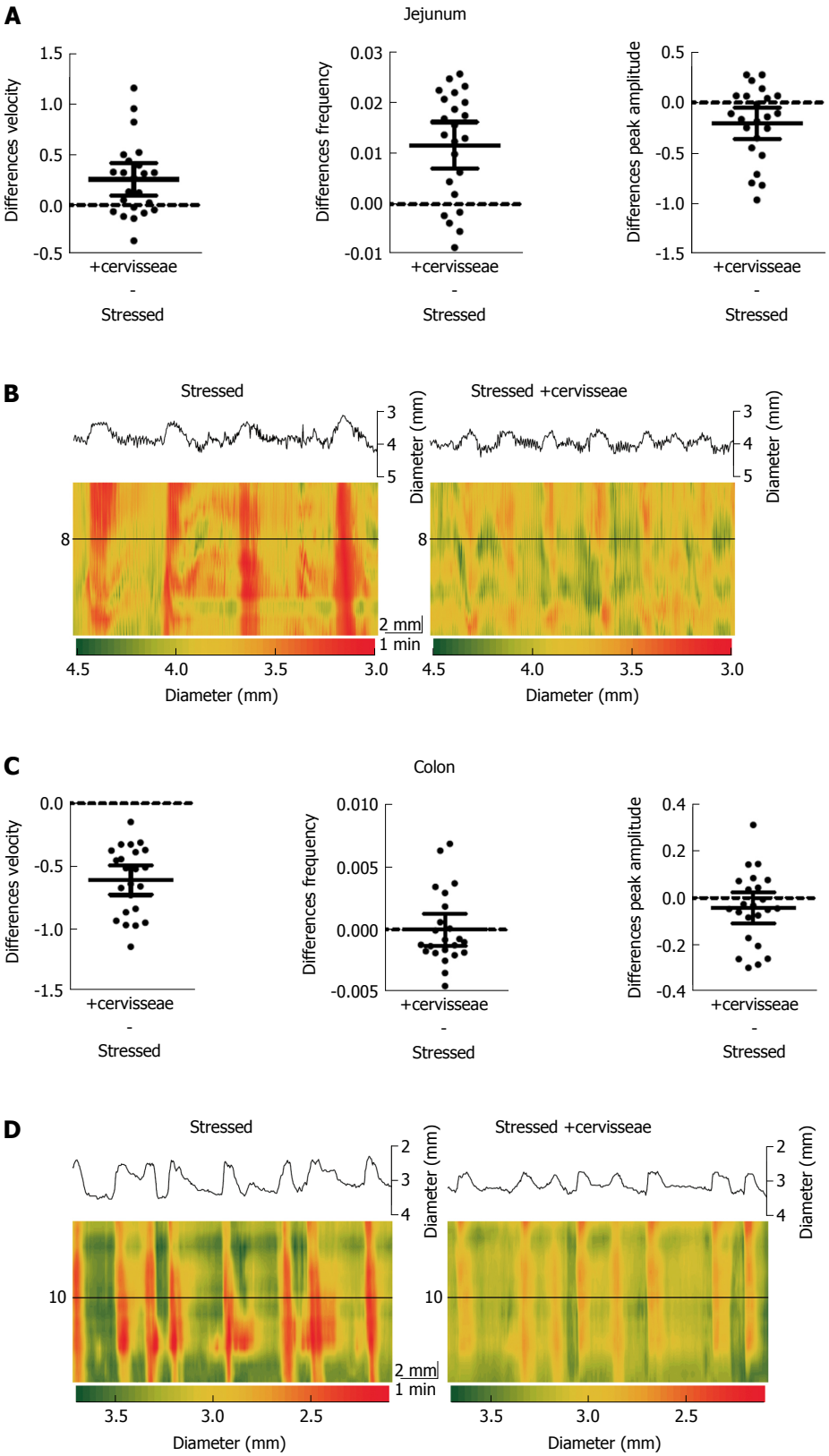

Figure 5 *Saccharomyces cerevisiae* helped to reduce dysmotility in the stressed gut, with most visible effects on the jejunum. A and C: *Saccharomyces cerevisiae* (*S. cerevisiae*) increased stressed jejunal propagating contractile clusters velocity and decreased velocity in the stressed colon. *S. cerevisiae* increased jejunal frequency and slightly decreased peak amplitude in both the colon and jejunum. 95%CIs straddled 0 for mean paired differences in colonic frequency; B and D: Dmaps representing the stressed gut before and after addition of *S. cerevisiae*. *S. cerevisiae* helped to restore jejunal motility after stress. *S. cerevisiae* had less potent effects on the stressed colon, but helped to ease some of the dysmotility.

Table 7 Effects of *Saccharomyces cerevisiae* supernatant on propagating contractile clusters in stressed gut

		Stressed	+cerevisiae Snt	*P* value (*t* test)
Velocity (mm/s)	Jejunum	0.713 ± 0.109 (6)	0.466 ± 0.122 (6)	0.97
	Colon	1.639 ± 0.18 (6)	1.018 ± 0.245 (6)	0.002
Frequency (Hz)	Jejunum	0.018 ± 0.004 (6)	0.029 ± 0.004 (6)	0.003
	Colon	0.0076 ± 0.001 (6)	0.0074 ± 0.001 (6)	0.83
Peak amplitude (mm)	Jejunum	0.713 ± 0.109 (6)	0.466 ± 0.122 (6)	0.0016
	Colon	0.741 ± 0.177 (6)	0.510 ± 0.202 (6)	0.10

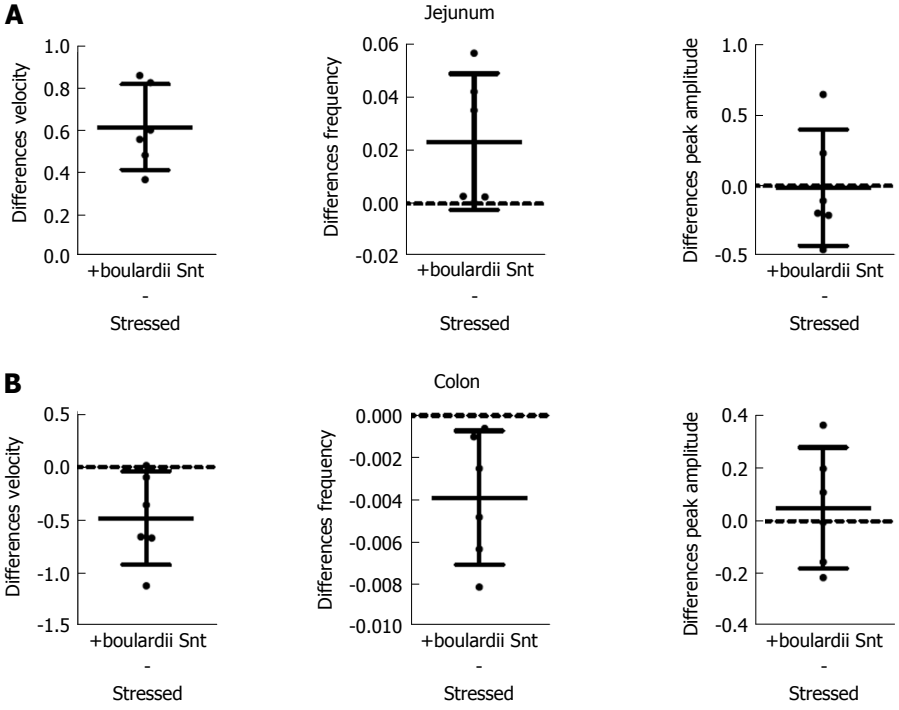

Figure 6 Addition of *Saccharomyces boulardii* supernatant to the stressed lumen recapitulated much of the effect of the boulardii yeast. A: *Saccharomyces boulardii* (*S. boulardii*) Snt increased PCC velocity and frequency in the stressed jejunum. 95%CIs straddled 0 for mean paired differences in jejunal peak amplitude, showing no effect; B: *S. boulardii* Snt decreased PCC velocity and frequency in the stressed colon. *S. boulardii* Snt had little effect (very little increase) on stressed colonic peak amplitude and again the 95%CIs straddled 0 for mean paired differences. PCC: Propagating contractile clusters.

locally within the intestine[13]. Therefore, it could not have involved the hypothalamic-pituitary-adrenal axis or other central structures. The relative short latency approximately 10 min) for the onset of the therapeutic effect is consistent with a drug-like pharmacological mode of action, however, fast indirect modes of action involving immune cells cannot be excluded, although we consider the latter possibility to be less likely than the former.

There have been previous reports of the acute *ex vivo* actions of bacterial, but not fungal microbes on intestinal motility (see discussion in[20]). Such effects appear to be region specific with different actions for small *vs* large intestine[20]. It is important to note that others have also reported a short (approximately 10 min) latency in onset for microbial effects on intestinal propulsive motility[18,26], consistent with a drug-like pharmacological action of the microbes on the neuromuscular machinery. This supporting finding as well as the region-specific, local effect on the intestine leads us to believe the effect is most likely pharmacological in action.

Wrap restraint has been reported to increase the contractile amplitudes in the small intestine and colon, while decreasing frequency in small intestine and increasing frequency in colon[13,27]. As far as we are aware the only other publication to date referring to treatment effects of beneficial microbes on stress-induced dysmotility is West *et al*[13] in which *Lactobacillus rhamnosus* JB-1™ reversed the effects of prior stress. *S. boulardii* or *S. cerevisiae* were also effective in reversing most, but not all of the stress-induced dysmotility. *S. boulardii* was particularly effective in reversing the effects of stress in both the jejunum and colon. *S. boulardii* also had similar effects in the unstressed gut as the stressed, increasing PCC frequency and velocity in the jejunum and decreasing PCC frequency and velocity in the colon. These effects on the unstressed gut were predictive of the restoration of the stressed gut towards unstressed

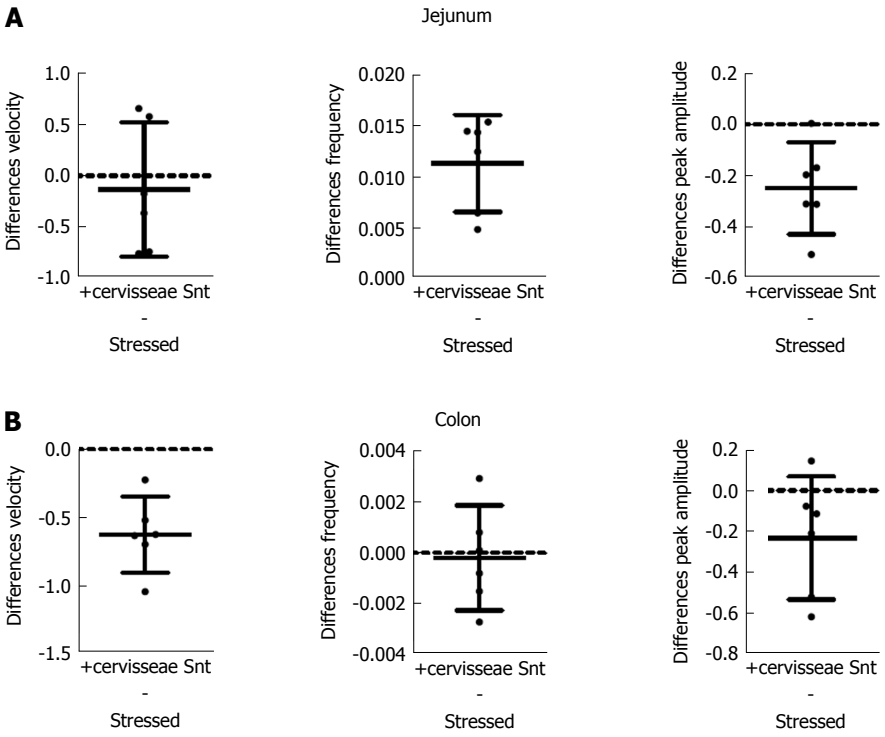

Figure 7 Addition of *Saccharomyces cerevisiae* supernatant to the stressed lumen had some therapeutic effect, but not across parameters. A: *Saccharomyces cerevisiae* (*S. cerevisiae*) Snt had little effect on stressed jejunal PCC velocity. *S. cerevisiae* Snt increased stressed jejunal PCC frequency and decreased peak amplitude; B: *S. cerevisiae* Snt decreased both stressed colonic PCC velocity and peak amplitude. There was no effect on stressed colon PCC frequency.

measures.

S. boulardii and *S. cerevisiae* are nearly identical genetically, but differ in resistance to temperature and acidic stressors and growth characteristics[2]. Similarly, the treatment effects of *S. boulardii* and *S. cerevisiae* were not identical, indicating that despite their genetic similarity the functional interaction of these microbes or their Snt with the host intestine is functionally different and region specific. There is clearly a need to identify the bioactive molecules released by the yeasts and those that mediate the motility modifying effects on the host tissue. This is likely a difficult task since there is no reason to suppose that only a single molecule is the mediator for anyone strain; yeasts like other microbes produce a multitude of molecules, of which any combination could potentially be effective. A similar logic applies to bacterial microbes with motility modifying effects. Previous research on *Lactobacillus rhamnosus* JB-1™ isolated the molecule-containing microvesicles of the bacteria and tested them for their individual effect. Application of the microvesicles to the gut epithelium replicated the effects of the JB-1™ bacteria on enteric neurons[28]. In similar fashion, further isolating components of the Snt and evaluating their effects may help to narrow down the underlying bioactive molecules.

The results of the present paper suggest that there may be other yeasts with potential therapeutic actions in animal models of stress. Our *ex vivo* perfusion setup may provide a relatively simple, though not high throughput, method to screen yeasts or fungi for beneficial effects on the host intestine. Previously discussed results of *S. cerevisiae* and *S. boulardii* clinical trials further support the therapeutic potential of *Saccharomyces* yeasts in humans[7,11]. We predict that the beneficial or probiotic potential of fungi and yeasts is set to expand with increased research, initially in animal models followed by human trials.

COMMENTS

Background

Stress has adverse effects on intestinal motility causing irregular propagating contractile clusters (PCCs) that decrease motility in the jejunum and increase motility in the colon. *Saccharomyces* yeasts have been shown to have potential beneficial and probiotic effects in the intestine and in the treatment of some gastrointestinal disorders.

Research frontiers

Most stress and probiotic research studies focus on the prevention of stress-related symptoms on the intestinal tract. The use of probiotics, including yeasts, is an emerging treatment option for gastrointestinal disorders, particularly in exchange for antibiotics.

Innovations and breakthroughs

This is first evidence of *Saccharomyces* yeasts acting in the pre-clinical treatment of stress-related gut dysmotility. It is also the first evidence of *Saccharomyces* supernatant (yeast microbes removed) having a beneficial effect on gut motility and the treatment of stress.

Applications

The data suggests that *Saccharomyces* yeasts may be potential therapeutic

treatments for stress-related dysmotility in the intestine. Additionally, *Saccharomyces* supernatants cause similar effects as their respective yeasts, and might also play a therapeutic role. However, more animal experiments are needed to better support these results.

Terminology

PCCs or propagating contractile clusters are sweeping bands of intestinal contraction that move in the oral to anal direction and are likely stimulated by the ENS.

Peer-review

The authors investigated the possible effects of *Saccharomyces boulardii* or *Saccharomyces cerevisiae* on reducing the stress-related intestinal dysmotility. The study is well designed and well implemented. The manuscript should be accepted for publication with the following revision.

REFERENCES

1 **Moslehi-Jenabian S**, Pedersen LL, Jespersen L. Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients* 2010; **2**: 449-473 [PMID: 22254033 DOI: 10.3390/nu2040449]

2 **Fietto JL**, Araújo RS, Valadão FN, Fietto LG, Brandão RL, Neves MJ, Gomes FC, Nicoli JR, Castro IM. Molecular and physiological comparisons between Saccharomyces cerevisiae and Saccharomyces boulardii. *Can J Microbiol* 2004; **50**: 615-621 [PMID: 15467787 DOI: 10.1139/w04-050]

3 **Edwards-Ingram L**, Gitsham P, Burton N, Warhurst G, Clarke I, Hoyle D, Oliver SG, Stateva L. Genotypic and physiological characterization of Saccharomyces boulardii, the probiotic strain of Saccharomyces cerevisiae. *Appl Environ Microbiol* 2007; **73**: 2458-2467 [PMID: 17293506 DOI: 10.1128/AEM.02201-06]

4 **McFarland LV**. Saccharomyces boulardii is not Saccharomyces cerevisiae. *Clin Infect Dis* 1996; **22**: 200-201 [PMID: 8825013 DOI: 10.1093/clinids/22.1.200]

5 **Czerucka D**, Piche T, Rampal P. Review article: yeast as probiotics -- Saccharomyces boulardii. *Aliment Pharmacol Ther* 2007; **26**: 767-778 [PMID: 17767461 DOI: 10.1111/j.1365-2036.2007.03442.x]

6 **Barnes D**, Yeh AM. Bugs and Guts: Practical Applications of Probiotics for Gastrointestinal Disorders in Children. *Nutr Clin Pract* 2015; **30**: 747-759 [PMID: 26538058 DOI: 10.1177/0884533615610081]

7 **Htwe K**, Yee KS, Tin M, Vandenplas Y. Effect of Saccharomyces boulardii in the treatment of acute watery diarrhea in Myanmar children: a randomized controlled study. *Am J Trop Med Hyg* 2008; **78**: 214-216 [PMID: 18256417]

8 **Canonici A**, Siret C, Pellegrino E, Pontier-Bres R, Pouyet L, Montero MP, Colin C, Czerucka D, Rigot V, André F. Saccharomyces boulardii improves intestinal cell restitution through activation of the α2β1 integrin collagen receptor. *PLoS One* 2011; **6**: e18427 [PMID: 21483797 DOI: 10.1371/journal.pone.0018427]

9 **Legras JL**, Merdinoglu D, Cornuet JM, Karst F. Bread, beer and wine: Saccharomyces cerevisiae diversity reflects human history. *Mol Ecol* 2007; **16**: 2091-2102 [PMID: 17498234 DOI: 10.1111/j.1365-294X.2007.03266.x]

10 **Ochigava I**, Kalandarishvili L, Zhvania M. Immunological and central nervous system changes in mice suffering from Staphyloccocus aureus and treated with Saccharomyces cerevisiae var. vini living cells. *Folia Microbiol* (Praha) 2006; **51**: 659-663 [PMID: 17455807]

11 **Spiller R**, Pélerin F, Cayzeele Decherf A, Maudet C, Housez B, Cazaubiel M, Jüsten P. Randomized double blind placebo-controlled trial of Saccharomyces cerevisiae CNCM I-3856 in irritable bowel syndrome: improvement in abdominal pain and bloating in those with predominant constipation. *United European Gastroenterol J* 2016; **4**: 353-362 [PMID: 27403301 DOI: 10.1177/2050640615602571]

12 **Martins FS**, Elian SD, Vieira AT, Tiago FC, Martins AK, Silva FC, Souza EL, Sousa LP, Araújo HR, Pimenta PF, Bonjardim CA, Arantes RM, Teixeira MM, Nicoli JR. Oral treatment with Saccharomyces cerevisiae strain UFMG 905 modulates immune responses and interferes with signal pathways involved in the activation of inflammation in a murine model of typhoid fever. *Int J Med Microbiol* 2011; **301**: 359-364 [PMID: 21236729 DOI: 10.1016/j.ijmm.2010.11.002]

13 **West C**, Wu RY, Wong A, Stanisz AM, Yan R, Min KK, Pasyk M, McVey Neufeld KA, Karamat MI, Foster JA, Bienenstock J, Forsythe P, Kunze WA. Lactobacillus rhamnosus strain JB-1 reverses restraint stress-induced gut dysmotility. *Neurogastroenterol Motil* 2016; Epub ahead of print [PMID: 27381257 DOI: 10.1111/nmo.12903]

14 **Kunze WA**, Mao YK, Wang B, Huizinga JD, Ma X, Forsythe P, Bienenstock J. Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *J Cell Mol Med* 2009; **13**: 2261-2270 [PMID: 19210574 DOI: 10.1111/j.1582-4934.2009.00686.x]

15 **Ma X**, Mao YK, Wang B, Huizinga JD, Bienenstock J, Kunze W. Lactobacillus reuteri ingestion prevents hyperexcitability of colonic DRG neurons induced by noxious stimuli. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G868-G875 [PMID: 19179624 DOI: 10.1152/ajpgi.90511.2008]

16 **Mao YK**, Kasper DL, Wang B, Forsythe P, Bienenstock J, Kunze WA. Bacteroides fragilis polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. *Nat Commun* 2013; **4**: 1465 [PMID: 23403566 DOI: 10.1038/ncomms2478]

17 **Perez-Burgos A**, Wang B, Mao YK, Mistry B, McVey Neufeld KA, Bienenstock J, Kunze W. Psychoactive bacteria Lactobacillus rhamnosus (JB-1) elicits rapid frequency facilitation in vagal afferents. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G211-G220 [PMID: 23139216 DOI: 10.1152/ajpgi.00128.2012]

18 **Wang B**, Mao YK, Diorio C, Pasyk M, Wu RY, Bienenstock J, Kunze WA. Luminal administration ex vivo of a live Lactobacillus species moderates mouse jejunal motility within minutes. *FASEB J* 2010; **24**: 4078-4088 [PMID: 20519636 DOI: 10.1096/fj.09-153841]

19 **Wang B**, Mao YK, Diorio C, Wang L, Huizinga JD, Bienenstock J, Kunze W. Lactobacillus reuteri ingestion and IK(Ca) channel blockade have similar effects on rat colon motility and myenteric neurones. *Neurogastroenterol Motil* 2010; **22**: 98-107, e33 [PMID: 19788711 DOI: 10.1111/j.1365-2982.2009.01384.x]

20 **Wu RY**, Pasyk M, Wang B, Forsythe P, Bienenstock J, Mao YK, Sharma P, Stanisz AM, Kunze WA. Spatiotemporal maps reveal regional differences in the effects on gut motility for Lactobacillus reuteri and rhamnosus strains. *Neurogastroenterol Motil* 2013; **25**: e205-e214 [PMID: 23316914 DOI: 10.1111/nmo.12072]

21 **Kakunaga T**. The role of cell division in the malignant transformation of mouse cells treated with 3-methylcholanthrene. *Cancer Res* 1975; **35**: 1637-1642 [PMID: 1169110 DOI: 10.1046/j.1365-2982.2001.00282.x]

22 **Fida R**, Lyster DJ, Bywater RA, Taylor GS. Colonic migrating motor complexes (CMMCs) in the isolated mouse colon. *Neurogastroenterol Motil* 1997; **9**: 99-107 [PMID: 9198085 DOI: 10.1046/j.1365-2982.1997.d01-25.x]

23 **Smith TK**, Oliver GR, Hennig GW, O'Shea DM, Vanden Berghe P, Kang SH, Spencer NJ. A smooth muscle tone-dependent stretch-activated migrating motor pattern in isolated guinea-pig distal colon. *J Physiol* 2003; **551**: 955-969 [PMID: 12847208 DOI: 10.1113/jphysiol.2003.049163]

24 **Simpson JL**, Nadler HL. Maternal serum alpha-fetoprotein screening in 1987. *Obstet Gynecol* 1987; **69**: 134-135 [PMID: 2432449 DOI: 10.3389/fpsyg.2013.00863]

25 **Cumming G**, Finch S. Inference by eye: confidence intervals and how to read pictures of data. *Am Psychol* 2005; **60**: 170-180 [PMID: 15740449 DOI: 10.1037/0003-066X.60.2.170]

26 **Massi M**, Ioan P, Budriesi R, Chiarini A, Vitali B, Lammers KM, Gionchetti P, Campieri M, Lembo A, Brigidi P. Effects of probiotic

bacteria on gastrointestinal motility in guinea-pig isolated tissue. *World J Gastroenterol* 2006; **12**: 5987-5994 [PMID: 17009397 DOI: 10.3748/wjg.v12.i37.5987]

27 **Burks TF**, Peterson JM, Williams, CL, Kramer TH. Restraint stress and intestinal transit in rats. In: Bueno L, Collins, S, Junien JL. Stress and digestive motility. London: John Libbey, 1989: 115-122

28 **Al-Nedawi K**, Mian MF, Hossain N, Karimi K, Mao YK, Forsythe P, Min KK, Stanisz AM, Kunze WA, Bienenstock J. Gut commensal microvesicles reproduce parent bacterial signals to host immune and enteric nervous systems. *FASEB J* 2015; **29**: 684-695 [PMID: 25392266 DOI: 10.1096/fj.14-259721]

P- Reviewer: Bi X, Garcia-Olmo D, Islek A, Soyturk M, Welch MG, Zhang ZJ

S- Editor: Yu J **L- Editor**: A **E- Editor**: Zhang FF

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.v22.i48.10545

World J Gastroenterol 2016 December 28; 22(48): 10545-10556
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Effects of asymmetric dimethylarginine on renal arteries in portal hypertension and cirrhosis

Gloria Segarra, Belén Cortina, María Dolores Mauricio, Susana Novella, Paloma Lluch, Javier Navarrete-Navarro, Inmaculada Noguera, Pascual Medina

Gloria Segarra, Belén Cortina, María Dolores Mauricio, Susana Novella, Javier Navarrete-Navarro, Inmaculada Noguera, Pascual Medina, Department of Physiology, University of Valencia and Institute of Health Research INCLIVA, Hospital Clínico Universitario Valencia, 46010 Valencia, Spain

Paloma Lluch, Department of Gastroenterology and Hepatology and Institute of Health Research INCLIVA, Hospital Clínico Universitario Valencia, 46010 Valencia, Spain

Author contributions: Segarra G, Cortina B and Medina P designed and supervised the study; Segarra G, Cortina B, Mauricio MD, Novella S, Navarrete-Navarro J and Noguera I collected samples and performed the experiments; Segarra G, Cortina B, Mauricio MD, Novella S, Lluch P, Noguera I and Medina P acquired and analyzed the data; all authors drafted the article, made critical revisions related to the intellectual content of the manuscript and approved the final version of the article for publication.

Supported by Consellería de Sanidad of the Generalitat Valenciana, No. AP-052/08.

Institutional review board statement: The study was reviewed and approved by the University of Valencia Institutional Ethics Committee.

Institutional animal care and use committee statement: All protocols were approved by the Institutional Ethics Committee at the University of Valencia (No. UV20121124), and conformed to the Guide for the Care and Use of Laboratory Animals published in Directive 2010/63/EU of the European Parliament.

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

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Pascual Medina, PhD, Department of Physiology, University of Valencia and Institute of Health Research INCLIVA, Hospital Clínico Universitario Valencia, Avenida Blasco Ibáñez 15, 46010 Valencia, Spain. pascual.medina@uv.es
Telephone: +34-96-3864983
Fax: +34-96-3864642

Received: July 27, 2016
Peer-review started: July 30, 2016
First decision: August 29, 2016
Revised: September 17, 2016
Accepted: October 10, 2016
Article in press: October 10, 2016
Published online: December 28, 2016

Abstract

AIM

To evaluate the effects of asymmetric dimethylarginine (ADMA) in renal arteries from portal hypertensive and cirrhotic rats.

METHODS

Rat renal arteries from Sham (n = 15), pre-hepatic portal hypertension (PPVL; n = 15) and bile duct ligation and excision-induced cirrhosis (BDL; n = 15) were precontracted with norepinephrine, and additional contractions were induced with ADMA (10^{-6}-10^{-3} mol/L), an endogenous inhibitor of nitric oxide (NO) synthase. Concentration-response curves to acetylcholine (1 × 10^{-9}-3 × 10^{-6} mol/L) were determined in precontracted

renal artery segments with norepinephrine in the absence and in the presence of ADMA. Kidneys were collected to determine the protein expression and activity of dimethylarginine dimethylaminohydrolase (DDAH), an enzyme that catabolizes ADMA.

RESULTS

In renal arteries precontracted with norepinephrine, ADMA caused endothelium-dependent contractions. The pD_2 values to ADMA were similar in the Sham and PPVL groups (4.20 ± 0.08 and 4.11 ± 0.09, $P > 0.05$, respectively), but were lower than those of the BDL group (4.79 ± 0.16, $P < 0.05$). Acetylcholine-induced endothelium-dependent relaxation that did not differ, in terms of pD_2 and maximal relaxation, among the 3 groups studied. Treatment with ADMA (3×10^{-4} mol/L) inhibited acetylcholine-induced relaxation in the 3 groups, but the inhibition was higher ($P < 0.05$) in the BDL group compared with that for the Sham and PPVL groups. The mRNA and protein expression of DDAH-1 were similar in kidneys from the three groups. Conversely, DDAH-2 expression was increased ($P < 0.05$) in PPVL and further enhanced ($P < 0.05$) in the BDL group. However, renal DDAH activity was significantly decreased in the BDL group.

CONCLUSION

Cirrhosis increased the inhibitory effect of ADMA on basal- and induced-release of NO in renal arteries, and decreased DDAH activity in the kidney.

Key words: Portal hypertension; Cirrhosis; Nitric oxide; Asymmetric dimethylarginine; Nitric oxide inhibitors; Dimethylarginine dimethylaminohydrolase

Core tip: Cirrhosis is associated with renal dysfunction and renal vasoconstriction. This constriction leads to decreased renal blood flow and glomerular filtration. Decreased nitric oxide (NO) bioavailability is involved in these effects. Although plasma levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, are increased in cirrhosis, the effects of ADMA on renal arteries under this pathological condition are unknown. Therefore, the present work studied the effects of ADMA on basal- and stimulated-NO release in renal arteries from portal hypertensive and cirrhotic rats and the renal expression and activity of dimethylarginine dimethylaminohydrolase, an enzyme that catabolizes ADMA.

Segarra G, Cortina B, Mauricio MD, Novella S, Lluch P, Navarrete-Navarro J, Noguera I, Medina P. Effects of asymmetric dimethylarginine on renal arteries in portal hypertension and cirrhosis. *World J Gastroenterol* 2016; 22(48): 10545-10556 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10545.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10545

INTRODUCTION

The progression of cirrhosis is frequently associated with an impairment in renal function manifested by the appearance of sodium and water retention and the accumulation of fluid within the interstitial tissue and peritoneal cavity[1]. As the disease develops, vasoconstriction of the renal vascular bed commonly results in a reduced glomerular filtration rate and eventually in renal failure[2]. The mechanisms leading to renal dysfunction in cirrhosis involve the activation of the vasoconstrictor and sodium-retaining systems in an attempt to preserve the tubular function[1].

In the kidney, nitric oxide (NO) has numerous physiological roles including the regulation of renal hemodynamics[3,4]. Studies using NO synthase (NOS) inhibitors have demonstrated that NO plays a significant role in maintaining normal vascular tone in the renal vascular bed[4,5]. Basal release of NO from the vessel wall has been described in humans[6,7] and in human renal artery[8]. In the kidney, the basal release of NO induces a substantially lower vascular resistance compared to other organs[4,5].

The plasma levels of N^G,N^G-asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor[9], are significantly increased in various pathological conditions, including end-stage chronic renal failure[10,11], cirrhosis[12-14] and hepatorenal syndrome[15]. In human renal artery, ADMA induces a progressive inhibition of NO synthesis and a diminished response to endothelium-mediated relaxation[8]. In spite of the increased ADMA plasma levels in patients with cirrhosis and hepatorenal syndrome, the role of ADMA in renal dysfunction associated to cirrhosis has almost been overlooked, and no attempt has been made to determine the effects of ADMA on the vascular tone of renal arteries during portal hypertension and cirrhosis.

Dimethylarginine dimethylaminohydrolase (DDAHs) degrade ADMA to citrulline and dimethylamine, whereas N^G-nitro-L-arginine methyl ester (L-NAME), another inhibitor of NOS, is not degraded by DDAHs[16,17]. DDAHs are expressed as type 1 and 2 isoforms[18] and are widely distributed in various organs and tissues, including the kidney and renal vascular bed[18-20].

The present study hypothesized that one mechanism involved in the renal vasoconstriction associated with cirrhosis could be the elevated levels of ADMA in cirrhosis that may decrease basal- and induced-release of NO by the endothelium of renal vessels. To verify this, the present study investigated the effects of ADMA and L-NAME on both the basal, as well as the stimulated release of NO in the renal arteries of rats with pre-hepatic portal hypertension without cirrhosis and in rats with portal hypertension and secondary biliary cirrhosis induced by ligation and excision of the bile duct. Furthermore, it assessed the effects of portal hypertension and cirrhosis on the renal expression of DDAH-1, DDAH-2 and renal DDAH activity.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200-250 g) were acquired from Charles River, and housed according to institutional guidelines (constant room temperature 22 ℃, 12 h light/dark cycle, 60% humidity, standard rat chow and water *ad libitum*). All protocols were approved by the Institutional Ethics Committee at the University of Valencia (No. UV20121124), and conformed to the Guide for the Care and Use of Laboratory Animals published in Directive 2010/63/EU of the European Parliament.

Rats were assigned to a sham-operated (Sham) group (n = 15), partial portal vein ligation (PPVL) group (n = 15) or bile duct ligation and excision (BDL) group (n = 15) in a random way. After induction of anesthesia by isoflurane (5%, by induction chamber), rats received isoflurane 2%-3% by mask. To assess the adequacy of anesthesia during the surgery, parameters such as responsiveness (*e.g.*, no response to toe pinching), respiratory rate, and heart rate were monitored. Analgesia with Butorphanol was used pre-operatively for preemptive analgesia and post-operatively every 4-12 h during the day of the surgery.

Surgical procedures

Surgical procedures were performed as described previously[21]. Briefly, pre-hepatic portal hypertension induced by partial portal vein ligation was performed by placing a 20-gauge needle on the portal vein. A non-absorbable surgical thread ligature was placed around the needle and portal vein, and the needle was then withdrawn. The studies were performed 14-16 d after PPVL, when the hyperdynamic circulation accompanying portal hypertension was fully established. Secondary biliary cirrhosis was induced by bile duct ligation and excision. The bile duct was cut between a ligature close to the hilum of the liver and another one close to the duodenum. The studies were performed 28 d after BDL when secondary biliary cirrhosis had developed. For the sham operation, the duodenum, portal vein, and bile duct were exposed during laparotomy, and the abdomen was closed 15 min later.

On the day of the experiment, mean arterial pressure (MAP) and portal pressure (PP) measurements were performed while the rats were kept under isoflurane anesthesia, as previously described[21]. Briefly, MAP and PP were measured by catheterization of the right carotid artery and ileocolic vein, respectively. Pressure was transmitted through a Statham pressure transducer and recorded continuously. The external zero reference was placed at the midportion of the rat.

Biochemical analysis

Blood drawn from the carotid artery in the anesthetized rat was collected after hemodynamic assay. The plasma was separated and stored at -20 ℃ until total bilirubin and creatinine levels were assayed in an autoanalyzer, according to the manufacturer's instructions.

Isolated rat renal artery preparation

The renal arteries were isolated and cleaned of connective tissue under a dissecting microscope. Segments (4 mm in length) of renal artery were cut for isometric recording of tension. Outside diameter of the rings was measured using an ocular micrometer within a Wild M8 zoom microscope (Heerbrugg, Switzerland) and ranged from 0.8 to 1.4 mm. In some experiments the endothelium was removed mechanically by inserting a roughened stainless-steel wire into the lumen and gently rolling the vessel ring on wet filter paper.

Two stainless-steel holders (100 μm in diameter) were introduced through the arterial lumen and placed in a 5 mL tissue bath containing modified Krebs-Henseleit solution of the following mmol/L composition: NaCl 115; KCl 4.6; KH_2PO_4 1.2; $MgCl_2$ 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25; glucose 11.1; EDTA 0.01, pH 7.3-7.4. Indomethacin (10^{-5} mol/L) was added to the Krebs-Henseleit solution in order to block the cyclooxygenase-derived substances that could interfere with the effects of the NOS inhibitors. The solution was continuously gassed with 95% O_2-5% CO_2 while the temperature was maintained at 37 ℃ with a circulating water jacket and a heat pump. One holder was fixed to the organ bath wall and the other was connected to a strain gauge (model FT03; Grass Instruments Division of Astro-Med Inc, United States). Changes in isometric force were recorded by use of Chart v. 4.2.3 software and a MacLab/8e data acquisition system (ADInstruments, Australia). Once the optimal resting tension was reached (1 g), each ring was allowed to attain this steady level of tension during a 1-h accommodation period before testing. Following this, smooth muscle function was assessed by exposing the arterial rings to receptor-independent depolarizing agent KCl (60 mmol/L) until the contraction reached a stable plateau. After washout and return to stable baseline, functional integrity of the endothelium was confirmed routinely by the presence of relaxation induced by acetylcholine (10^{-6} mol/L) during contraction obtained with norepinephrine (3×10^{-7} to 1×10^{-6} mol/L). Arteries in which acetylcholine reversed the norepinephrine-induced tone by more than 70% were designated as endothelium intact and arteries in which acetylcholine caused less than 15% relaxation were designated as without endothelium.

To assess the effects of portal hypertension and cirrhosis on renal artery contractility, we performed in artery rings from each group cumulative concentration-response curves to KCl (10-120 mmol/L), an agent that induces contraction by facilitating Ca^{2+} entry through voltage-dependent Ca^{2+} channels.

The basal release of NO is revealed when endothelium-intact artery rings are precontracted and an

additional contraction is induced by NOS inhibitor. This additional contraction provides a functional indication of NO release. Therefore, the ability of ADMA (10^{-6}-10^{-3} mol/L) or L-NAME (10^{-6}-10^{-3} mol/L) to inhibit basal activity of NO was assessed from its enhancement of low-levels of contraction (approximately 200-300 mg) induced by norepinephrine (1×10^{-7}-3×10^{-7} mol/L) in endothelium-containing renal artery rings. The ability of L-arginine (10^{-3} mol/L) to either protect against or reverse the enhancement by ADMA or L-NAME was also assessed. Additionally, the effects of both ADMA and L-NAME were examined on norepinephrine-induced tone in endothelium-denuded rings.

Concentration-response curves to acetylcholine (1×10^{-9}-3×10^{-6} mol/L), an endothelium-dependent vasorelaxant, were determined in precontracted segments with norepinephrine (3×10^{-6} mol/L), in the absence and in the presence of ADMA (3×10^{-4} mol/L) or L-NAME (3×10^{-4} mol/L) that were added to the organ bath 20 min before starting the concentration-response curve.

All substances and drugs were purchased from Sigma-Aldrich Chemical Co. (United States). Drugs were prepared and diluted in distilled water except for indomethacin, which was dissolved in absolute ethanol. Stock solutions of the drugs were freshly prepared every day.

Real Time PCR analyses

Samples of cortical tissue from the kidney of each rat were immediately collected into RNAlater RNA stabilization reagent (Thermo Fisher Scientific, United States) following the manufacturer's instructions. Total RNA was isolated and reverse transcribed as previously described[21]. Ready-to-use primers and probes from the Assay-on-demand service of Applied Biosystems were used for the quantification of DDAH-1 and DDAH-2 (Rn00574200_m1 and Rn01525775_g1, respectively) and endogenous reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 4352338E). The qRT-PCR was carried out using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, United States). Samples were run in triplicate and fold changes were generated for each sample by calculating $2^{-\Delta\Delta Ct}$[22].

Western blotting

Equal amounts of protein from renal cortical homogenates (100 μg total protein) were resolved in SDS-PAGE on 12% gels and electroblotted onto polyvinylidene difluoride membranes. After 1 h blocking with 5% milk in phosphate-buffered saline with 0.1% (v/v) Tween 20 (PBST), membranes were incubated in PBST containing 0.1% milk with a specific primary antibody: monoclonal goat anti-rat DDAH1 antibody (Santa Cruz Biotechnology, United States; 1:500 dilution, overnight incubation at 4 ℃) or goat anti-rat DDAH2 antibody (Santa Cruz Biotechnology, United States, 1:200 dilution, overnight incubation at 4 ℃). After 4 washes with PBST, membranes were incubated for 1 h with a horseradish peroxidase-labeled antibody at a 1:2000 dilution in PBST containing 1% milk. After 4 additional washes, the membranes were incubated with chemiluminescent reagent according to the manufacturer's protocols (Inmuno-Star™ HRP Substrate Kit, Bio-Rad, United States), and the chemiluminescent signal was visualized by the LAS-1000 imaging system (Fujifilm, Japan). Densitometric analyses of Western blots were performed using Image Reader LAS-1000 Pro v2.3 software. All membranes were reblogged using a monoclonal antibody anti β-actin (1:2500, Sigma-Aldrich, United States) as a loading control. Data were normalized to corresponding values of β-actin densitometry.

DDAH activity

DDAH activity was measured as described previously by a colorimetric assay[23]. Kidney cortex was homogenized in 5 volumes of 0.1 mol/L sodium phosphate buffer (pH 6.5). Protein concentration of the homogenate was determined using the BCA protein assay (Thermo Fisher, United States) according to the manufacturer's instructions. The final protein concentration of the homogenate was adjusted to 20 mg/mL with sodium phosphate buffer. Then, 100 μL of homogenate were preincubated with urease (100 U/mL) for 15 min at 37 ℃, and then incubated with 1 mmol/L ADMA for 60 min at 37 ℃. After deproteinization with 0.5 mL of 4% sulfosalicylic acid, 100 μL of supernatant was incubated with 100 μL of a mixture composed by one part of diacetyl monoxime (0.8% wt/v in 5% acetic acid) and two parts of antipyrine (0.5% wt/v in 50% sulfuric acid) at 90 ℃ for 1 h. Each sample was analyzed with a paired blank, in which ADMA was omitted. The amounts of L-citrulline formed were determined by spectrophotometry at 466 nm. The DDAH activity was represented as μmol L-citrulline formatted/g protein/min at 37 ℃.

Statistical analysis

All values are expressed as mean ± SEM. The contractile effects were expressed as absolute tension (milligrams-force). Relaxation was expressed as a percentage of the norepinephrine-induced contraction. The pD_2 (negative logarithm of the molar concentration at which half-maximum response occurs) was determined from individual concentration-response curves by non-linear regression analysis. Area under the concentration-response curve (AUC) was calculated from each individual concentration-response curve to acetylcholine and was expressed as arbitrary units. The contribution of NO to the vascular relaxation induced by acetylcholine was calculated by subtracting from the AUC for acetylcholine the AUC for acetylcholine in the presence of L-NAME or ADMA. All *n* values are presented as the number of rats.

Table 1 Morphological characteristics, hemodynamic and biochemical parameters of the Sham, partial portal vein ligation, and bile duct ligation groups

	Sham	PPVL	BDL
Body weight gain (g)	39 ± 6	35 ± 6	10 ± 8[a,c]
Spleen weight (g)	0.7± 0.1	1.3 ± 0.1[a]	1.4 ± 0.2[a]
Liver weight (g)	11.6 ± 0.6	10.9 ± 0.7	16.9 ± 0.7[a,c]
Mean arterial pressure (mmHg)	116 ± 9	95 ± 5[a]	92 ± 6[a]
Portal pressure (mmHg)	7 ± 1	17 ± 2[a]	19 ± 3[a]
Bilirubin (mg/dL)	0.12 ± 0.03	0.15 ± 0.03	9.91 ± 0.07[a,c]
Creatinine (mg/dL)	0.75 ± 0.07	0.73 ± 0.06	0.78 ± 0.06

[a]$P < 0.05$ *vs* Sham group and [c]$P < 0.05$ *vs* PPVL group. PPVL: Partial portal vein ligation; BDL: Bile duct ligation.

Table 2 pD_2 values and maximal responses of the concentration-response curves to KCl (10-120 mmol/L) in renal arteries from Sham, partial portal vein ligation and bile duct ligation groups

	n	pD_2	Emax (mg)
Sham	8	1.49 ± 0.01	1018 ± 83
PPVL	8	1.46 ± 0.01	1050 ± 131
BDL	8	1.46 ± 0.01	762 ± 59[a,c]

pD_2, - log M of KCl causing 50% of the maximal contraction; Emax, maximal contraction; *n* = number of rats; [a]$P < 0.05$ *vs* Sham group and [c]$P < 0.05$ *vs* PPVL group.

One- or two-way analyses of variance (ANOVA) were performed followed by Bonferroni's post-test. The level of statistical significance was $P < 0.05$. The statistical analysis was carried out using Prism 4 software (GraphPad Software Inc., United States).

RESULTS

Morphological features, hemodynamic and biochemical parameters

Morphological characteristics, hemodynamic, and biochemical parameters of the Sham, PPVL, and BDL groups are summarized in Table 1. Both the PPVL and BDL groups led to the characteristic hemodynamic changes found in portal hypertension, with higher values in PP and lower MAP compared to the Sham rats, suggesting the presence of a hyperdynamic state. As expected, the PPVL and BDL groups exhibited higher spleen weights than did Sham rats. In the BDL group, the rats became visibly icteric by the 3rd wk following surgery, weight gain was decreased, and they had higher total bilirubin values than the Sham or PPVL rats. Creatinine concentrations were within the normal range in the three groups. The Sham rats displayed normal post-operative recovery.

Effects of KCl

In the Sham group, KCl caused concentration-dependent contractions with a pD_2 of 1.49 ± 0.01 and a maximal contraction of 1018 ± 83 mg (Figure 1 and Table 2). In the PPVL group, neither maximal contraction nor pD_2 values to KCl were affected (Figure 1 and Table 2). In the renal artery rings of the BDL group, maximal contraction to KCl was decreased ($P < 0.05$) compared to the Sham and PPVL groups (Figure 1 and Table 2). There were no differences among groups in the sensitivity to KCl as demonstrated by similar pD_2 values (Table 2).

Table 3 pD_2 values and maximal responses of the concentration-response curves to N^G-nitro-L-arginine methyl ester and asymmetric dimethylarginine in renal arteries from Sham, partial portal vein ligation, and bile duct ligation groups, after precontraction with norepinephrine

	n	pD_2	Emax (mg)
Sham			
L-NAME	8	5.35 ± 0.17	370 ± 36
ADMA	8	4.20 ± 0.08[e]	400 ± 26
PPVL			
L-NAME	8	5.30 ± 0.16	365 ± 30
ADMA	8	4.11 ± 0.09[e]	388 ± 25
BDL			
L-NAME	8	5.24 ± 0.16	285 ± 29[a,c]
ADMA	8	4.79 ± 0.16[a,c,e]	310 ± 27[a,c]

pD_2, - log M of substance causing 50% of the maximal contraction; Emax, maximal contraction; *n* = number of rats; [a]$P < 0.05$ *vs* Sham group with the same treatment, [c]$P < 0.05$ *vs* PPVL group with the same treatment and [e]$P < 0.05$ *vs* L-NAME treatment in the same group. ADMA: Asymmetric dimethylarginine; L-NAME: N^G-nitro-L-arginine methyl ester.

Effects of NOS inhibitors on basal NO

At resting tension, the addition of L-NAME (10^{-6}-10^{-3} mol/L) or ADMA (10^{-6}-10^{-3} mol/L) did not show significant changes in tension (results not shown). Following the induction of a low level of contraction (210 ± 50 mg) with norepinephrine (1×10^{-7}-3×10^{-7} mol/L), the addition of L-NAME (10^{-6}-10^{-3} mol/L) or ADMA (10^{-6}-10^{-3} mol/L) led to concentration-dependent increases in tension (Figure 2). The pD_2 values for the concentration-response curves to L-NAME were similar in the Sham, PPVL and BDL groups (Table 3). The pD_2 values for the ADMA curves were similar in Sham and PPVL, but were lower ($P < 0.05$) than those for the BDL group, suggesting an increased sensitivity to ADMA in renal arteries from cirrhotic rats. In the Sham, PPVL and BDL groups, pD_2 values of the ADMA curves were lower ($P < 0.05$) than those for L-NAME, suggesting a decreased sensitivity to ADMA in all groups. The maximal responses to ADMA and L-NAME were similar in the Sham and PPVL groups (Figure 2 and Table 3). Conversely, in the BDL group the maximal responses to both ADMA and L-NAME were reduced ($P < 0.05$) compared with those for Sham and PPVL rats. The contractile effect induced by ADMA and L-NAME was prevented or reverted by L-arginine 10^{-3} mol/L, a precursor of NO synthesis, in all the groups studied (Figure 2).

Table 4 pD_2 and maximal response values for the concentration-response curves to acetylcholine in renal arteries from Sham, partial portal vein ligation, and bile duct ligation groups in the absence (Control) and in the presence of N^G-nitro-L-arginine methyl ester or asymmetric dimethylarginine

Acetylcholine	*n*	pD_2	Emax (%)
Sham			
Control	8	7.95 ± 0.08	93 ± 3
L-NAME (3×10^{-4} mol/L)	8	7.13 ± 0.28[a]	24 ± 7[a]
ADMA (3×10^{-4} mol/L)	8	7.27 ± 0.13[a,c]	60 ± 3[a,c]
PPVL			
Control	8	7.92 ± 0.08	94 ± 2
L-NAME (3×10^{-4} mol/L)	8	7.24 ± 0.11[a]	21 ± 3[a]
ADMA (3×10^{-4} mol/L)	8	7.18 ± 0.15[a]	65 ± 6[a,c]
BDL			
Control	8	7.91 ± 0.10	95 ± 3
L-NAME (3×10^{-4} mol/L)	8	7.13 ± 0.35[a]	24 ± 8[a]
ADMA (3×10^{-4} mol/L)	8	7.17 ± 0.22[a]	32 ± 6[a,c,e]

pD_2, - log M of acetylcholine causing 50% of the maximal relaxation; Emax, maximal relaxation expressed as a percentage of the contraction in response to 3×10^{-6} mol/L norepinephrine; *n* = number of rats. [a]*P* < 0.05 *vs* control group, [c]*P* < 0.05 *vs* L-NAME treated group and [e]*P* < 0.05 *vs* Sham and PPVL groups with the same treatment. ADMA: Asymmetric dimethylarginine; L-NAME: N^G-nitro-L-arginine methyl ester.

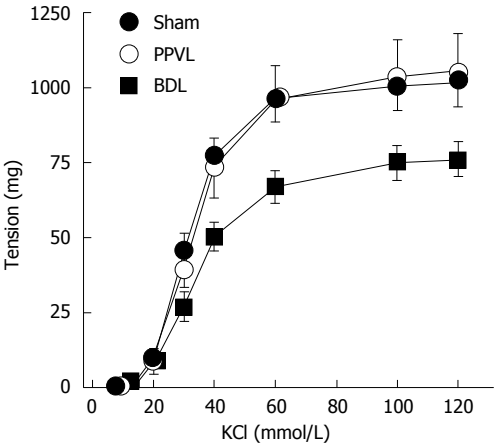

Figure 1 Effects of portal hypertension and cirrhosis on contractile effects induced by high extracellular concentrations of KCl in rat renal arteries. PPVL: Pre-hepatic portal hypertension; BDL: Bile duct ligation.

Effects of NOS inhibitors on acetylcholine-induced relaxation

In renal arteries from the Sham group, acetylcholine (1×10^{-9}-3×10^{-6} mol/L) caused endothelium-dependent relaxation (pD_2 = 7.95 ± 0.08 and Emax = 93% ± 3%) in rings precontracted with norepinephrine (Figure 3A). The relaxation induced by acetylcholine did not differ, in terms of pD_2 and maximal relaxation, among the 3 groups studied (Figure 3A and Table 4). No relaxation was observed in response to acetylcholine in renal arteries without endothelium (Figure 3A).

The relaxation induced by acetylcholine was inhibited by the treatment with L-NAME (3×10^{-4} mol/L) in renal arteries from the three groups. In the Sham, PPVL and BDL groups, the inhibitions of

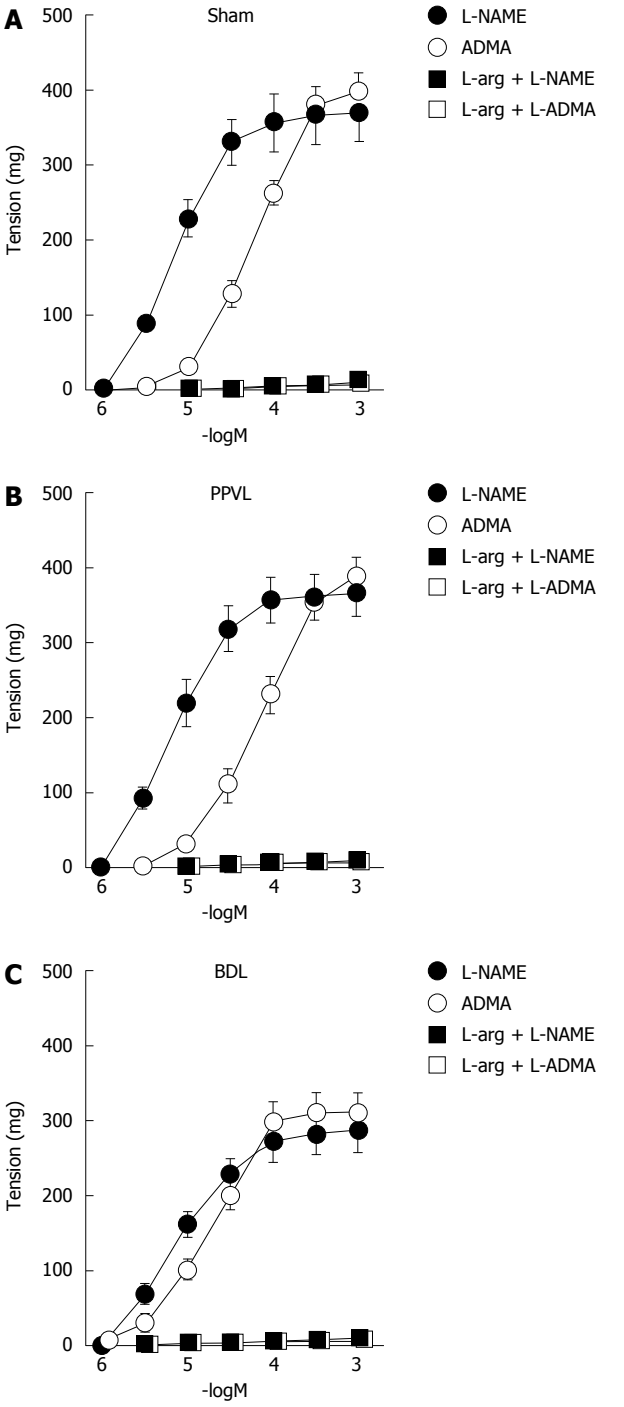

Figure 2 Effects of nitric oxide synthase inhibitors on basal nitric oxide release in renal artery. Contractions induced by L-NAME (*n* = 8) and ADMA (*n* = 8) on rings of rat renal artery with endothelium from Sham, PPVL, and BDL groups in the absence and in the presence of L-arginine (L-arg, 10^{-3} mol/L, *n* = 6). Contractions were determined after evoking submaximal tone with 10^{-7}-3×10^{-7} mol/L norepinephrine. PPVL: Pre-hepatic portal hypertension; BDL: Bile duct ligation; ADMA: Asymmetric dimethylarginine; L-NAME: N^G-nitro-L-arginine methyl ester.

maximal relaxations induced by acetylcholine in the presence of L-NAME were similar (*P* > 0.05) 69% ± 4%, 73% ± 3%, and 71% ± 5%, respectively (Figure 3A and Table 4). The pD_2 values decreased (*P* < 0.05) likewise in the presence of L-NAME compared to those for the untreated segments, providing evidence that

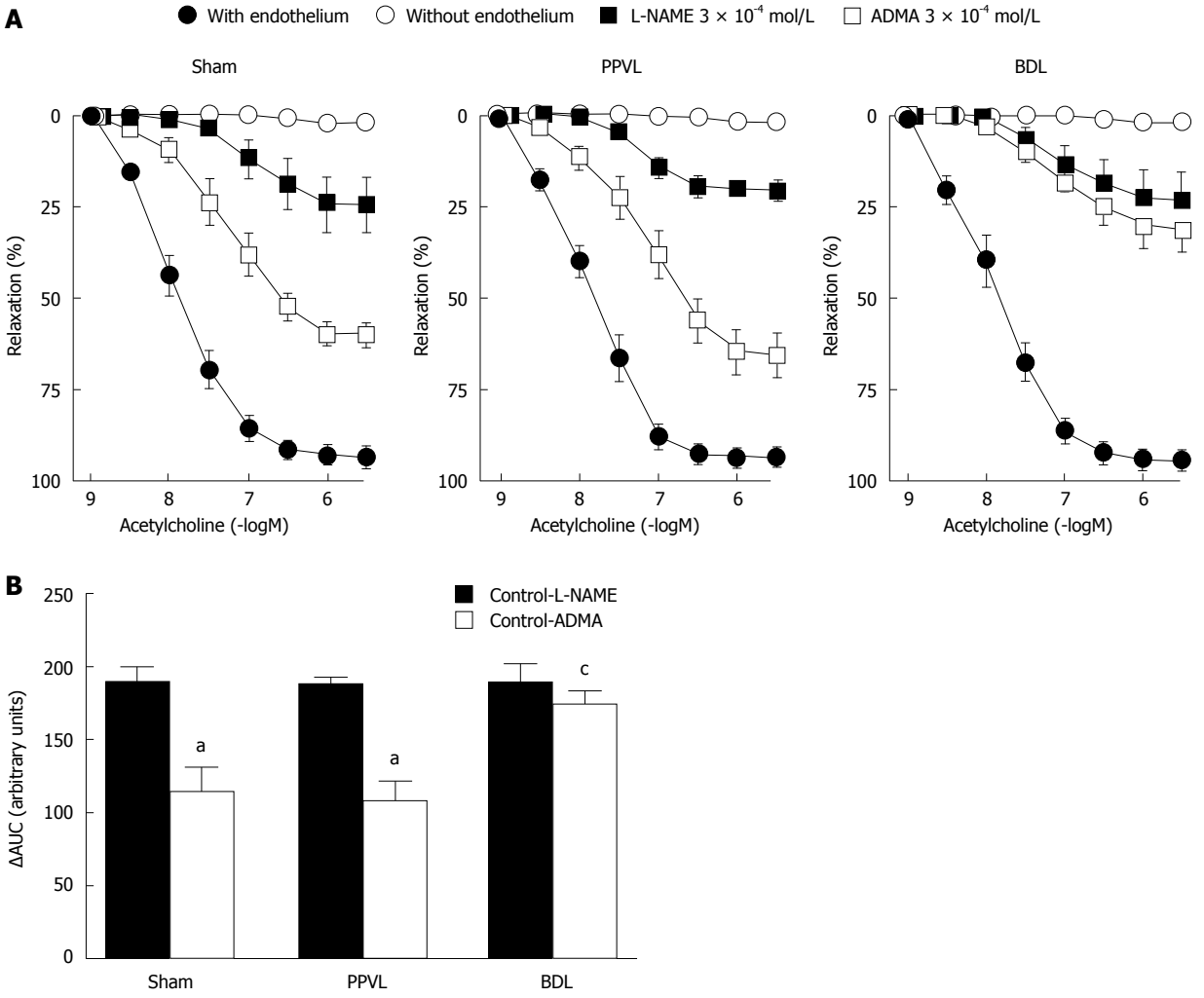

Figure 3 Effects of nitric oxide synthase inhibitors on acetylcholine-induced relaxation in renal artery. A: Concentration-response curves to acetylcholine on rings of rat renal artery from Sham, PPVL, and BDL groups in artery rings with endothelium (n = 8) and without endothelium (n = 6) and in artery rings with endothelium in the presence of L-NAME (3 × 10^{-4} mol/L; n = 8) or ADMA (3 × 10^{-4} mol/L; n = 8). Relaxation is expressed as a percentage of the contraction in response to norepinephrine; B: Difference between the areas under curves (AUCs) from artery rings with endothelium (Control) and treated with L-NAME or ADMA. $^{a}P < 0.05$ *vs* L-NAME treated group and $^{c}P < 0.05$ *vs* Sham and PPVL groups treated with ADMA. PPVL: Pre-hepatic portal hypertension; BDL: Bile duct ligation.

NO induced by acetylcholine is similar in the three groups. Treatment with ADMA (3 ×10^{-4} mol/L) inhibited acetylcholine-induced relaxation in the 3 groups, but the inhibition was higher ($P < 0.05$) in the BDL group compared with the Sham and PPVL groups (Figure 3A and Table 4). In renal arteries from the BDL group, ADMA induced a greater ($P < 0.05$) inhibition of maximal relaxation than it did in the Sham and PPVL groups, but sensitivity (evidenced by pD_2 values) was unchanged (Figure 3A and Table 4). When areas under the curve (AUC) were analyzed, L-NAME inhibited the NO-mediated relaxation similarly in the 3 groups (Figure 3B). Likewise, ADMA inhibited NO release in the Sham and PPVL groups; in BDL group the inhibitory effects of ADMA were increased (Figure 3B).

Expression of DDAHs

We performed real-time RT-PCR on kidneys from the Sham, PPVL, and BDL groups (n = 6 per group). The DDAH-1 mRNA expression was similar in kidneys from the three groups (Figure 4A). In contrast, the DDAH-2 mRNA expression was increased ($P < 0.05$) in the PPVL compared to that for the Sham group, and in the BDL group, it was further enhanced (Figure 4B). The level of DDAH-2 mRNA expression in PPVL and BDL rats increased 1.33- and 1.64-fold, respectively. Densitometry analysis of Western blot confirmed that DDAH 1 was equally expressed in the kidney of the three groups (Figure 4C and E). Conversely, DDAH-2 expression in kidney was increased in the PPVL group and further increased in the BDL group (Figure 4D and F).

DDAH activity

We determined the effects of portal hypertension and cirrhosis on renal DDAH activity in crude tissue lysates. Renal DDAH activity was increased, but not significantly ($P > 0.05$), in kidneys from the PPVL group. The DDAH activity in the BDL group, however, was significantly reduced (61% ± 7%, $P < 0.05$) compared to that for the Sham and PPVL groups (Figure 5).

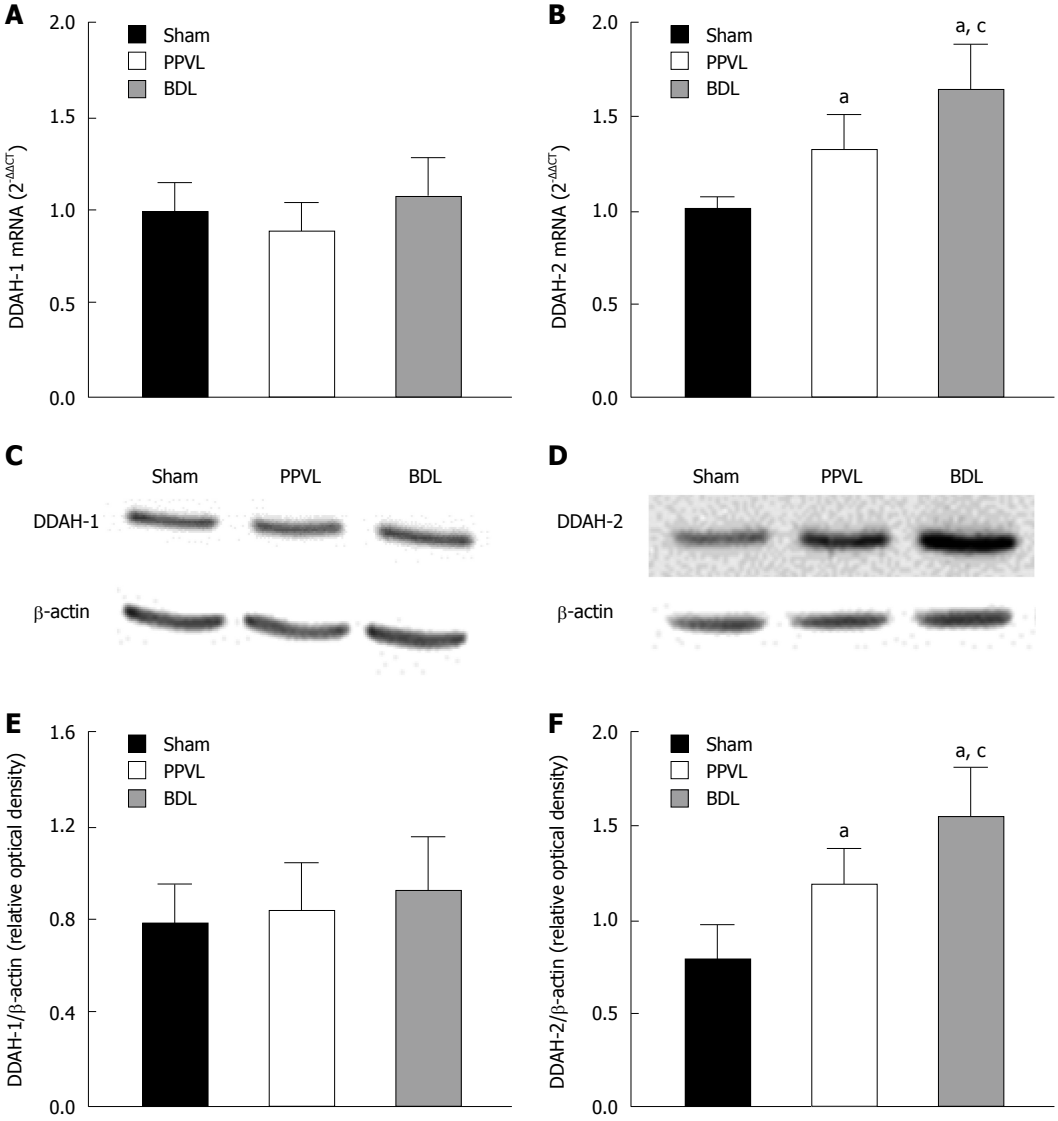

Figure 4 DDAH1 and DDAH2 expression in kidneys from portal hypertensive and cirrhotic rats. A and B: DDAH1 and DDAH2 mRNA expression in kidney from Sham, PPVL, and BDL groups normalized to the expression of GAPDH, which was used as an endogenous reference gene; C and D: Immunoblot analysis in single kidney probed with antibodies against DDAH1, DDAH2 or β-actin, as indicated; E and F: Graphs show the results of densitometric analyses from pooled data, plotted as optical densitometry relative to the signal obtained by β-actin. Each data set represents the mean ± SEM derived from 6 independent experiments. $^{a}P < 0.05$ *vs* Sham group and $^{c}P < 0.05$ *vs* PPVL group. DDAH: Dimethylarginine dimethylaminohydrolase.

DISCUSSION

The results of the present study demonstrate that both the basal- and induced-release of NO are inhibited by ADMA, with a higher effect in renal arteries from rats with secondary biliary cirrhosis. The increased effect of ADMA inhibiting NO synthesis together with decreased renal DDAH activity indicates that the accumulation of ADMA during cirrhosis could make the renal artery prone to vasoconstriction.

One finding of the present report demonstrates a decreased contraction in renal arteries from cirrhotic rats in response to a high extracellular K^+ concentration, which causes the depolarization and subsequent opening of voltage-dependent Ca^{2+} channels. Vascular hypocontractility in cirrhosis is a multifactorial phenomenon where several mechanisms have been identified and contribute to impaired vasoconstriction. These include the overproduction of vasodilators and decreased responsiveness to vasoconstrictors. Although NO overproduction is widely accepted as main culprit of vasodilation in cirrhosis[24-26], several studies have shown that other factors besides NO are involved in the pathogenesis of vascular hypocontractility. It is known that in cirrhosis, a component of hypocontractility is found in isolated vessels, even though the endothelium is removed and NOS is pharmacologically inhibited[27-29]. It is noteworthy that the cirrhosis-impaired Rho kinase pathway results in decreased phosphorylation of Ca^{2+} sensitizing proteins, increased myosin light chain phosphatase activity and decreased Ca^{2+} sensitivity[30]. Although our results *in vitro* show a reduced contraction of renal arteries during cirrhosis, the *in vivo* activation of vasoactive systems on renal circulation

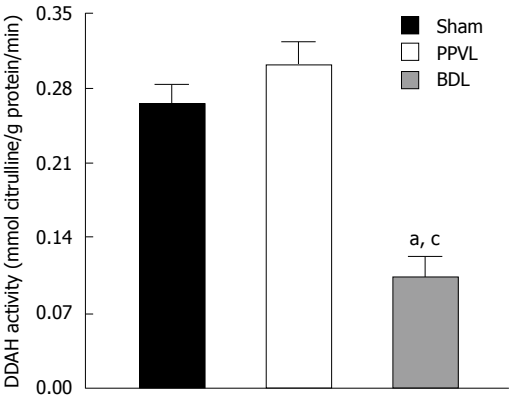

Figure 5 Effects of portal hypertension and cirrhosis on renal dimethylarginine dimethylaminohydrolase activity. Bar graphs represent DDAH activity in kidney from Sham, PPVL, and BDL groups. Each data set represents the mean ± SEM derived from 6 independent experiments. $^{a}P < 0.05$ *vs* Sham group and $^{c}P < 0.05$ *vs* PPVL group. DDAH: Dimethylarginine dimethylaminohydrolase.

during hyperdynamic circulation associated to portal hypertension and cirrhosis[1] could develop into an excessive contraction of renal artery.

The basal release of NO was determined indirectly by measuring the effects of ADMA and L-NAME in precontracted artery rings. We found that in renal arteries both NOS inhibitors markedly increased the vascular tone, suggesting an important basal NO synthesis. The contractile effects induced by NOS inhibitors were endothelium-dependent and reversed by L-arginine, the substrate for NO synthesis, thus demonstrating that ADMA and L-NAME increase arterial tone by inhibiting the basal release of endothelial NO.

The maximal contraction induced by NOS inhibitors in renal arteries from cirrhotic rats was lower than those in control or portal hypertensive rats. The hypocontractility cannot be attributed to a lower basal release of NO, since the smooth muscle of renal arteries from cirrhotic rats showed hypocontractility in response to KCl. Therefore, differences in the level of contraction in response to NOS inhibitors would not reflect changes in basal NO generation.

That notwithstanding, we found a similar sensitivity to L-NAME in the three groups studied. Furthermore, when comparing the sensitivity of the two inhibitors, a decreased sensitivity to ADMA as compared with that for L-NAME was observed in the three groups. The concentration-response curve to ADMA was significantly displaced to the left for BDL, as compared to those for Sham and PPVL rats; this represents indirect evidence of a decreased ability of DDAH to catabolize ADMA. Interestingly, no significant differences in the contractile response to ADMA were observed between Sham and PPVL rats. Since BDL and PPVL rats had similar increases in portal pressure, and liver damage was only present in the BDL group, it is conceivable that in renal arteries the liver dysfunction is the main factor causing the altered responses to the ADMA.

Since DDAH is highly specific for the degradation of ADMA, but not L-NAME[16], these changes in ADMA sensitivity could be related to changes in DDAH activity. Another study addressing the localization of DDAH and NOS in the rat kidney has shown co-localization of the two enzymes[20]. Therefore, the close relationship between DDAH and NOS in the kidney supports the idea that DDAH regulates ADMA levels and NOS activity[19,31]. The increased sensitivity to ADMA in renal arteries from the BDL group offers a reasonable indication that decreased DDAH activity and the accumulation of ADMA occur in the vessel wall, enhancing the inhibitory effect on NO biosynthesis.

Both ADMA and L-NAME inhibited acetylcholine-induced relaxation in renal arteries indicating that NO pathways contribute to this effect. As expected, L-NAME markedly inhibited the relaxation induced by acetylcholine. Although it has been demonstrated that ADMA preferentially blocks basal NO release, but it has little effect on acetylcholine-induced relaxation[7,21,32], ADMA markedly inhibited the acetylcholine-induced relaxation in renal arteries from cirrhotic rats. It has been demonstrated that ADMA inhibits basal- and stimulated-release of NO in human renal arteries[8] and other arterial beds where acetylcholine-induced relaxation is mainly dependent on endothelial NO, such as the human middle cerebral artery[33] and internal mammary artery[34].

The present functional analyses in renal arteries from cirrhotic rats demonstrates for the first time that the increased ability of ADMA to inhibit NOS could be related, at least in part, to a lower activity of DDAH and a lesser degradation of ADMA. This reinforces the role of DDAH in controlling the NO bioavailability, and its impairment during cirrhosis might be a mechanism involved in the increased renal artery contraction during cirrhosis.

The study shows that the mRNA and protein expressions of DDAH-1 were unchanged in kidneys from the PPVL and BDL groups, thus suggesting that portal hypertension and cirrhosis do not control renal DDAH-1 expression. The present results confirm previous studies demonstrating unchanged levels of DDAH-1 expression in kidneys from young cirrhotic rats[35]. In contrast, it has been demonstrated that there is a decreased hepatic DDAH-1 expression[36] and increased DDAH-1 expression in mesenteric arteries[21] from BDL rats. Although the mechanisms involved in this different regulation of DDAH-1 expression are not apparent, they could be related to the organ involved.

The results of the present work suggest an association between portal hypertension, cirrhosis and DDAH-2 expression. The kidneys of rats with portal hypertension exhibited a higher expression of DDAH-2 than the kidneys of control rats, and those of the BDL group exhibited a further increase of DDAH-2. In this case, a similar pattern of expression has been shown in mesenteric arteries[21] and liver[36] from BDL rats. Surprisingly, DDAH-2 protein expression was unaltered in kidneys from young BDL rats[35], suggesting an age-

dependent regulation of the expression of DDAH-2 induced by cirrhosis.

To determine whether the different patterns in DDAH protein expression were correlated with enzymatic activity, *in vitro* ADMA degradation by DDAH was measured. Renal DDAH activity was unchanged in the PPVL group, but was significantly reduced in the BDL group, pointing out the liver dysfunction as a main factor responsible for the decreased DDAH activity as opposed to the portal hypertension and hyperdynamic circulation. These data confirm previous findings that demonstrate the inhibitory effect of cirrhosis in the renal DDAH activity in young rats[35].

There is a growing body of evidence that DDAH activity is inhibited by superoxide[37,38]. It has been demonstrated that ADMA can uncouple endothelial NOS and initiate superoxide generation by NOS[39]. This finding suggests that an increased concentration of ADMA during liver dysfunction[12,13,15] could be an initial point for further NOS uncoupling, increased superoxide and DDAH inhibition, therefore a further increase of ADMA, thereby initiating a feed-forward reaction. Accordingly, it has been hypothesized that there is a possible role for ADMA in the development of hepatorenal syndrome[40], a pathology characterized by an excessive vasoconstriction of renal circulation[1].

In cholestatic patients, a correlation between oxidative stress during obstructive jaundice and renal dysfunction has recently been established[41]. The levels of bilirubin were progressively increased from benign to malignant evolution[41] which is in concordance with pro-oxidant capacity of toxic bile acids[42]. Therefore, it is possible that the hyperbilirubinemia associated with the BDL model of cirrhosis could increase the oxidative stress in the kidney and inhibit renal DDAH. Renal dysfunction in cirrhosis is a common complication, characterized by marked renal artery contraction as a consequence of the activation of several vasoactive pathways[1]. Therefore, the increased inhibitory effects of ADMA on NO synthesis in renal arteries from BDL rats could be another factor contributing to the vasoconstriction associated with cirrhosis. DDAH activators or ADMA-reducing agents may be a potential therapeutic approach to managing the vascular renal dysfunction associated with cirrhosis.

In conclusion, both basal- and induced-NO release are inhibited in renal arteries by ADMA, an effect that is increased in cirrhotic rats. The results of the present study confirm that liver dysfunction is the main factor in the decreased renal DDAH activity and supports the notion that the vascular renal system is highly exposed to ADMA during cirrhosis. Furthermore, our data show an increased DDAH-2 expression, but a reduced DDAH activity in the kidney, associated with cirrhosis.

COMMENTS

Background

Increased renal vascular contraction is a major cause for the development of renal dysfunction in cirrhosis. Several observations have shown that nitric oxide (NO) inhibition is associated with decreased renal plasma flow and increased renal vascular resistance, suggesting that NO exerts a tonic relaxing effect on the renal circulation. Therefore, the kidney is highly vulnerable to the accumulation of asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor. The plasma levels of ADMA are significantly increased in cirrhosis and hepatorenal syndrome. No attempts, however, have been made to determine the effects of ADMA on the vascular tone of renal arteries from portal hypertensive and cirrhotic rats.

Research frontiers

Evidence indicates that in the BDL group dimethylarginine dimethylaminohydrolase (DDAH) activity is reduced in kidneys and ADMA inhibits the basal and stimulated NO in renal arteries more efficiently. High levels of ADMA in the plasma of patients with cirrhosis and hepatorenal syndrome have been previously described and could be responsible, in part, for the contraction and decreased vasodilation of renal arteries during the development of cirrhosis.

Innovations and breakthroughs

This findings draw attention to the role of ADMA and DDAH in the renal vascular dysfunction associated with cirrhosis. Since the enhanced sensitivity to ADMA and inhibition of DDAH is observed in BDL rats but not in PPVL ones, these effects are related to liver dysfunction more than are the portal hypertension and hyperdynamic circulation.

Applications

DDAH emerges as an important regulator of NO bioavailability in the renal artery. The DDAH activators or ADMA-reducing agents may be a potential therapeutic approach to managing the vascular renal dysfunction associated with cirrhosis.

Terminology

Hepatorenal syndrome is defined as the development of renal failure in patients with severe liver disease, acute or chronic, in the absence of any other identifiable cause of renal pathology.

Peer-review

ADMA is a new molecule that its value as a marker is being tested for many diseases and situations; cardiovascular diseases, statin usage, *etc*. The study is a well designed and conducted one. It may contribute to the pathophysiology and to the development strategies to prevent/treat of hepatorenal syndrome.

REFERENCES

1 **Ginès P**, Guevara M, Arroyo V, Rodés J. Hepatorenal syndrome. *Lancet* 2003; **362**: 1819-1827 [PMID: 14654322 DOI: 10.1016/S0140-6736(03)14903-3]

2 **Ginès A**, Escorsell A, Ginès P, Saló J, Jiménez W, Inglada L, Navasa M, Clària J, Rimola A, Arroyo V. Incidence, predictive factors, and prognosis of the hepatorenal syndrome in cirrhosis with ascites. *Gastroenterology* 1993; **105**: 229-236 [PMID: 8514039 DOI: 10.1016/0016-5085(93)90031-7]

3 **Bachmann S**, Mundel P. Nitric oxide in the kidney: synthesis, localization, and function. *Am J Kidney Dis* 1994; **24**: 112-129 [PMID: 7517625 DOI: 10.1016/S0272-6386(12)80170-3]

4 **Majid DS**, Navar LG. Nitric oxide in the control of renal hemodynamics and excretory function. *Am J Hypertens* 2001; **14**: 74S-82S [PMID: 11411769 DOI: 10.1016/S0895-7061(01)02073-8]

5 **Mount PF**, Power DA. Nitric oxide in the kidney: functions and regulation of synthesis. *Acta Physiol* (Oxf) 2006; **187**: 433-446 [PMID: 16866775 DOI: 10.1111/j.1748-1716.2006.01582.x]

6 **Vallance P**, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 1989; **2**: 997-1000 [PMID: 2572793 DOI: 10.1016/S0140-6736(89)91013-1]

7 **Mittermayer F**, Schaller G, Pleiner J, Vychytil A, Sunder-Plassmann G, Hörl WH, Wolzt M. Asymmetrical dimethylarginine plasma concentrations are related to basal nitric oxide release but

not endothelium-dependent vasodilation of resistance arteries in peritoneal dialysis patients. *J Am Soc Nephrol* 2005; **16**: 1832-1838 [PMID: 15857920 DOI: 10.1681/ASN.2004121109]

8 **Segarra G**, Medina P, Vila JM, Chuan P, Domenech C, Torondel B, Lluch A. Inhibition of nitric oxide activity by arginine analogs in human renal arteries. *Am J Hypertens* 2001; **14**: 1142-1148 [PMID: 11724214 DOI: 10.1016/S0895-7061(01)02208-7]

9 **Vallance P**, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol* 1992; **20** Suppl 12: S60-S62 [PMID: 1282988 DOI: 10.1097/00005344-199204002-00018]

10 **Vallance P**, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992; **339**: 572-575 [PMID: 1347093 DOI: 10.1016/0140-6736(92)90865-Z]

11 **Kielstein JT**, Böger RH, Bode-Böger SM, Schäffer J, Barbey M, Koch KM, Frölich JC. Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. *J Am Soc Nephrol* 1999; **10**: 594-600 [PMID: 10073610]

12 **Lluch P**, Torondel B, Medina P, Segarra G, Del Olmo JA, Serra MA, Rodrigo JM. Plasma concentrations of nitric oxide and asymmetric dimethylarginine in human alcoholic cirrhosis. *J Hepatol* 2004; **41**: 55-59 [PMID: 15246208 DOI: 10.1016/j.jhep.2004.03.016]

13 **Lluch P**, Segarra G, Medina P. Asymmetric dimethylarginine as a mediator of vascular dysfunction in cirrhosis. *World J Gastroenterol* 2015; **21**: 9466-9475 [PMID: 26327755 DOI: 10.3748/wjg.v21.i32.9466]

14 **Ferrigno A**, Di Pasqua LG, Berardo C, Richelmi P, Vairetti M. Liver plays a central role in asymmetric dimethylarginine-mediated organ injury. *World J Gastroenterol* 2015; **21**: 5131-5137 [PMID: 25954086 DOI: 10.3748/wjg.v21.i17.5131]

15 **Lluch P**, Mauricio MD, Vila JM, Segarra G, Medina P, Del Olmo JA, Rodrigo JM, Serra MA. Accumulation of symmetric dimethylarginine in hepatorenal syndrome. *Exp Biol Med* (Maywood) 2006; **231**: 70-75 [PMID: 16380646]

16 **Ogawa T**, Kimoto M, Sasaoka K. Purification and properties of a new enzyme, NG,NG-dimethylarginine dimethylaminohydrolase, from rat kidney. *J Biol Chem* 1989; **264**: 10205-10209 [PMID: 2722865]

17 **Jacobi J**, Sydow K, von Degenfeld G, Zhang Y, Dayoub H, Wang B, Patterson AJ, Kimoto M, Blau HM, Cooke JP. Overexpression of dimethylarginine dimethylaminohydrolase reduces tissue asymmetric dimethylarginine levels and enhances angiogenesis. *Circulation* 2005; **111**: 1431-1438 [PMID: 15781754 DOI: 10.1161/01.CIR.0000158487.80483.09]

18 **Leiper JM**, Santa Maria J, Chubb A, MacAllister RJ, Charles IG, Whitley GS, Vallance P. Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. *Biochem J* 1999; **343** Pt 1: 209-214 [PMID: 10493931 DOI: 10.1042/0264-6021: 3430209]

19 **Kimoto M**, Tsuji H, Ogawa T, Sasaoka K. Detection of NG,NG-dimethylarginine dimethylaminohydrolase in the nitric oxide-generating systems of rats using monoclonal antibody. *Arch Biochem Biophys* 1993; **300**: 657-662 [PMID: 8434946 DOI: 10.1006/abbi.1993.1091]

20 **Tojo A**, Welch WJ, Bremer V, Kimoto M, Kimura K, Omata M, Ogawa T, Vallance P, Wilcox CS. Colocalization of demethylating enzymes and NOS and functional effects of methylarginines in rat kidney. *Kidney Int* 1997; **52**: 1593-1601 [PMID: 9407505 DOI: 10.1038/ki.1997.490]

21 **Serna E**, Mauricio MD, Lluch P, Segarra G, Cortina B, Lluch S, Medina P. Basal release of nitric oxide in the mesenteric artery in portal hypertension and cirrhosis: role of dimethylarginine dimethylaminohydrolase. *J Gastroenterol Hepatol* 2013; **28**: 880-886 [PMID: 23302093 DOI: 10.1111/jgh.12119]

22 **Livak KJ**, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**: 402-408 [PMID: 11846609 DOI: 10.1006/meth.2001.1262]

23 **Tain YL**, Baylis C. Determination of dimethylarginine dimethylaminohydrolase activity in the kidney. *Kidney Int* 2007; **72**: 886-889 [PMID: 17653133 DOI: 10.1038/sj.ki.5002446]

24 **Lee FY**, Colombato LA, Albillos A, Groszmann RJ. N omega-nitro-L-arginine administration corrects peripheral vasodilation and systemic capillary hypotension and ameliorates plasma volume expansion and sodium retention in portal hypertensive rats. *Hepatology* 1993; **17**: 84-90 [PMID: 8423045 DOI: 10.1002/hep.1840170116]

25 **Sieber CC**, Groszmann RJ. Nitric oxide mediates hyporeactivity to vasopressors in mesenteric vessels of portal hypertensive rats. *Gastroenterology* 1992; **103**: 235-239 [PMID: 1612331 DOI: 10.1016/0016-5085(92)91118-N]

26 **Sieber CC**, Lopez-Talavera JC, Groszmann RJ. Role of nitric oxide in the in vitro splanchnic vascular hyporeactivity in ascitic cirrhotic rats. *Gastroenterology* 1993; **104**: 1750-1754 [PMID: 8388839 DOI: 10.1016/0016-5085(93)90655-V]

27 **Heller J**, Trebicka J, Shiozawa T, Schepke M, Neef M, Hennenberg M, Sauerbruch T. Vascular, hemodynamic and renal effects of low-dose losartan in rats with secondary biliary cirrhosis. *Liver Int* 2005; **25**: 657-666 [PMID: 15910503 DOI: 10.1111/j.1478-3231.2005.01053.x]

28 **Hennenberg M**, Biecker E, Trebicka J, Jochem K, Zhou Q, Schmidt M, Jakobs KH, Sauerbruch T, Heller J. Defective RhoA/Rho-kinase signaling contributes to vascular hypocontractility and vasodilation in cirrhotic rats. *Gastroenterology* 2006; **130**: 838-854 [PMID: 16530523 DOI: 10.1053/j.gastro.2005.11.029]

29 **Lin HC**, Yang YY, Huang YT, Lee TY, Hou MC, Lee FY, Lee SD. Vascular contractile response and signal transduction in endothelium-denuded aorta from cirrhotic rats. *World J Gastroenterol* 2005; **11**: 2306-2312 [PMID: 15818743 DOI: 10.3748/WJG.v11.i15.2306]

30 **Hennenberg M**, Trebicka J, Biecker E, Schepke M, Sauerbruch T, Heller J. Vascular dysfunction in human and rat cirrhosis: role of receptor-desensitizing and calcium-sensitizing proteins. *Hepatology* 2007; **45**: 495-506 [PMID: 17256744 DOI: 10.1002/hep.21502]

31 **MacAllister RJ**, Parry H, Kimoto M, Ogawa T, Russell RJ, Hodson H, Whitley GS, Vallance P. Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. *Br J Pharmacol* 1996; **119**: 1533-1540 [PMID: 8982498 DOI: 10.1111/j.1476-5381.1996.tb16069.x]

32 **Al-Zobaidy MJ**, Craig J, Martin W. Differential sensitivity of basal and acetylcholine-induced activity of nitric oxide to blockade by asymmetric dimethylarginine in the rat aorta. *Br J Pharmacol* 2010; **160**: 1476-1483 [PMID: 20590637 DOI: 10.1111/j.1476-5381.2010.00802.x]

33 **Segarra G**, Medina P, Ballester RM, Lluch P, Aldasoro M, Vila JM, Lluch S. Effects of some guanidino compounds on human cerebral arteries. *Stroke* 1999; **30**: 2206-2211 [PMID: 10512930 DOI: 10.1161/01.STR.30.10.2206]

34 **Segarra G**, Medina P, Vila JM, Martínez-León JB, Ballester RM, Lluch P, Lluch S. Contractile effects of arginine analogues on human internal thoracic and radial arteries. *J Thorac Cardiovasc Surg* 2000; **120**: 729-736 [PMID: 11003756 DOI: 10.1067/mtc.2000.109537]

35 **Tain YL**, Hsieh CS, Chen CC, Sheen JM, Lee CT, Huang LT. Melatonin prevents increased asymmetric dimethylarginine in young rats with bile duct ligation. *J Pineal Res* 2010; **48**: 212-221 [PMID: 20210851 DOI: 10.1111/j.1600-079X.2010.00745.x]

36 **Mookerjee RP**, Mehta G, Balasubramaniyan V, Mohamed Fel Z, Davies N, Sharma V, Iwakiri Y, Jalan R. Hepatic dimethylarginine-dimethylaminohydrolase1 is reduced in cirrhosis and is a target for therapy in portal hypertension. *J Hepatol* 2015; **62**: 325-331 [PMID: 25152204 DOI: 10.1016/j.jhep.2014.08.024]

37 **Sydow K**, Munzel T. ADMA and oxidative stress. *Atheroscler Suppl* 2003; **4**: 41-51 [PMID: 14664902 DOI: 10.1016/S1567-5688(03)00033-3]

38 **Palm F**, Onozato ML, Luo Z, Wilcox CS. Dimethylarginine

dimethylaminohydrolase (DDAH): expression, regulation, and function in the cardiovascular and renal systems. *Am J Physiol Heart Circ Physiol* 2007; **293**: H3227-H3245 [PMID: 17933965 DOI: 10.1152/ajpheart.00998.2007]

39 **Böger RH**, Bode-Böger SM, Tsao PS, Lin PS, Chan JR, Cooke JP. An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes. *J Am Coll Cardiol* 2000; **36**: 2287-2295 [PMID: 11127475 DOI: 10.1016/S0735-1097(00)01013-5]

40 **Nijveldt RJ**, Teerlink T, van Leeuwen PA. The asymmetrical dimethylarginine (ADMA)-multiple organ failure hypothesis. *Clin Nutr* 2003; **22**: 99-104 [PMID: 12553957 DOI: 10.1054/clnu.2002.0614]

41 **Martínez-Cecilia D**, Reyes-Díaz M, Ruiz-Rabelo J, Gomez-Alvarez M, Villanueva CM, Álamo J, Muntané J, Padillo FJ. Oxidative stress influence on renal dysfunction in patients with obstructive jaundice: A case and control prospective study. *Redox Biol* 2016; **8**: 160-164 [PMID: 26774750 DOI: 10.1016/j.redox.2015.12.009]

42 **Tan KP**, Yang M, Ito S. Activation of nuclear factor (erythroid-2 like) factor 2 by toxic bile acids provokes adaptive defense responses to enhance cell survival at the emergence of oxidative stress. *Mol Pharmacol* 2007; **72**: 1380-1390 [PMID: 17724089 DOI: 10.1124/mol.107.039370]

P- Reviewer: Bayraktar Y, Fernandez-Rodriguez CM, Fierbinteanu-Braticevici C, Ozen H, Thomopoulos KC
S- Editor: Gong ZM **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.v22.i48.10557

World J Gastroenterol 2016 December 28; 22(48): 10557-10565
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Clinical and epidemiological characteristics of norovirus gastroenteritis among hospitalized children in Lebanon

Nada M Melhem, Hassan Zaraket, Khalil Kreidieh, Zeinab Ali, Moza Hammadi, Soha Ghanem, Farah Hajar, Amjad Haidar, Adlette Inati, Mariam Rajab, Hassan Fakhouri, Bassam Ghanem, Ghassan Baasiri, Ghassan Dbaibo

Nada M Melhem, Hassan Zaraket, Zeinab Ali, Moza Hammadi, Soha Ghanem, Farah Hajar, Amjad Haidar, Ghassan Dbaibo, Center for Infectious Diseases Research, Faculty of Medicine, American University of Beirut, Beirut 1107-2020, Lebanon

Nada M Melhem, Khalil Kreidieh, Medical Laboratory Sciences Program, Faculty of Health Sciences, American University of Beirut, Beirut 1107-2020, Lebanon

Hassan Zaraket, Department of Experimental Pathology, Immunology and Microbiology, American University of Beirut, Faculty of Medicine, Beirut 1107-2020, Lebanon

Zeinab Ali, Moza Hammadi, Soha Ghanem, Farah Hajar, Ghassan Dbaibo, Department of Pediatrics and Adolescent Medicine, Faculty of Medicine, American University of Beirut Medical Center, Beirut 1107-2020, Lebanon

Adlette Inati, Department of Pediatrics, Nini Hospital, Tripoli 1300, Lebanon

Mariam Rajab, Department of Pediatrics, Makassed General Hospital, Beirut 1107-2020, Lebanon

Hassan Fakhouri, Department of Pediatrics, Rafic Hariri University Hospital, Beirut 1107-2020, Lebanon

Bassam Ghanem, Department of Pediatrics, Nabatieh Governmental Hospital, Nabatieh 1700, Lebanon

Ghassan Baasiri, Department of Pediatrics, Hammoud Hospital, Saida 1600, Lebanon

Author contributions: Melhem NM designed the study, supervised the experimental work, performed the analysis and wrote the paper; Zaraket H contributed to the analysis; Kreidieh K performed the experiments and contributed to the analysis; Ali Z, Hammadi M, Ghanem S, Hajar F, Haidar A, Inati A, Rajab M, Fakhouri H, Ghanem B and Baasiri G helped in the recruitment of participants and approval of the final version of the article to be published; Dbaibo G critically read and revised the manuscript; Melhem NM and Dbaibo G are co-corresponding authors of this manuscript.

Supported by an investigator-initiated research grant from Merck Sharpe and Dohme (MSD); and University Review Board Grant, American University of Beirut.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Board of the American University of Beirut.

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

Data sharing statement: Not applicable.

Manuscript source: Invited manuscript

Correspondence to: Nada M Melhem, PhD, Associate Professor, Medical Laboratory Sciences Program, Faculty of Health Sciences, American University of Beirut, PO Box 11-0236 Riad El Solh, 325 Van Dyck Hall, Beirut 1107-2020, Lebanon. melhemn@aub.edu.lb
Telephone: +961-1-3500004699
Fax: +961-1-744470

Received: June 28, 2016
Peer-review started: June 29, 2016
First decision: October 11, 2016
Revised: October 20, 2016
Accepted: October 27, 2016

Article in press: October 27, 2016
Published online: December 28, 2016

Abstract

AIM

To assess the burden of norovirus (NoV) and to determine the diversity of circulating strains among hospitalized children in Lebanon.

METHODS

Stool samples were collected from children presenting with acute gastroenteritis to six major hospitals in Lebanon. A total of 739 eligible stool samples, testing negative for diarrhea caused by rotavirus as a possible viral pathogen, were collected between January 2011 and June 2013. A standardized questionnaire including demographic, epidemiological and clinical observations was used at the time of hospitalization of children presenting with diarrhea. Viral RNA was extracted from stool samples followed by reverse transcription polymerase chain reaction and nucleotide sequencing of a fragment of the viral protein 1 capsid gene. Multiple sequence alignments were carried out and phylogenetic trees were constructed using the MEGA 6 software.

RESULTS

Overall, 11.2% of stool samples collected from children aged < 5 years tested positive for NoV genogroups Ⅰ (GⅠ) and Ⅱ (GⅡ). GⅡ accounted for 10.6% of the gastroenteritis cases with only five samples being positive for GⅠ (0.7%). The majority of hospitalized children showed symptoms of diarrhea, dehydration, vomiting and fever. Upon sequencing of positive samples and based on their clustering in the phylogenetic tree, 4/5 of GⅠ gastroenteritis cases were designated GⅠ.3 and one case as GⅠ.4. GⅡ.4 was predominantly detected in stool of our study participants (68%). We report a JB-15/KOR/2008 GⅡ.4 Apeldoorn 2008-like variant strain circulating in 2011; this strain was replaced between 2012 and 2013 by a variant sharing homology with the Sydney/NSW0514/2012/AUS GⅡ.4 Sydney 2012 and Sydney 2012/FRA GⅡ.4 strains. We also report the co-circulation of non-GⅡ.4 genotypes among hospitalized children. Our data show that NoV gastroenteritis can occur throughout the year with the highest number of cases detected during the hot months.

CONCLUSION

The majority of NoV-associated viral gastroenteritis cases among our participants are attributable to GⅡ.4, which is compatible with results reported worldwide.

Key words: Norovirus; Reverse transcription polymerase chain reaction; Sequencing; Norovirus genogroup Ⅰ; Norovirus genogroup Ⅱ; Lebanon

Core tip: We report the results of a large study of norovirus (NoV)-associated gastroenteritis among children aged < 5 years in Lebanon. The majority of viral gastroenteritis cases were attributable to NoV GⅡ.4, which is compatible with results reported worldwide. Our data support a peak incidence in July, while reports from other countries show peaks during the cold months. We report NoV A JB-15/KOR/2008 GⅡ.4 Apeldoorn 2008-like variant strain circulating in 2011. This strain was replaced between 2012 and 2013 by a variant sharing homology with the Sydney/NSW0514/2012/AUS GⅡ.4 Sydney 2012 and Sydney 2012/FRA GⅡ.4 strains.

Melhem NM, Zaraket H, Kreidieh K, Ali Z, Hammadi M, Ghanem S, Hajar F, Haidar A, Inati A, Rajab M, Fakhouri H, Ghanem B, Baasiri G, Dbaibo G. Clinical and epidemiological characteristics of norovirus gastroenteritis among hospitalized children in Lebanon. *World J Gastroenterol* 2016; 22(48): 10557-10565 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10557.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10557

INTRODUCTION

Gastroenteritis caused by norovirus (NoVs) has been recently reported to be the second most common cause of acute viral gastroenteritis worldwide following rotavirus and a major cause of foodborne illness[1,2]. NoV is the leading cause of acute gastroenteritis across all age groups seeking medical care in emergency departments, outpatient clinics, and the community[1]. Recent reviews of the literature on community, outpatient and hospital-based studies in developing and developed countries report that NoV gastroenteritis accounted for 10%-15% of severe cases in children aged < 5 years and 9%-15% of cases of mild to moderate diarrhea among individuals of all ages[3,4]. Fecal-oral spread is the primary mode of NoV transmission. The average incubation period is 24-48 h. The symptoms include vomiting (≥ 50% of cases), diarrhea, nausea, abdominal cramps, malaise and low-grade fever. Illness usually resolves in 12-72 h; however, it may last longer in young children, elderly people, and hospitalized and immunocompromised individuals. Several factors contribute to the high communicability of NoV, including, most importantly, the low infectious dose of the virus (18-100 particles); the high levels of virus shedding (> 10^9 particles/mL of feces during the first days following infection) known to precede illness and to be prolonged in immunosuppressed persons; stability of the virus at 0-60 ℃; and finally, the high rate of mutation and recombination leading to antigenic diversity[5-7]. While 75% of NoV cases have been reported during the cooler months, geographic variability and annual fluctuations have also been described[8].

NoVs are non-enveloped, polyadenylated, single stranded, positive-sense RNA viruses of the family *Caliciviridae.* The RNA genome of NoVs is composed of three large open reading frames designated as ORF-1, ORF-2 and ORF-3. ORF-1 encodes six non-structural proteins including the protease and the RNA-dependent RNA-polymerase (RdRp). ORF-2 and ORF-3 encode the structural viral components viral protein 1 (VP1) (major capsid protein) and VP2 (minor capsid protein), respectively. Based on the amino-acid sequence of VP1, NoVs are divided into six genogroups (GⅠ-GⅥ). GⅠ, GⅡ and GⅣ are known to infect humans[9,10]. Genogroups are further subdivided into genotypes based on the RdRp sequence or capsid sequence. At the genomic level, strains of the same genogroups are 51%-56% similar, whereas genotypes have 69%-87% similarity[11,12]. At least eight and 21 genotypes belong to GⅠ and GⅡ, respectively[1]. The genogroup Ⅱ, genotype 4 NoVs, designated GII.4, are responsible for the majority of NoV outbreaks in the United States, Australia and many European countries[13,14]. GⅡ.4 NoVs are continuously changing and viral variants emerge every couple of years and every 2-7 years as a result of genetic drift; an observation compatible with the immune escape mechanism observed with influenza A virus[15-19]. Globally and during the past decade, GⅡ.3 and GⅡ.6 were reported as the second and third most predominant genotypes after GⅡ.4, respectively[13].

To the best of our knowledge, there have been no large studies conducted in Lebanon on NoV and its association with acute gastroenteritis. The aim of this study was to determine the prevalence of NoV gastroenteritis as well as the genotypic characterization of the virus among hospitalized children aged < 5 years.

MATERIALS AND METHODS

Study population and specimen collection

The study was conducted in accordance with the ethical guidelines of the Helsinki Declaration and after approval of the Institution Review Board of the American University of Beirut. Written informed consent was obtained from the legal guardians of hospitalized children, and consequently, stool samples and medical data were collected. A standardized questionnaire including demographic, epidemiological and clinical observations was used at the time of hospitalization of children presenting with diarrhea. Stool samples were collected from children presenting with acute gastroenteritis to six major hospitals in Lebanon. A total of 739 eligible stool samples, testing negative for diarrhea caused by rotavirus as a possible viral pathogen, were collected over a 2-year period (January 2011 to June 2013).

Viral RNA extraction and NoV detection

Stool specimens (0.5-1.0 mL) were suspended in 5 mL 0.89% NaCl. The fecal suspension was centrifuged at 4000 × *g*; following which, the supernatant was filtered and 140 μL of the filtrate was used for viral RNA extraction. QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used for viral RNA extraction. Viral RNA was stored at -20 ℃.

PCR and sequencing

Reverse transcription polymerase chain reaction (RT-PCR) was performed using genogroup-specific primers as previously described[20-22]. RT-PCR targeted the 5′ end of the capsid region in ORF2 using: G1-SKF (Forward CTG CCC GAA TTY GTA AATGA) and G1-SKR (Reverse CCA ACC CAR CCA TTR TACA and primers GoG2F (Forward CAR GAR BCN ATG TTY AGR TGG ATGAG) and G2-SKR (Reverse CCR CCN GCA TRH CCR TTR TACAT) for amplifying 330- and 387-bp PCR products of GⅠ and GⅡ genogroups, respectively. Qiagen OneStep RT-PCR Kit was used under the following conditions: 42 ℃ for 30 min; initial PCR activation step at 95 ℃ for 15 min; denaturation at 94 ℃ for 30 s, annealing at 52-54 ℃ for 30 s, extension at 72 ℃ for 45 s (30 cycles); and final extension at 72 ℃ for 7 min. Synthetic Norovirus G1 (I) RNA (ATCC VR3199SD) and Synthetic Norovirus G2 (II) RNA (ATCC VR3200SD) were used as positive controls. The PCR products were analyzed by gel electrophoresis and stored at -20 ℃ until analysis. Nucleotide sequencing of NoV-positive samples was performed by Macrogen (Seoul, South Korea) using the PCR primers. A total of 19 full-length human NoV capsid protein sequences were downloaded from GenBank and used as reference strains. These included six GⅠ and 16 GII with the following accession numbers: AAS86780.1 (GⅠ), ACN32270.1 (GⅠ.1), ACU56258.1 (GⅠ.2), ACX33982.1 (GⅠ.3), ACV41096.1 (GⅠ.4), ADB54834.1(GⅠ.8), aAIO11150.1 (GⅡ), ABC96332.1 (GⅡ), AFA55174.1 (GⅡ.1), BAG68716.1 (GⅡ.2), ADK23787.1 (GⅡ.3), AEG79292.1 (GⅡ.4), ABL74397.1 (GⅡ.4), ABL74391.1 (GⅡ.4), AGT95930.1 (GⅡ.4), KM245069.1 (GⅡ.4 Yerseke/2006a), KF361437.1 (GⅡ.4 Minerva/2006b), KP762437.1 (GⅡ.4 Den Haag 2006b), ADE28721.1 (GⅡ.6), ACX85810.1 (GⅡ.7), ADZ24003.1 (GⅡ.12), and ACX81355.1 (GⅡ.14). Multiple sequence alignments were carried out using CLUSTALL or BioEdit out and phylogenetic trees were constructed using the MEGA 6 software. The phylogenetic tree was generated using the neighbor-joining method validated by 1000 bootstrap replicates.

Nucleotide sequence accession numbers

The partial nucleotide sequences determined in this study were deposited in GenBank with the following accession numbers: GⅠ KU950315-KU950319 and GⅡ KU963412-KU963487.

Statistical analysis

Data was analyzed using SPSS version 22. For comparisons of demographic and clinical symptoms, χ^2 analysis and Pearson χ^2 test were used.

Table 1 Demographic characteristics of study participants *n* (%)

	n	NoV positive	NoV negative
Participants	739	83 (11.2)	656 (88.8)
Gender			
Male	413	47 (11.4)	366 (88.6)
Female	326	36 (11.0)	290 (89.0)
Age group (mo)			
0-11	376	34 (9.0)	342 (91.0)
12-23	226	35 (15.5)	191 (84.5)
24-35	79	10 (12.7)	69 (87.3)
36-47	30	3 (10.0)	27 (90.0)
48-59	27	1 (3.7)	26 (96.3)
Region			
Beirut	217	20 (9.2)	197 (90.8)
North Lebanon	315	35 (11.1)	280 (88.9)
South Lebanon	207	28 (13.5)	179 (86.5)

Table 2 Clinical characteristics of NoV-positive cases *n* (%)

	NoV positive	G Ⅰ	G Ⅱ
Fever			
Yes	56 (67.5)	5 (100.0)	51 (65.4)
No	27 (32.5)	0 (0.0)	27 (34.6)
Vomiting			
Yes	63 (75.9)	3 (60.0)	60 (76.9)
No	20 (24.1)	2 (40.0)	18 (23.1)
Diarrhea			
Yes	79 (95.2)	5 (100.0)	74 (94.9)
No	4 (4.8)	0 (0.0)	4 (5.1)
Assessed dehydration			
Severe	10 (12.0)	1 (20.0)	9 (11.5)
Mild to moderate	64 (77.1)	3 (60.0)	61 (78.2)
No dehydration	9 (10.8)	1 (20.0)	8 (10.3)
Vesikari score			
Severe	76 (91.6)	4 (80.0)	72 (92.3)
Mild to moderate	7 (8.4)	1 (20.0)	6 (7.7)

RESULTS

Seven hundred and thirty-nine eligible rotavirus-negative stool samples were assayed for NoV by RT-PCR during January 2011 to June 2013. Stool samples were collected from children aged < 5 years presenting to six hospitals in Lebanon due to acute gastroenteritis. Tables 1 and 2 summarize the demographic and clinical characteristics of our study participants. Overall, 11.26% (*n* = 83/739) of the samples tested positive for NoV (Table 1). The majority of cases were NoV genogroup GII (*n* = 78/83) (Table 2), with a total incidence rate of 10.6%, while only five samples tested positive for NoV genogroup GI, with a total incidence rate of 0.7%. We did not have mixed infections with NoV G Ⅰ and G Ⅱ among our study participants. Males accounted for 55.9% (413/739) of hospitalized children and females for 44% (326/739) while 11.4% of the former and 11% of the latter were NoV positive (Table 1). Gender was not significantly associated with NoV infection (*P* = 0.887). The mean age of the study participants testing positive for NoV and presenting with gastroenteritis symptoms upon admission was 16.2 ± 9.5 mo. Fifteen point five percent of samples testing positive for NoV and presenting to hospitals with acute gastroenteritis symptoms were children aged 12-23 mo (35/376), followed by children aged 24-35 mo (12.7%; 10/79) (Table 1). Our results showed, however, that there was no association between age and NoV infection among our study participants (*P* = 0.729).

As expected, the majority of our study participants testing positive for NoV had symptoms of diarrhea (95%), dehydration (90%), vomiting (76%) and fever (67.5%). The Vesikari Clinical Severity Scoring System was used to assess the severity of acute gastroenteritis. Severe gastroenteritis (*i.e.*, score > 11) was reported in 92% of NoV-positive participants (Table 2). The average hospital stay of children admitted ranged between 3 and 5 d. Ninety-five percent of NoV-positive cases received intravenous rehydration, whereas only 18% received oral rehydration during hospitalization.

NoV incidence was similar across different geographic regions. Incidence in hospitalized children was 9%, 13% and 11% in Beirut, and the Southern and Northern parts of Lebanon, respectively (*P* = 0.371). Overall, 11.23% (83/739) of our study participants tested positive for NoV, of whom, 45 (54%) were detected in 2011 and 36 (43%) in 2012, and two samples tested positive during the first half of 2013. The seasonal onset of NoV cases was similar during 2011 and 2012 (Figure 1). While our data show that NoV infection can occur throughout the year, the highest percentage of NoV-positive samples was detected in July 2011 (24%) and July 2012 (27%), *i.e.*, in the hot months. Fewer infections were observed between October and February, which are the cooler months in Lebanon.

In order to analyze the extent of the genetic diversity and to designate the genotypes of NoVs detected among our cohort of children aged < 5 years, we inferred the phylogenetic relationship of the major capsid protein gene (*orf2*) along with subgenotype reference isolates. We sequenced 81 samples rather than the total number of NoV-positive samples (*n* = 83) due to the lack of sufficient volume of purified RNA for two samples. Among five G Ⅰ samples, four were designated G Ⅰ.3 and one as G Ⅰ.4, based on their clustering in the phylogenetic tree. Eight different NoV G Ⅱ genotypes were detected among our study participants, and 68% (52/76) of positive cases were attributed to G Ⅱ.4. G Ⅱ.4 diversified into two distinct subclusters distinguished by an A151T substitution. These subclusters co-circulated between 2011 and 2013 (Figure 2). While GII.4 was predominantly associated with gastroenteritis among our study participants, circulation of more than one sub-genotype during the same year was also recorded. The following non-G Ⅱ.4 genotypes were also detected among hospitalized children during the study period: G Ⅱ.6

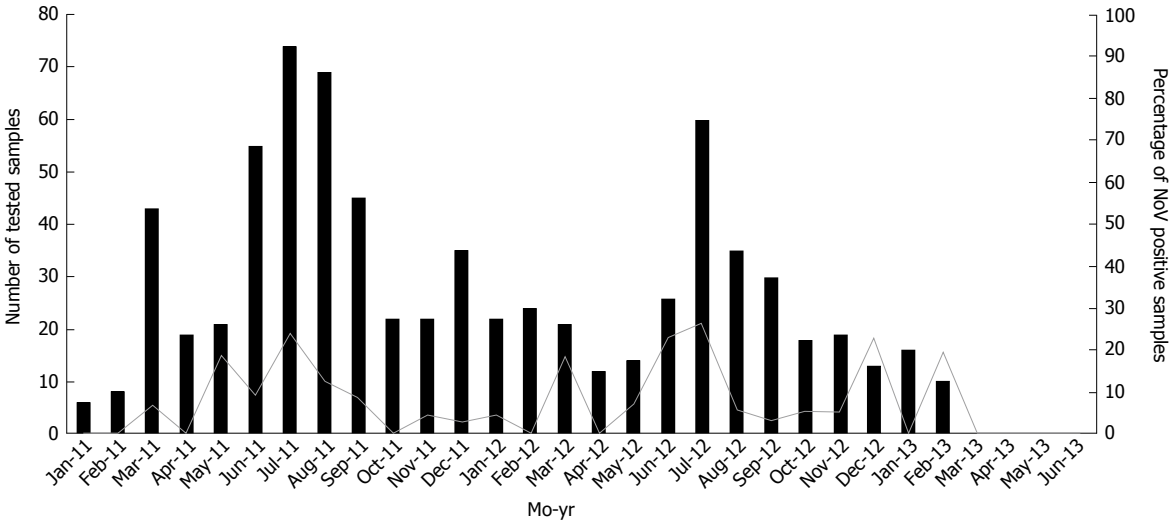

Figure 1 Seasonal distribution of NoV cases among children aged < 5 years in Lebanon. The absolute number (left Y axis) and the percentage (right Y axis) of NoV-positive cases isolated from stool samples and collected from children presenting with acute gastroenteritis to six major hospitals in Lebanon are depicted against month and year of circulation (X axis).

(7/76, 9.2%), GⅡ.21 (5/76, 6.6%), GⅡ.3 (5/76, 6.6%), GⅡ.13 (3/76, 3.95), GⅡ.9 (1/76, 1.3%), GⅡ.1 (1/76, 1.3%), and GⅡ.2 (1/76, 1.3%) (Figure 2).

DISCUSSION

We report the results of a large study on NoV-associated gastroenteritis in Lebanon. Our study reflects the predominance of GII strains among children aged < 5 years who were hospitalized due to acute gastroenteritis. We detected a broad genetic diversity of NoVs causing acute gastroenteritis among our study participants. Overall, GⅡ.4 (68%) was the most prevalent genotype isolated from hospitalized children in Lebanon during the study period. Our results are compatible with global reports in which most cases of NoV-associated gastroenteritis were attributable to GⅡ.4[23-25], and co-circulating with other genotypes[13,26-28]. Locally, NoV GII has been previously reported in five Lebanese children less than ten years old[29]. Regionally, in the Middle East and North Africa (MENA), several studies have recently assessed the prevalence of NoV among hospitalized children aged < 5 years (hospitalized due to signs of acute gastroenteritis). These studies were performed on a variable sample size in Egypt[30], Israel[31,32], Iran[33], Jordan[34], Kuwait[35], Libya[36,37], Morocco[38], Tunisia[39,40], Turkey[41-45] and Yemen[46]. NoV was detected in stool samples of 6%-30% of hospitalized children aged < 5 years, with GⅡ.4 and GⅡ.3 predominantly reported in these studies.

We reported A JB-15/KOR/2008 GⅡ.4 Apeldoorn 2008-like variant strain circulating in 2011 among children aged < 5 years in Lebanon. This strain was replaced between 2012 and 2013 by a variant sharing homology with the Sydney/NSW0514/2012/AUS GⅡ.4 Sydney 2012 and Sydney 2012/FRA GⅡ.4 strains. The latter emerged in Australia in March 2012 and was later isolated from the United States, Belgium, Denmark, Scotland, and Japan[47]. The co-circulation of several GⅡ.4 lineages is well described[18,48] and is suggested to be a mechanism of positive selection of mutations to generate new NoV variants[49]. The variants of the NoV GⅡ.4 lineage have been associated with 62%-80% of cases of NoV gastroenteritis worldwide, as well as explosive outbreaks occurring in community settings[11,50]. Global epidemics of NoV gastroenteritis have been associated with the following strains: US 1995/96 in 1996[51], Farmington Hills in 2002[52,53], Hunter in 2004[54], 2006b virus in 2007 and 2008[55], New Orleans virus during 2009-2012[56] and Sydney 2012[57]. Other GⅡ.4 variants have also been associated with localized types of epidemics such as Henry 2001, Japan 2001, Asia 2003, and 2006a and Apeldoorn 2008[18].

While GⅡ.3 was reported to be the second most predominant genotype in many countries, it ranked third along with GⅡ.21 among our study participants after GⅡ.4 and GⅡ.6. GⅡ.6 and GⅡ.2 are reported to account for 5% of the globally reported strains. The prevalence of GⅡ.6 ,the second most predominant cause of gastroenteritis among our study participants ,was similar to reports in several countries including Brazil[58], Japan[59], Africa[60] and Finland[61]. GⅡ.21, previously reported in Brazil[62], has been described as a recombinant product between GⅡ.4/2006b and GⅡ.18 strains[63]. In our study, this genotype was similar to the Salisbury150/2011/United States GⅡ.21. GⅡ.13, previously described as an uncommon cause of gastroenteritis, is increasingly being reported in many Asian countries[13]. Our results show that GⅡ.13 ranked fourth as a causative agent of gastroenteritis among hospitalized children in Lebanon. Among G Ⅰ, GⅠ.3 was predominantly detected, albeit less

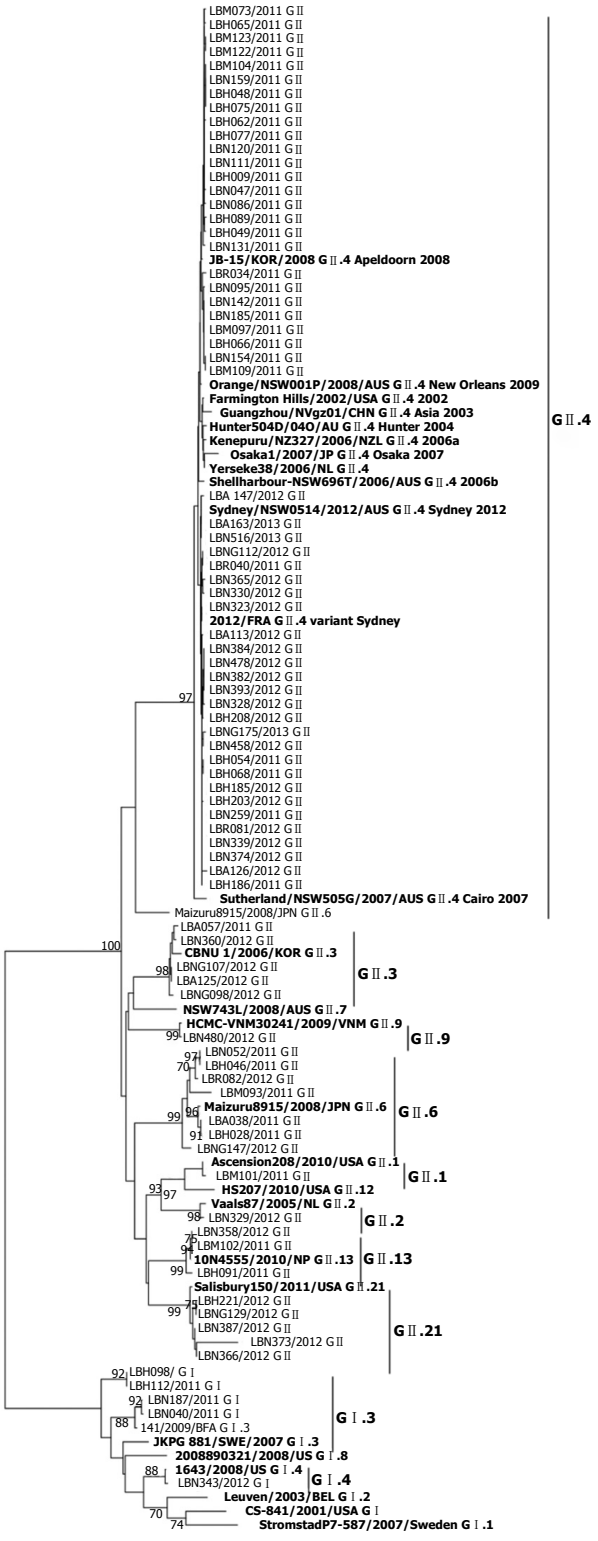

Figure 2 Phylogenetic analysis of norovirus viral protein 1 major capsid gene. Nucleotide sequences spanning nucleotides 5385–5657 (length = 273 nt) of NoV isolated in Lebanon were aligned with reference strains obtained from GenBank. The trees were then constructed based on the nucleotide sequences using the neighbor-joining method with bootstrap analysis of 1000 replicates using MEGA version 6.0. Bootstrap values > 70% are shown. Reference strains are in bold. LBM: Lebanon Makassed Hospital; LBH: Lebanon, Hammoud Hospital; LBN: Lebanon, Nini Hospital; LBR: Lebanon, Hariri Governmental Hospital; LBA: Lebanon, American University of Beirut Medical Center; LBNG: Lebanon, Nabatiyeh Hospital; NoV: Norovirus.

often compared to GⅡ. Our results are compatible with global reports in which GⅡ is the most prevalent genogroup causing approximately 96% of infections, with GⅠ constituting the remaining genogroup and causing on average 3.6% of NoV infections[13].

Reports show that NoV infection peaks during the cold months in parts of Europe and North America, with sporadic cases detected all year round, as well as outbreaks during the summer months[59,64-66]. Similar results are reported from a few other countries[33,42,43,67]. New GII.4 variants have been reported to emerge with unusual spring/summer seasonality[52], along with a total increase in wintertime disease in Europe. The former was suggested to be due to the lack of effective immunity to the newly emergent GⅡ.4 variant. Recently, a systematic review reported a variable global seasonality of NoV[8]. A peak in winter months was reported in the Northern Hemisphere, while cases peaks and outbreaks were reported in the hot months in the Southern Hemisphere. Little or Limited data exist from Africa, the tropical regions and the MENA region, and when present, studies report on short duration NoV infections detected in diverse settings. Consequently, the ability to associate climate and demographic factors with the seasonality of NoV in these areas is difficult. Although our data support a peak incidence in July, the seasonal pattern of NoV infection in Lebanon is to be further investigated in comparison with existing reports. In summary, the genotypic characterization of NoV-positive samples revealed a wide diversity of circulating genotypes, with GⅡ.4 predominantly being associated with gastroenteritis. Globally, GⅡ.4 circulating strains have been responsible for a large number of outbreaks in many countries including China, India, Japan, Egypt, Turkey and Italy[13]. The evolution of GⅡ.4, described as being epochal (long periods of status quo followed by outbursts of variation) over time generates escape mutants that are periodically selected for by herd immunity[11]. Further studies are needed to assess the extent of NoV molecular diversity in Lebanon among different age groups.

While we partially genotyped our circulating strains, we realize that we cannot suggest any recombination event to elaborate on the diverse genotypes detected among our study participants. Nevertheless, our study confirms the significant role of NoV as a causative agent of gastroenteritis among children less than 5 years old in Lebanon. Our results are compatible with global reports in which most cases of NoV-associated gastroenteritis are attributable to GII.4. The continuous monitoring of NoV infection among different age groups is needed in Lebanon to support intervention strategies and to detect new circulating variants possibly associated with increased rates of morbidity.

In conclusion, we report the results of a large-scale study on NoV-associated gastroenteritis in Lebanon. Our results are compatible with global reports in which

most cases of viral gastroenteritis among children aged < 5 years are attributable to GⅡ.4. Moreover, our data support a peak incidence in July, whereas other reports show peak incidences during the cold months (*e.g.*, North America, parts of Europe). The seasonal pattern of NoV in Lebanon should be further investigated. Efforts should be made to introduce the clinical diagnosis of the virus due to its impact on the community as well as health care institutions.

COMMENTS

Background

Norovirus (NoV) is one of the most common causes of acute gastroenteritis among children. To the best of our knowledge, there have been no large scale studies conducted in Lebanon on NoV and its association with acute gastroenteritis among children aged < 5 years. Moreover, the authors have no data on the genotypic characterization of the predominantly circulating NoV strains in Lebanon as compared to other countries. This study is important to support intervention strategies.

Research frontiers

NoV is the leading cause of acute gastroenteritis across all age groups seeking medical care in emergency departments, outpatient clinics and the community. Recent reviews of the literature on community, outpatient and hospital-based studies in developing and developed countries report that NoV gastroenteritis accounts for 10%-15% of severe cases in children aged < 5 years and 9%-15% of cases of mild to moderate diarrhea among individuals of all ages. This data are compatible with global reports in which NoV Genogroup 2 genotype 4 are the most prevalent strains associated with gastroenteritis.

Innovations and breakthroughs

To the best of our knowledge, there have been no large studies conducted in Lebanon on NoV and its association with acute gastroenteritis. The aim of this study was to determine the prevalence of NoV gastroenteritis, as well as the genotypic characterization of the virus among hospitalized children < 5 years old. The authors believe that this study is the first in Lebanon to report on the circulating strains of NoV GⅠ and GⅡ among children hospitalized due to acute gastroenteritis.

Applications

This study is believed to be the first to report on the clinical epidemiology, seasonality and genotypic characterization of NoV as a causative agent of acute gastroenteritis leading to hospitalization among children < 5 years old in Lebanon. This study is important to guide intervention strategies in Lebanon as well as the national introduction of clinical diagnosis of the virus as a major cause of gastroenteritis.

Peer-review

Important work on an interesting topic, the clinical and epidemiologic characteristics of NoV gastroenteritis among hospitalized children in Lebanon.

REFERENCES

1 **Division of Viral Diseases**; National Center for Immunization and Respiratory Diseases; Centers for Disease Control and Prevention. Updated norovirus outbreak management and disease prevention guidelines. *MMWR Recomm Rep* 2011; **60**: 1-18 [PMID: 21368741]

2 **Lanata CF**, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, Black RE. Global causes of diarrheal disease mortality in children & lt; 5 years of age: a systematic review. *PLoS One* 2013; **8**: e72788 [PMID: 24023773 DOI: 10.1371/journal.pone.0072788]

3 **Patel MM**, Hall AJ, Vinjé J, Parashar UD. Noroviruses: a comprehensive review. *J Clin Virol* 2009; **44**: 1-8 [PMID: 19084472 DOI: 10.1016/j.jcv.2008.10.009]

4 **Patel MM**, Widdowson MA, Glass RI, Akazawa K, Vinjé J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis* 2008; **14**: 1224-1231 [PMID: 18680645 DOI: 10.3201/eid1408.071114]

5 **Lee BE**, Pang XL. New strains of norovirus and the mystery of viral gastroenteritis epidemics. *CMAJ* 2013; **185**: 1381-1382 [PMID: 24003105 DOI: 10.1503/cmaj.130426]

6 **Barclay L**, Park GW, Vega E, Hall A, Parashar U, Vinjé J, Lopman B. Infection control for norovirus. *Clin Microbiol Infect* 2014; **20**: 731-740 [PMID: 24813073 DOI: 10.1111/1469-0691.12674]

7 **Glass RI**, Parashar UD, Estes MK. Norovirus gastroenteritis. *N Engl J Med* 2009; **361**: 1776-1785 [PMID: 19864676 DOI: 10.1056/NEJMra0804575]

8 **Ahmed SM**, Lopman BA, Levy K. A systematic review and meta-analysis of the global seasonality of norovirus. *PLoS One* 2013; **8**: e75922 [PMID: 24098406]

9 **Zheng DP**, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. Norovirus classification and proposed strain nomenclature. *Virology* 2006; **346**: 312-323 [PMID: 16343580]

10 **Vinjé J**, Green J, Lewis DC, Gallimore CI, Brown DW, Koopmans MP. Genetic polymorphism across regions of the three open reading frames of "Norwalk-like viruses". *Arch Virol* 2000; **145**: 223-241 [PMID: 10752550]

11 **Donaldson EF**, Lindesmith LC, Lobue AD, Baric RS. Viral shape-shifting: norovirus evasion of the human immune system. *Nat Rev Microbiol* 2010; **8**: 231-241 [PMID: 20125087 DOI: 10.1038/nrmicro2296]

12 **Katayama K**, Shirato-Horikoshi H, Kojima S, Kageyama T, Oka T, Hoshino F, Fukushi S, Shinohara M, Uchida K, Suzuki Y, Gojobori T, Takeda N. Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. *Virology* 2002; **299**: 225-239 [PMID: 12202225]

13 **Hoa Tran TN**, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. *J Clin Virol* 2013; **56**: 185-193 [PMID: 23218993 DOI: 10.1016/j.jcv.2012.11.011]

14 **Desai R**, Hembree CD, Handel A, Matthews JE, Dickey BW, McDonald S, Hall AJ, Parashar UD, Leon JS, Lopman B. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. *Clin Infect Dis* 2012; **55**: 189-193 [PMID: 22491335 DOI: 10.1093/cid/cis372]

15 **Siebenga JJ**, Vennema H, Renckens B, de Bruin E, van der Veer B, Siezen RJ, Koopmans M. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. *J Virol* 2007; **81**: 9932-9941 [PMID: 17609280]

16 **Allen DJ**, Gray JJ, Gallimore CI, Xerry J, Iturriza-Gómara M. Analysis of amino acid variation in the P2 domain of the GII-4 norovirus VP1 protein reveals putative variant-specific epitopes. *PLoS One* 2008; **3**: e1485 [PMID: 18213393 DOI: 10.1371/journal.pone.0001485]

17 **Allen DJ**, Noad R, Samuel D, Gray JJ, Roy P, Iturriza-Gómara M. Characterisation of a GII-4 norovirus variant-specific surface-exposed site involved in antibody binding. *Virol J* 2009; **6**: 150 [PMID: 19781066 DOI: 10.1186/1743-422X-6-150]

18 **Eden JS**, Tanaka MM, Boni MF, Rawlinson WD, White PA. Recombination within the pandemic norovirus GII.4 lineage. *J Virol* 2013; **87**: 6270-6282 [PMID: 23536665 DOI: 10.1128/JVI.03464-12]

19 **Lindesmith LC**, Donaldson EF, Lobue AD, Cannon JL, Zheng DP, Vinje J, Baric RS. Mechanisms of GII.4 norovirus persistence in human populations. *PLoS Med* 2008; **5**: e31 [PMID: 18271619 DOI: 10.1371/journal.pmed.0050031]

20 **Chen Y**, Li Z, Han D, Cui D, Chen X, Zheng S, Yu F, Liu J, Lai S, Yan Y, Lin Z, Shi Z, Wu T, Li L, Yang W. Viral agents associated with acute diarrhea among outpatient children in southeastern China. *Pediatr Infect Dis J* 2013; **32**: e285-e290 [PMID: 23407102 DOI: 10.1097/INF.0b013e31828c3de4]

21 **Gao Y**, Jin M, Cong X, Duan Z, Li HY, Guo X, Zuo Y, Zhang Y, Zhang Y, Wei L. Clinical and molecular epidemiologic analyses of

norovirus-associated sporadic gastroenteritis in adults from Beijing, China. *J Med Virol* 2011; **83**: 1078-1085 [PMID: 21503924 DOI: 10.1002/jmv.22077]

22 **Kojima S**, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, Natori K, Takeda N, Katayama K. Genogroup-specific PCR primers for detection of Norwalk-like viruses. *J Virol Methods* 2002; **100**: 107-114 [PMID: 11742657]

23 **Bull RA**, White PA. Mechanisms of GII.4 norovirus evolution. *Trends Microbiol* 2011; **19**: 233-240 [PMID: 21310617 DOI: 10.1016/j.tim.2011.01.002]

24 **Siebenga JJ**, Vennema H, Duizer E, Koopmans MP. Gastroenteritis caused by norovirus GGII.4, The Netherlands, 1994-2005. *Emerg Infect Dis* 2007; **13**: 144-146 [PMID: 17370531]

25 **Guo L**, Song J, Xu X, Ren L, Li J, Zhou H, Wang M, Qu J, Wang J, Hung T. Genetic analysis of norovirus in children affected with acute gastroenteritis in Beijing, 2004-2007. *J Clin Virol* 2009; **44**: 94-98 [PMID: 19062336 DOI: 10.1016/j.jcv.2008.10.002]

26 **Pang XL**, Preiksaitis JK, Wong S, Li V, Lee BE. Influence of novel norovirus GII.4 variants on gastroenteritis outbreak dynamics in Alberta and the Northern Territories, Canada between 2000 and 2008. *PLoS One* 2010; **5**: e11599 [PMID: 20661286 DOI: 10.1371/journal.pone.0011599]

27 **Siebenga JJ**, Lemey P, Kosakovsky Pond SL, Rambaut A, Vennema H, Koopmans M. Phylodynamic reconstruction reveals norovirus GII.4 epidemic expansions and their molecular determinants. *PLoS Pathog* 2010; **6**: e1000884 [PMID: 20463813 DOI: 10.1371/journal.ppat.1000884]

28 **Puustinen L**, Blazevic V, Huhti L, Szakal ED, Halkosalo A, Salminen M, Vesikari T. Norovirus genotypes in endemic acute gastroenteritis of infants and children in Finland between 1994 and 2007. *Epidemiol Infect* 2012; **140**: 268-275 [PMID: 21489338 DOI: 10.1017/S0950268811000549]

29 **Al-Ali RM**, Chehadeh W, Hamze M, Dabboussi F, Hlais S, Mallat H. First description of gastroenteritis viruses in Lebanese children: a pilot study. *J Infect Public Health* 2011; **4**: 59-64 [PMID: 21663874 DOI: 10.1016/j.jiph.2011.01.002]

30 **El-Mohammady H**, Mansour A, Shaheen HI, Henien NH, Motawea MS, Raafat I, Moustafa M, Adib-Messih IA, Sebeny PJ, Young SY, Klena JD. Increase in the detection rate of viral and parasitic enteric pathogens among Egyptian children with acute diarrhea. *J Infect Dev Ctries* 2012; **6**: 774-781 [PMID: 23277502 DOI: 10.3855/jidc.2349]

31 **Muhsen K**, Kassem E, Rubinstein U, Schachter Y, Kremer A, Goren S, Zilberstein I, Ephros M, Cohen D, Shulman LM. Incidence and characteristics of sporadic norovirus gastroenteritis associated with hospitalization of children less than 5 years of age in Israel. *Pediatr Infect Dis J* 2013; **32**: 688-690 [PMID: 23411623 DOI: 10.1097/INF.0b013e318287fc81]

32 **Leshem E**, Givon-Lavi N, Vinjé J, Gregoricus N, Parashar U, Dagan R. Differences in Norovirus-Associated Hospital Visits Between Jewish and Bedouin Children in Southern Israel. *Pediatr Infect Dis J* 2015; **34**: 1036-1038 [PMID: 26107344 DOI: 10.1097/INF.0000000000000786]

33 **Najafi A**, Najafi S, Vahdat K, Kargar M, Javdani N. Importance of viral pathogens in children with acute gastroenteritis in the south of Iran. *Ann Saudi Med* 2013; **33**: 124-129 [PMID: 23562998 DOI: 10.5144/0256-4947.2013.124]

34 **Kaplan NM**, Kirby A, Abd-Eldayem SA, Dove W, Nakagomi T, Nakagomi O, Cunliffe NA. Detection and molecular characterisation of rotavirus and norovirus infections in Jordanian children with acute gastroenteritis. *Arch Virol* 2011; **156**: 1477-1480 [PMID: 21503643 DOI: 10.1007/s00705-011-0996-x]

35 **Al-Rashidi A**, Chehadeh W, Szücs GG, Albert MJ. Different norovirus genotypes in patients with gastroenteritis in Kuwait. *J Med Virol* 2013; **85**: 1611-1618 [PMID: 23852687]

36 **Abugalia M**, Cuevas L, Kirby A, Dove W, Nakagomi O, Nakagomi T, Kara M, Gweder R, Smeo M, Cunliffe N. Clinical features and molecular epidemiology of rotavirus and norovirus infections in Libyan children. *J Med Virol* 2011; **83**: 1849-1856 [PMID: 21837804 DOI: 10.1002/jmv.22141]

37 **Rahouma A**, Klena JD, Krema Z, Abobker AA, Treesh K, Franka E, Abusnena O, Shaheen HI, El Mohammady H, Abudher A, Ghenghesh KS. Enteric pathogens associated with childhood diarrhea in Tripoli-Libya. *Am J Trop Med Hyg* 2011; **84**: 886-891 [PMID: 21633024 DOI: 10.4269/ajtmh.2011.11-0116]

38 **El Qazoui M**, Oumzil H, Baassi L, El Omari N, Sadki K, Amzazi S, Benhafid M, El Aouad R. Rotavirus and norovirus infections among acute gastroenteritis children in Morocco. *BMC Infect Dis* 2014; **14**: 300 [PMID: 24894194 DOI: 10.1186/1471-2334-14-300]

39 **Sdiri-Loulizi K**, Hassine M, Gharbi-Khelifi H, Aouni Z, Chouchane S, Sakly N, Neji-Guédiche M, Pothier P, Ambert-Balay K, Aouni M. Molecular detection of genogroup I sapovirus in Tunisian children suffering from acute gastroenteritis. *Virus Genes* 2011; **43**: 6-12 [PMID: 21461589 DOI: 10.1007/s11262-011-0600-1]

40 **Ben Salem-Ben Nejma I**, Hassine Zaafrane M, Hassine F, Sdiri-Loulizi K, Ben Said M, Aouni M, Mzoughi R. Etiology of Acute Diarrhea in Tunisian Children with Emphasis on Diarrheagenic Escherichia coli: Prevalence and Identification of E. coli Virulence Markers. *Iran J Public Health* 2014; **43**: 947-960 [PMID: 25909062]

41 **Bicer S**, Col D, Erdag GC, Giray T, Gurol Y, Yilmaz G, Vitrinel A, Ozelgun B. A retrospective analysis of acute gastroenteritis agents in children admitted to a university hospital pediatric emergency unit. *Jundishapur J Microbiol* 2014; **7**: e9148 [PMID: 25147694 DOI: 10.5812/jjm.9148]

42 **Cöl D**, Biçer S, Ciler Erdağ G, Giray T, Gürol Y, Yilmaz G, Küçük Ö, Vitrinel A. Annual report on norovirus in children with acute gastroenteritis in 2009 and their genotypes in Turkey. *Infez Med* 2013; **21**: 261-269 [PMID: 24335456]

43 **Altay A**, Bozdayı G, Meral M, Dallar Bilge Y, Dalgıç B, Ozkan S, Ahmed K. [Investigation of norovirus infection incidence among 0-5 years old children with acute gastroenteritis admitted to two different hospitals in ankara, Turkey]. *Mikrobiyol Bul* 2013; **47**: 98-108 [PMID: 23390907]

44 **Mitui MT**, Bozdayi G, Ahmed S, Matsumoto T, Nishizono A, Ahmed K. Detection and molecular characterization of diarrhea causing viruses in single and mixed infections in children: a comparative study between Bangladesh and Turkey. *J Med Virol* 2014; **86**: 1159-1168 [PMID: 24105741 DOI: 10.1002/jmv.23744]

45 **Ozkul AA**, Kocazeybek BS, Turan N, Reuter G, Bostan K, Yilmaz A, Altan E, Uyunmaz G, Karaköse AR, Muratoglu K, Elevli M, Helps CR, Yilmaz H. Frequency and phylogeny of norovirus in diarrheic children in Istanbul, Turkey. *J Clin Virol* 2011; **51**: 160-164 [PMID: 21592853 DOI: 10.1016/j.jcv.2011.03.004]

46 **Kirby A**, Al-Eryani A, Al-Sonboli N, Hafiz T, Beyer M, Al-Aghbari N, Al-Moheri N, Dove W, Cunliffe NA, Cuevas LE. Rotavirus and norovirus infections in children in Sana'a, Yemen. *Trop Med Int Health* 2011; **16**: 680-684 [PMID: 21392189 DOI: 10.1111/j.1365-3156.2011.02756.x]

47 **Rahman M**, Nahar S, Afrad MH, Faruque AS, Azim T. Norovirus variant GII.4/Sydney/2012, Bangladesh. *Emerg Infect Dis* 2013; **19**: 1347-1348 [PMID: 23880583 DOI: 10.3201/eid1908.130227]

48 **Eden JS**, Hewitt J, Lim KL, Boni MF, Merif J, Greening G, Ratcliff RM, Holmes EC, Tanaka MM, Rawlinson WD, White PA. The emergence and evolution of the novel epidemic norovirus GII.4 variant Sydney 2012. *Virology* 2014; **450-451**: 106-113 [PMID: 24503072 DOI: 10.1016/j.virol.2013.12.005]

49 **Medici MC**, Tummolo F, De Grazia S, Calderaro A, De Conto F, Terio V, Chironna M, Bonura F, Pucci M, Bányai K, Martella V, Giammanco GM. Epidemiological dynamics of norovirus GII.4 variant New Orleans 2009. *J Gen Virol* 2015; **96**: 2919-2927 [PMID: 26025873 DOI: 10.1099/vir.0.000204]

50 **Siebenga JJ**, Vennema H, Zheng DP, Vinjé J, Lee BE, Pang XL, Ho EC, Lim W, Choudekar A, Broor S, Halperin T, Rasool NB, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreier E, Ratcliff RM, White PA, Iritani N, Reuter G, Koopmans M. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001-2007. *J Infect Dis* 2009; **200**: 802-812 [PMID: 19627248 DOI: 10.1086/605127]

51 **White PA**, Hansman GS, Li A, Dable J, Isaacs M, Ferson M, McIver CJ, Rawlinson WD. Norwalk-like virus 95/96-US strain is a major cause of gastroenteritis outbreaks in Australia. *J Med Virol* 2002; **68**: 113-118 [PMID: 12210438]

52 **Lopman B**, Vennema H, Kohli E, Pothier P, Sanchez A, Negredo A, Buesa J, Schreier E, Reacher M, Brown D, Gray J, Iturriza M, Gallimore C, Bottiger B, Hedlund KO, Torvén M, von Bonsdorff CH, Maunula L, Poljsak-Prijatelj M, Zimsek J, Reuter G, Szücs G, Melegh B, Svennson L, van Duijnhoven Y, Koopmans M. Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. *Lancet* 2004; **363**: 682-688 [PMID: 15001325]

53 **Widdowson MA**, Cramer EH, Hadley L, Bresee JS, Beard RS, Bulens SN, Charles M, Chege W, Isakbaeva E, Wright JG, Mintz E, Forney D, Massey J, Glass RI, Monroe SS. Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus--United States, 2002. *J Infect Dis* 2004; **190**: 27-36 [PMID: 15195240]

54 **Bull RA**, Tu ET, McIver CJ, Rawlinson WD, White PA. Emergence of a new norovirus genotype II.4 variant associated with global outbreaks of gastroenteritis. *J Clin Microbiol* 2006; **44**: 327-333 [PMID: 16455879]

55 **Eden JS**, Bull RA, Tu E, McIver CJ, Lyon MJ, Marshall JA, Smith DW, Musto J, Rawlinson WD, White PA. Norovirus GII.4 variant 2006b caused epidemics of acute gastroenteritis in Australia during 2007 and 2008. *J Clin Virol* 2010; **49**: 265-271 [PMID: 20888289 DOI: 10.1016/j.jcv.2010.09.001]

56 **Yen C**, Wikswo ME, Lopman BA, Vinje J, Parashar UD, Hall AJ. Impact of an emergent norovirus variant in 2009 on norovirus outbreak activity in the United States. *Clin Infect Dis* 2011; **53**: 568-571 [PMID: 21832262 DOI: 10.1093/cid/cir478]

57 **van Beek J**, Ambert-Balay K, Botteldoorn N, Eden JS, Fonager J, Hewitt J, Iritani N, Kroneman A, Vennema H, Vinjé J, White PA, Koopmans M. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. *Euro Surveill* 2013; **18**: 8-9 [PMID: 23305715]

58 **de Andrade Jda S**, Rocha MS, Carvalho-Costa FA, Fioretti JM, Xavier Mda P, Nunes ZM, Cardoso J, Fialho AM, Leite JP, Miagostovich MP. Noroviruses associated with outbreaks of acute gastroenteritis in the State of Rio Grande do Sul, Brazil, 2004-2011. *J Clin Virol* 2014; **61**: 345-352 [PMID: 25223919 DOI: 10.1016/j.jcv.2014.08.024]

59 **Thongprachum A**, Khamrin P, Maneekarn N, Hayakawa S, Ushijima H. Epidemiology of gastroenteritis viruses in Japan: Prevalence, seasonality, and outbreak. *J Med Virol* 2016; **88**: 551-570 [PMID: 26387663 DOI: 10.1002/jmv.24387]

60 **Mans J**, Armah GE, Steele AD, Taylor MB. Norovirus Epidemiology in Africa: A Review. *PLoS One* 2016; **11**: e0146280 [PMID: 27116615 DOI: 10.1371/journal.pone]

61 **Puustinen L**, Blazevic V, Salminen M, Hämäläinen M, Räsänen S, Vesikari T. Noroviruses as a major cause of acute gastroenteritis in children in Finland, 2009-2010. *Scand J Infect Dis* 2011; **43**: 804-808 [PMID: 21696253 DOI: 10.3109/00365548.2011.588610]

62 **Ferreira MS**, Cubel Garcia Rde C, Xavier Mda P, Ribeiro RL, Assis RM, Mota Mdo C, Leite JP, Miagostovich MP, de Oliveira SA. Genotyping of gastroenteric viruses in hospitalised children: first report of norovirus GII.21 in Brazil. *Mem Inst Oswaldo Cruz* 2012; **107**: 1064-1067 [PMID: 23295760]

63 **Chhabra P**, Walimbe AM, Chitambar SD. Molecular characterization of three novel intergenotype norovirus GII recombinant strains from western India. *Virus Res* 2010; **147**: 242-246 [PMID: 19941918 DOI: 10.1016/j.virusres.2009.11.007]

64 **Mounts AW**, Ando T, Koopmans M, Bresee JS, Noel J, Glass RI. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *J Infect Dis* 2000; **181** Suppl 2: S284-S287 [PMID: 10804139]

65 **Rohayem J**. Norovirus seasonality and the potential impact of climate change. *Clin Microbiol Infect* 2009; **15**: 524-527 [PMID: 19604277 DOI: 10.1111/j.1469-0691.2009.02846.x]

66 **Verhoef L**, Depoortere E, Boxman I, Duizer E, van Duynhoven Y, Harris J, Johnsen C, Kroneman A, Le Guyader S, Lim W, Maunula L, Meldal H, Ratcliff R, Reuter G, Schreier E, Siebenga J, Vainio K, Varela C, Vennema H, Koopmans M; Food Borne Viruses in Europe Network. Emergence of new norovirus variants on spring cruise ships and prediction of winter epidemics. *Emerg Infect Dis* 2008; **14**: 238-243 [PMID: 18258116 DOI: 10.3201/eid1402.061567]

67 **Ayukekbong JA**, Andersson ME, Vansarla G, Tah F, Nkuo-Akenji T, Lindh M, Bergström T. Monitoring of seasonality of norovirus and other enteric viruses in Cameroon by real-time PCR: an exploratory study. *Epidemiol Infect* 2014; **142**: 1393-1402 [PMID: 24047516 DOI: 10.1017/S095026881300232X]

P- Reviewer: Solano R **S- Editor**: Yu J **L- Editor**: Kerr C
E- Editor: Zhang FF

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.v22.i48.10566

World J Gastroenterol 2016 December 28; 22(48): 10566-10574
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Helicobacter pylori inhibits the cleavage of TRAF1 *via* a CagA-dependent mechanism

Xiu-Kun Wan, Sheng-Ling Yuan, Yan-Chun Wang, Hao-Xia Tao, Wei Jiang, Zhang-Yan Guan, Cheng Cao, Chun-Jie Liu

Xiu-Kun Wan, Sheng-Ling Yuan, Yan-Chun Wang, Hao-Xia Tao, Wei Jiang, Zhang-Yan Guan, Cheng Cao, Chun-Jie Liu, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Biotechnology, Beijing 100071, China

Author contributions: Liu CJ designed the research; Wan XK performed the majority of experiments and analyzed the data; Yuan SL, Wang YC, Tao HX, Jiang W, Guan ZY and Cao C participated equally in the research; Wan XK and Liu CJ wrote and revised the paper.

Supported by Ministry of Science and Technology of the People's Republic of China and Major New Drug Creation, No. 2012ZX09301003-001-005.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at liucj@nic.bmi.ac.cn. All participants provided informed consent for data sharing.

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Chun-Jie Liu, Professor, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Biotechnology, 20 Dongdajie Street, Fengtai District, Beijing 100071, China. liucj@nic.bmi.ac.cn
Telephone: + 86-10-66948834
Fax: + 86-10-66948815

Received: June 28, 2016
Peer-review started: June 29, 2016
First decision: August 19, 2016
Revised: September 5, 2016
Accepted: October 10, 2016
Article in press: October 10, 2016
Published online: December 28, 2016

Abstract

AIM

To study the impact on cleavage of tumor necrosis factor receptor-associated factor 1 (TRAF1) regulated by *Helicobacter pylori* (*H. pylori*).

METHODS

Cleavage of TRAF1 was detected by western blotting in the human gastric cancer cell line AGS following treatment with an apoptosis inducer. Cleavage of TRAF1 mediated by caspase was examined *in vitro* using specific caspase inhibitors. The effect of the COOH-terminal TRAF1 fragment on gastric cell apoptosis during *H. pylori* infection was measured using flow cytometry. The impact of *H. pylori* infection on TRAF1 cleavage was detected in the presence of apoptosis inducer. The roles of *H. pylori* virulence factors that may regulate TRAF1 cleavage were analyzed using isogenic *cagA*-, *vacA*- and *cagE*-null mutants.

RESULTS

TRAF1 was found to be cleaved in AGS cells treated with the apoptosis inducer, and caspase-8 was the major caspase involved in the cleavage of TRAF1. The COOH-terminal TRAF1 fragment significantly induced cell apoptosis ($P < 0.05$) as well as promoted *H. pylori*-induced cell apoptosis ($P < 0.05$). *H. pylori* infection was found to significantly inhibit the cleavage of TRAF1 and to inhibit the activation of caspase-8 in the

presence of the apoptosis inducer at specific infection times and different cell/bacteria ratios. We also found that the effects of *cagE*- and *cagA*-null mutants on the inhibition of TRAF1 cleavage and activation of caspase-8 were significantly attenuated, compared with wild-type *H. pylori*, in the presence of the apoptosis inducer, showing that the virulence factor CagA was mainly involved in the inhibition of TRAF1 cleavage.

CONCLUSION

H. pylori infection significantly inhibits the cleavage of TRAF1 *via* a CagA-dependent mechanism, which would increase the relative amounts of full-length TRAF1 and exert an antiapoptotic effect on *H. pylori*-infected cells.

Key words: *Helicobacter pylori*; Tumor necrosis factor receptor-associated factor 1; CagA; Cleavage; Apoptosis

Core tip: We report our first results from a study of *Helicobacter pylori* (*H. pylori*)-mediated tumor necrosis factor receptor-associated factor 1 (TRAF1) cleavage. The impact of *H. pylori* infection and its virulence factors on TRAF1 cleavage were detected in the presence of an apoptosis inducer. This study demonstrates, for the first time, that the cleavage of TRAF1 and the activation of caspase-8 were significantly inhibited by *H. pylori* infection in the presence of an apoptosis inducer. In addition, the virulence factor CagA was mainly involved in the inhibition of TRAF1 cleavage.

Wan XK, Yuan SL, Wang YC, Tao HX, Jiang W, Guan ZY, Cao C, Liu CJ. *Helicobacter pylori* inhibits the cleavage of TRAF1 *via* a CagA-dependent mechanism. *World J Gastroenterol* 2016; 22(48): 10566-10574 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10566.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10566

INTRODUCTION

Gastric cancer is one of the most common malignant tumors and the third leading cause of cancer-related deaths worldwide[1]. *Helicobacter pylori* (*H. pylori*) is a gram-negative, spiral shaped pathogen that successfully colonizes human gastric mucosa and is a strong risk factor for chronic gastritis, peptic ulcer, mucosa-associated lymphoid tissue lymphoma and gastric cancer[2-4]. *H. pylori* pathogenesis is determined largely by interactions between bacterial factors and host cells. The best-characterized virulence determinants of *H. pylori* are the cytotoxin-associated gene pathogenicity island (*cag* PAI) and the vacuolating cytotoxin A (*vacA*). The *cag* PAI encodes a type Ⅳ secretion system (T4SS), which is responsible for injecting virulence factors, such as CagA protein, directly into host cells. Once injected into cells, the CagA protein will induce complex cell changes involving various host signaling pathways[5,6]. Though numerous studies have identified the association between *H. pylori* infection and the development of gastric carcinoma, the mechanisms underlying the carcinogenic potential of *H. pylori* are still not completely understood.

TRAF1 is a member of the TRAF family and is characterized by its diverse biological functions, acting through direct or indirect interactions with multiple tumor necrosis factor receptor (TNFR) family members and intracellular proteins[7]. Several studies have demonstrated that TRAF1 might exert an antiapoptotic role in lymphoma cells through regulation of the activation of NF-κB[8-10]. Wang *et al*[11] reported that TRAF1 expression was up-regulated in the human gastric mucosal samples infected with *H. pylori* in a clinical immunohistochemical analysis. Moreover, the up-regulation of TRAF1 was increased as the gastric disease progressed from chronic gastritis to gastric cancer.

Our previous study showed that the expression of TRAF1 is up-regulated by *H. pylori* infection in both gastric epithelial cells and mice. The up-regulation of TRAF1 inhibited cell apoptosis as well as increased the viability of infected cells, which suggested that TRAF1 is an important protein that contributes to the pathogenesis of *H. pylori*-related gastric cancer[12]. Interestingly, several studies have shown that TRAF1 could be transformed into a pro-apoptotic form after cleavage by caspase-8 in the presence of Fas ligand or TNF-α-induced apoptosis[13-15]. Caspase-8 cleaves TRAF1 into two fragments and overexpression of the COOH-terminal fragment could enhance Fas or TNF-α mediated apoptosis. However, whether the cleavage of TRAF1 could be influenced by *H. pylori* and the mechanisms are not known.

The aim of our work was to elucidate the effect on TRAF1 cleavage regulated by *H. pylori* and the roles of *H. pylori* virulence factors regulating TRAF1 cleavage. To gain a better understanding of the role of *H. pylori* infection related to TRAF1 cleavage, in the present study we detected the cleavage of TRAF1 in AGS cells co-cultured with *H. pylori* or sterile saline alone in the presence of an apoptosis inducer. We also analyzed the roles of *H. pylori* virulence factors that may regulate TRAF1 cleavage using isogenic *cagA*-, *vacA*- and *cagE*-null mutants.

MATERIALS AND METHODS

Cell line and H. pylori strains

The human gastric cancer cell line AGS was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). AGS cells were grown in F-12 medium (Invitrogen, United States) supplemented with 10% (vol/vol) fetal bovine serum and were cultured at 37 ℃ in a humidified 5% CO_2 atmosphere. *H. pylori*

strain NCTC11637, obtained from Chinese Center for Disease Control and Prevention (Beijing, China) was grown on Columbia agar plates with 5% sheep blood. The plates were incubated in a mixed atmosphere of 10% CO_2, 5% O_2, and 85% N_2, at 37 ℃ for 24 h, after which they were harvested by centrifugation at 1000 × *g* for 5 min at 4 ℃, washed 3 times with sterile saline, resuspended in sterile saline and added to gastric cells as indicated. Isogenic *cagA*-, *cagE*- and *vacA*-null mutants of *H. pylori* strain NCTC11637 were constructed by insertional mutagenesis using homologous recombination and selected with 25 μg/mL kanamycin.

Antibodies and reagents

The following primary antibodies were used: mouse anti-HA tag from Zhongshan Golden Bridge Biotechnology (Beijing, China); rabbit anti-GAPDH from Bioworld Technology (United States); rabbit anti-caspase-8 from Beyotime Biotechnology (Jiangsu, China). The following secondary antibodies were used: peroxidase-conjugated goat anti-mouse IgG (H + L) and peroxidase-conjugated goat anti-rabbit IgG (H + L) from Zhongshan Golden Bridge Biotechnology.

The pharmacological inhibitors were as follows: caspase-8 inhibitor Z-IETD-FMK from BioVision (United States); pan caspase inhibitor Z-VAD-FMK and caspase-3 inhibitor Ac-DEVD-CHO from Beyotime Biotechnology. The apoptosis inducer cycloheximide (CHX) and TNF-α were purchased from BioVision and Peprotech (United States) respectively.

Plasmids and transfection

The plasmids pcDNA-TRAF1-HA, pcDNA-TRAF1(D163A)-HA, pcDNA- TRAF1-N-HA, pcDNA-TRAF1-C-HA were constructed by inserting the specific cDNA and HA-tag sequences into pcDNA3.1 (+) (conserved in our laboratory) respectively.

In brief, AGS cells were seeded in a 24-well plate at a density of 1 × 10^5 cells/well and cultured for 24 h, with a target of 60%-80% confluency at the time of transfection. Cells were transfected with 0.5 μg of plasmids using jetPRIME Transfection Reagent (Polyplus, France) according to the manufacturer's protocol. After 24 h of transfection, cells were co-cultured with *H. pylori* for the indicated experiments.

Western blot analysis

Proteins were extracted from the cultured cells and homogenized in a lysis buffer containing a protease inhibitor cocktail (CWBiotech, Beijing, China) according to the instructions. Then, the proteins were separated on 12% SDS-PAGE gels and transferred to nitrocellulose membranes (GE Healthcare Life Sciences, United States). The membranes were blocked in phosphate-buffered saline plus Tween-20 (PBST) containing 5% skim milk powder for 2 h at 37 ℃, and then incubated with the primary antibodies for 1.5 h at 37 ℃. Next, the membranes were incubated with peroxidase-conjugated secondary antibodies for 1 h at 37 ℃. The membranes were treated with ECL reagents (Engreen Biosystem, Beijing, China) and visualized using a Minichemi Lane1D imager (Sagecreation, Beijing, China), according to the manufacturer's instructions.

Cell apoptosis analysis

Cell apoptosis was measured according to the instructions of the Annexin V-FITC Apoptosis Detection Kit purchased from Beyotime Biotechnology. Briefly, AGS cells were transfected with the respective plasmids for 24 h and followed by co-culture with *H. pylori* for 24 h. Then, the cells were collected, washed in sterile PBS, and resuspended at a density of 1 × 10^6 cells/mL. After that, the cells were stained with Annexin V-FITC and propidium iodide (PI) for 10 min prior to the analysis using a FACScan flow cytometer (Bio-Rad, United States).

Statistical analysis

Statistical analyses in our paper were performed using the software GraphPad Prism 5. The results are presented as mean ± SD. Differences among groups were compared using variance analysis, and comparisons between two groups were performed using an unpaired Student's *t*-test. Differences were considered to be significant at $P < 0.05$.

RESULTS

TRAF1 is cleaved via caspase-8 in AGS cells infected with H. pylori

To confirm that TRAF1 could be cleaved under the condition of *H. pylori* infection, AGS cells transfected with HA-tagged TRAF1 were treated with the apoptosis inducer (0.3 μg/mL CHX and 80 ng/mL TNF-α) for 6 h, and then analyzed for the cleavage of TRAF1. Our results showed that TRAF1 could be significantly cleaved in AGS cells treated with the apoptosis inducer (Figure 1A). To confirm that the cleavage site of TRAF1 is located at aspartic acid 163 in the ^{160}LVED^{163} motif, AGS cells were transfected with HA-tagged TRAF1 mutation (D163A) and treated with the apoptosis inducer. The result showed that the TRAF1 mutation (D163A) could not be cleaved by the apoptosis inducer, which suggested the cleavage site of TRAF1 is located at aspartic acid 163 (Figure 1B). To further confirm the cleavage of TRAF1 mediated by caspase-8, TRAF1 cleavage was examined *in vitro* using specific caspase inhibitors in the presence of the apoptosis inducer treatment or *H. pylori* infection. As shown in Figure 1C and 1D, TRAF1 cleavage was blocked completely by the addition of Z-VAD-FMK, a broad specificity inhibitor of caspases, and was mainly blocked by Z-IETD-FMK, a caspase-8-specific inhibitor. However, Z-YVAD-FMK, a caspase-3-specific inhibitor, had no apparent effect on TRAF1 cleavage. The results show caspase-8 is the

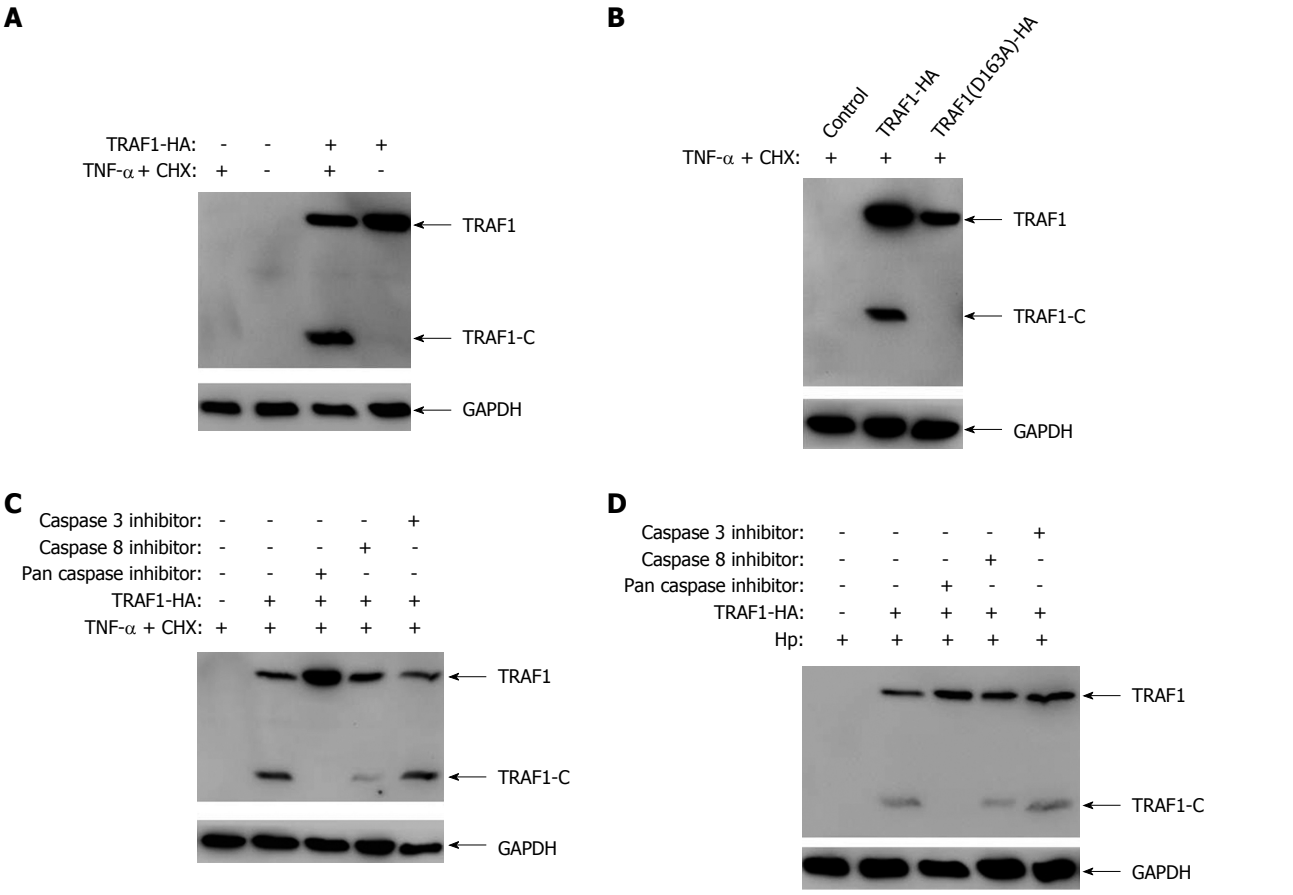

Figure 1 Tumor necrosis factor receptor-associated factor 1 is cleaved *via* caspase-8 in AGS cells infected with *Helicobacter pylori*. A: AGS cells transfected with either HA-tagged tumor necrosis factor receptor-associated factor 1 (TRAF1) or empty vector were treated with the apoptosis inducer (0.3 μg/mL CHX and 80 ng/mL TNF-α) for 6 h, and the cleavage of TRAF1 was detected by western blotting; B: AGS cells were transfected with wild-type or D163A mutant of TRAF1 and analyzed for the cleavage of TRAF1 by western blotting; C: AGS cells transfected with the HA-tagged TRAF1 were pretreated with 20 μmol/L of Z-VAD-FMK, Z-IETD-FMK, or Z-YVAD-FMK for 30 min, followed by treatment with the apoptosis inducer for 6 h and then analyzed for the cleavage of TRAF1 by western blotting; D: AGS cells transfected with the HA-tagged TRAF1 were pretreated with 20 μmol/L of Z-VAD-FMK, Z-IETD-FMK, or Z-YVAD-FMK for 30 min, followed by infection with *Helicobacter pylori* strain NCTC11637 for 6 h and then analyzed for the cleavage of TRAF1 by western blotting.

major caspase involved in the cleavage of TRAF1 in cells infected with *H. pylori.*

COOH-terminal TRAF1 fragment promoted cell apoptosis in H. pylori infected cells

To confirm the pro-apoptotic role of the COOH-terminal TRAF1 fragment, AGS cells were transfected with HA-tagged TRAF1, TRAF1 (D163A), the NH2-terminal TRAF1 (TRAF1-N), the COOH-terminal TRAF1 (TRAF1-C), or empty vector respectively and cell apoptosis was analyzed by flow cytometry. The results showed transfection with TRAF1, TRAF1 (D163A), or TRAF1-N had no significant effect on cell apoptosis compared with empty vector control, whereas transfection with TRAF1-C significantly promoted cell apoptosis (Figure 2A). In addition, to confirm the pro-apoptotic role of the COOH-terminal TRAF1 fragment in AGS cells infected with *H. pylori*, AGS cells were also transfected with the above five plasmids respectively and infected with *H. pylori* for 24 h and analyzed for cell apoptosis (Figure 2B). The results showed transfection with TRAF1-N has no significant effect on cell apoptosis. Transfection with TRAF1 or TRAF1 (D163A) significantly inhibited *H. pylori*-induced apoptosis, whereas transfection with TRAF1-C significantly promoted *H. pylori*-induced apoptosis, thereby indicating the pro-apoptotic role of the COOH-terminal TRAF1 fragment in *H. pylori* infected cells.

H. pylori infection significantly inhibits the cleavage of TRAF1

To investigate the effect of *H. pylori* infection on TRAF1 cleavage in cells, the expression of TRAF1 and its cleavage were examined in AGS cells when co-cultured with *H. pylori* or sterile saline alone in the presence of the apoptosis inducer for the specific infection times and cell/bacteria ratios. The cleavage of TRAF1 in AGS cells decreased significantly 4 h after *H. pylori* infection compared with the uninfected cells (Figure 3A). The effect of cell/bacteria ratios (1:10-1:500) on TRAF1 cleavage was then tested. A significantly decreased cleavage was observed at a ratio of 1:50, and the cleavage of TRAF1 gradually decreased as the bacteria amounts increased (Figure 3B). As caspase-8 is the

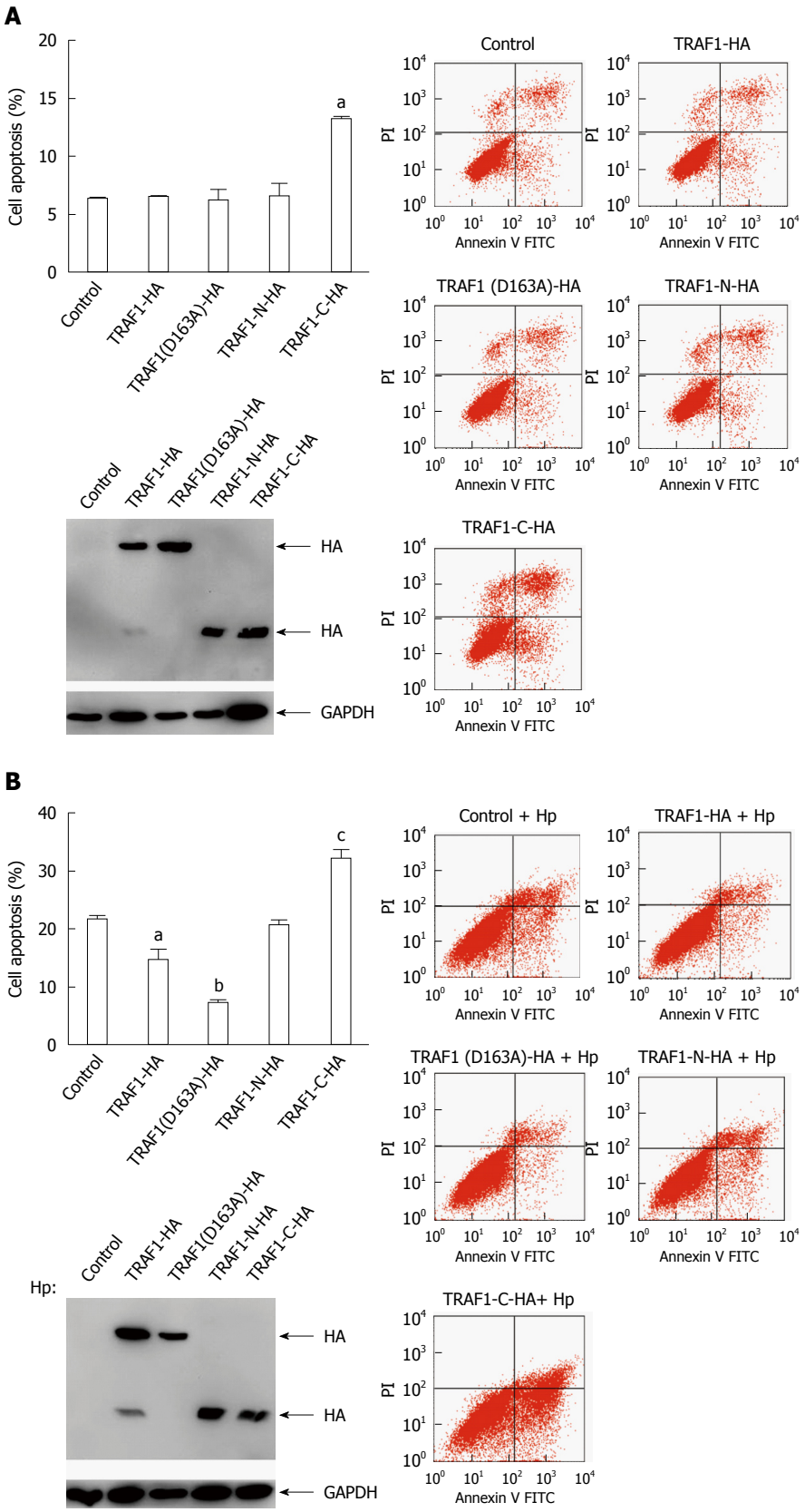

Figure 2 COOH-terminal tumor necrosis factor receptor-associated factor 1 fragment promoted cell apoptosis in *Helicobacter pylori* infected cells. A: AGS cells were transfected with HA-tagged tumor necrosis factor receptor-associated factor 1 (TRAF1), TRAF1(D163A), TRAF1-N, TRAF1-C for 24 h and then analyzed for cell apoptosis by flow cytometry using annexin V-FITC and propidium iodide staining ($^{a}P < 0.05$ *vs* control). The expression levels of these proteins in the analyzed cells were shown (Lower); B: AGS cells were transfected with HA-tagged TRAF1, TRAF1(D163A), TRAF1-N, TRAF1-C for 24 h respectively, followed by infection with *Helicobacter pylori* strain NCTC11637 for 24 h and then analyzed for cell apoptosis ($^{a}P < 0.05$ *vs* control, $^{b}P < 0.01$ *vs* Control, $^{c}P < 0.05$ *vs* control). The expression levels of these proteins in the analyzed cells are shown (Lower).

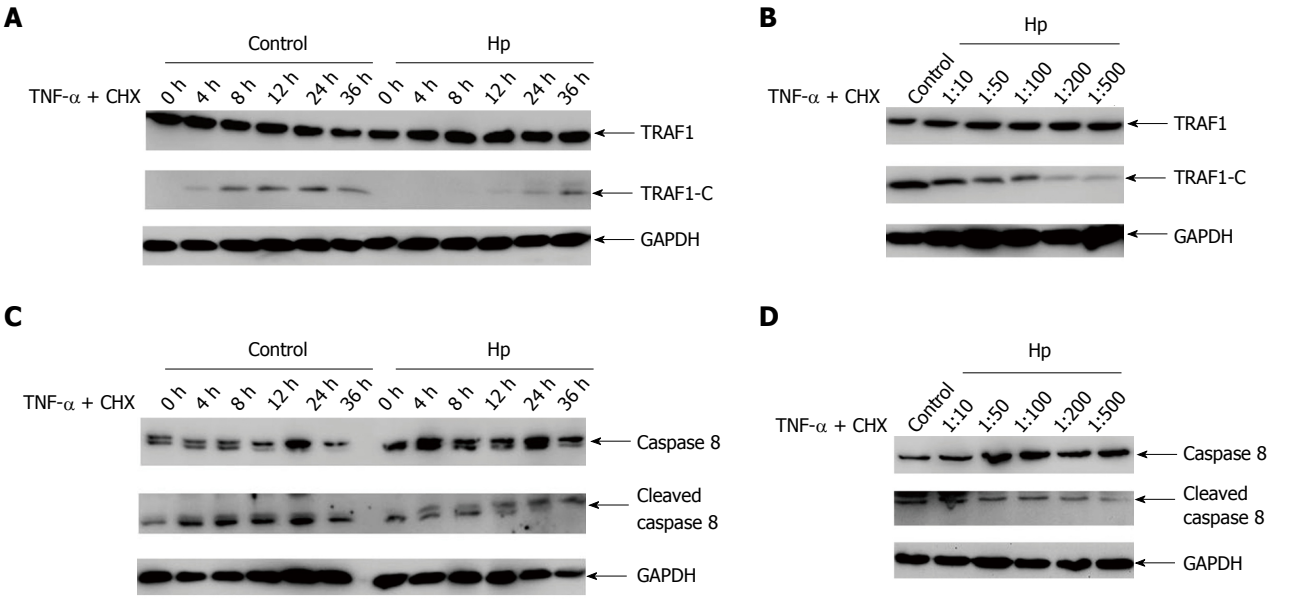

Figure 3 ***Helicobacter pylori*** **inhibits the cleavage of tumor necrosis factor receptor-associated factor 1.** A: AGS cells transfected with HA-tagged tumor necrosis factor receptor-associated factor 1 (TRAF1) were treated with the apoptosis inducer (0.3 μg/mL CHX and 80 ng/mL TNF-α) and co-cultured with *Helicobacter pylori* (*H. pylori*) strain NCTC11637, or left untreated for the infection time indicated, and analyzed for the cleavage of TRAF1 by western blotting; B: AGS cells transfected with HA-tagged TRAF1 were treated with the apoptosis inducer and co-cultured with *H. pylori* strain NCTC11637, or left untreated, at the indicated cell/bacteria ratios and analyzed for the cleavage of TRAF1 by western blotting; C: The same as shown in (A), but analyzed for the uncleaved and cleaved caspase-8 by western blotting; D: The same as shown in (B), but analyzed for the uncleaved and cleaved caspase-8 by western blotting.

major caspase involved in the cleavage of TRAF1, we next analyzed the activation of caspase-8. As shown in Figure 3C and D, the cleaved caspase-8 was significantly decreased when co-cultured with *H. pylori* in the presence of the apoptosis inducer for the specific infection times as well as the indicated cell/bacteria ratios, suggesting that the activity of caspase-8 was inhibited. These results indicate that *H. pylori* infection significantly inhibits the cleavage of TRAF1.

CagA is the major virulence factor involved in inhibiting the cleavage of TRAF1

As CagA is a major virulence factor in *H. pylori*-related gastric pathogenesis, we next investigated whether CagA could affect TRAF1 cleavage. The isogenic *cagA*-, *vacA*- and *cagE*-null mutants were used. *H. pylori* lacking CagE cannot form an effective type Ⅳ secretion system and inhibits the translocation of CagA into cells, whereas knocking-out of *cagA* and *vacA* leads to no expression of the VacA and CagA virulence factors, respectively.

AGS cells were infected with the wild-type *H. pylori* or the isogenic *H. pylori* mutants in the presence of the apoptosis inducer, and analyzed for the TRAF1 cleavage. The results showed the TRAF1 cleavage significantly increased in 6 h and 12 h after infection with *cagE*- and *cagA*-null mutants compared with wild-type *H. pylori*, while the *vacA*-null mutant had no significant changes compared with wild-type *H. pylori* (Figure 4A). A significantly increased cleavage was also observed in *cagE*- and *cagA*-null mutants at the indicated cell/bacteria ratios (1:100-1:500) (Figure 4B). This suggested that the effect of *cagE*- and *cagA*-null mutants on the inhibition of TRAF1 cleavage was significantly attenuated compared with wild-type *H. pylori* in the presence of the apoptosis inducer. We also analyzed the uncleaved and cleaved caspase-8 forms at the indicated infection time and cell/bacteria ratios. As shown in Figure 4C and D, the activity of caspase-8 significantly increased in the *cagE*- and *cagA*-null mutants compared with wild-type *H. pylori*. Thus, these results suggested that the virulence factor CagA plays an important role in the *H. pylori*-mediated inhibition of TRAF1 cleavage.

DISCUSSION

In the present study, we show the previously unidentified role of TRAF1 cleavage regulated by *H. pylori* infection. TRAF1 was found to be cleaved *via* caspase-8 in AGS cells treated with an apoptosis inducer, and the COOH-terminal TRAF1 fragment was found to promote *H. pylori*-induced cell apoptosis. However, *H. pylori* infection was found to inhibit the cleavage of TRAF1 as well as inhibit the activation of caspase-8 in the presence of the apoptosis inducer. We also found that the *H. pylori* virulence factor CagA is mainly involved in the *H. pylori*-mediated inhibition of TRAF1 cleavage.

TRAF1 is a member of the TRAF family and is dysregulated in various diseases, such as atheroma, lymphoma, and solid tumors[16-19]. Some studies suggested that TRAF1 plays an antiapoptotic role[8,9] and could suppress TNFR-induced cell apoptosis[20,21]. Immunohistochemistry of gastric mucosal specimens from *H. pylori*-positive patients with chronic gastritis (CG), intestinal metaplasia (IM), dysplasia, and

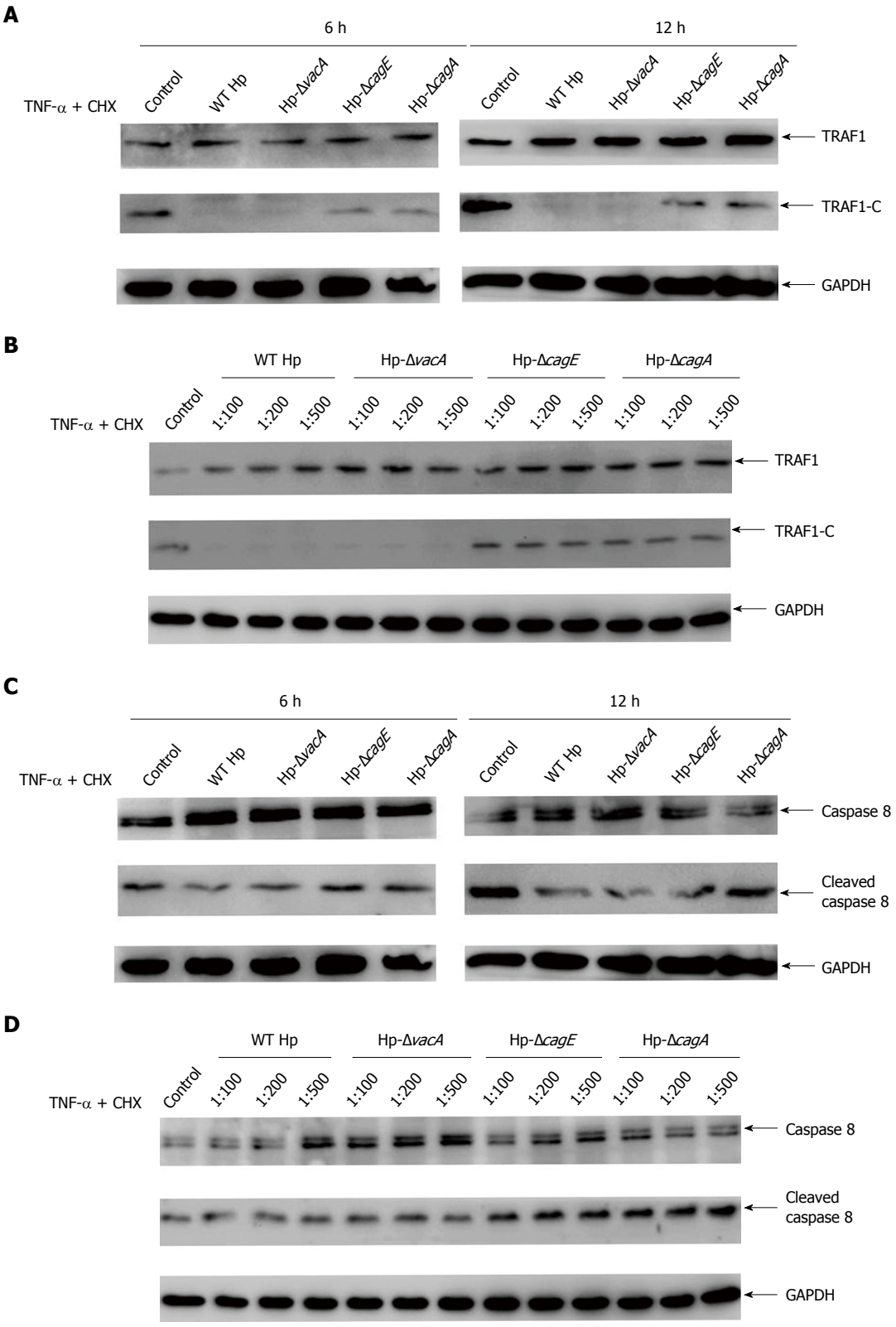

Figure 4 CagA is the major factor involved in inhibiting the cleavage of tumor necrosis factor receptor-associated factor 1. A: AGS cells transfected with tumor necrosis factor receptor-associated factor 1 (TRAF1) were treated with the apoptosis inducer (0.3 μg/mL CHX and 80 ng/mL TNF-α), and co-cultured with *Helicobacter pylori* (*H. pylori*) strain NCTC11637 or its isogenic *vacA*-, *cagE*- or *cagA*-null mutants, and analyzed for the cleavage of TRAF1 at 6 h and 12 h by western blotting; B: AGS cells transfected with TRAF1 were treated with the apoptosis inducer, and co-cultured with *H. pylori* strain NCTC11637, or its isogenic *vacA*-, *cagE*- or *cagA*-null mutants at the indicated cell/bacteria ratios, and analyzed for the cleavage of TRAF1 by western blotting; C: The same as shown in (A), but analyzed for uncleaved and cleaved caspase-8 by western blotting; D: The same as shown in (B), but analyzed for uncleaved and cleaved caspase-8 by western blotting.

gastric carcinoma (GC) showed that positive TRAF1 expression was detected in 34.8%, 53.3%, 72.7%, and 88.9% specimens of CG, IM, dysplasia, and GC, respectively, suggesting that TRAF1 may be involved in *H. pylori*-induced gastric carcinogenesis[11]. Our previous study showed that the up-regulation of TRAF1 could be induced by *H. pylori* in both gastric epithelial cells and mice. The up-regulation of TRAF1 inhibited cell apoptosis and increased the viability of gastric epithelial cells infected with *H. pylori*. We also determined that *H. pylori* infection significantly enhanced the formation of the antiapoptotic complex containing TRAF1, TRAF2, cIAP1, and cIAP2, which might play a major role in suppressing the activation of caspase-8 and in inhibiting cell apoptosis[12].

Though the full-length TRAF1 protein exerts an anti-apoptotic role during *H. pylori* infection, TRAF1 could be converted into a pro-apoptotic protein after cleavage[13-15]. So, it is important to explore the regulation of TRAF1 cleavage by *H. pylori*. In our study, we found the cleavage of TRAF1 as well as the activation of caspase-8 induced by the apoptosis inducer could be significantly inhibited by *H. pylori* infection. As the COOH-terminal TRAF1 fragment has a pro-apoptotic role, the inhibition of TRAF1 cleavage may further protect *H. pylori*-infected cells from apoptosis. Our results showed that the cleavage of TRAF1 decreased significantly after 4 h following *H. pylori* infection and the cleavage of TRAF1 gradually decreased as the bacteria amounts increased, which suggests the inhibition of TRAF1 cleavage is related to the infection time and the bacterial amounts.

Another important finding is that the inhibition of TRAF1 cleavage was mainly dependent on the *H. pylori* virulence factor CagA. Our results suggested that the effects of *cagE*- and *cagA*-null mutants on the inhibition of TRAF1 cleavage were significantly attenuated compared with wild-type *H. pylori* or the *vacA*-null mutant in the presence of the apoptosis inducer. However, the mechanisms are not completely understood. It is possible that CagA-positive *H. pylori* up-regulates the expression of TRAF1, TRAF2, cIAP1, and cIAP2, which can increase formation of the antiapoptotic complex to suppress caspase-8 activation and then inhibit TRAF1 cleavage. It is also possible that *H. pylori* inhibits caspase-8 activation *via* other signaling pathways mediated by activation of NF-κB, thereby inhibiting TRAF1 cleavage. NF-κB is a major transcription regulator involved in inflammation, cell proliferation, and apoptosis, which are considered to be anti-apoptotic and to have an oncogenic role[22]. *H. pylori* infection could activate NF-κB through host-bacterial interactions *via* multiple signaling pathways[23,24]. Among these pathways, CagA is the major bacterial component to induce activation of NF-κB through interaction with multiple signaling regulators, such as receptor tyrosine kinase c-Met, guanine exchange factor α-Pix, and Ras, thereby leading to activation of phosphatidylinositol-3-kinase (PI3K)/Akt kinase, transforming growth factor beta-activated kinase 1 (TAK1), and MAPK/ERK signaling pathways[25-28].

In summary, our study suggested that during its evolutionary adaptation to the host gastric environment and to counteract detrimental conditions, *H. pylori* infection induced the up-regulation of TRAF1 as well as inhibited its cleavage. Therefore, the dysregulation and increase of TRAF1 may be advantageous for *H. pylori* by allowing this pathogen long-term habitation in the gastric mucosa without triggering cell apoptosis. However, this change may increase the risk of gastric carcinoma.

ACKNOWLEDGMENTS

We would like to express our great appreciation to Weiwei Liu for his statistical review of our data.

COMMENTS

Background

Our previous study showed that tumor necrosis factor receptor-associated factor 1 (TRAF1) is up-regulated by *Helicobacter pylori* (*H. pylori*) infection in both gastric epithelial cells and mice. The up-regulation of TRAF1 has been shown to inhibit cell apoptosis and to increase the viability of infected cells, suggesting that TRAF1 may be an important molecule during the pathogenesis of *H. pylori*-related gastric cancer. Interestingly, several studies have shown that TRAF1 can be converted into a pro-apoptotic protein after cleavage by caspase-8 in the presence of Fas ligand or TNF-α-induced apoptosis. However, whether the cleavage of TRAF1 could be influenced by *H. pylori* infection and the related mechanism are not known.

Research frontiers

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer-related deaths worldwide. Though numerous studies have identified an association between *H. pylori* infection and the development of gastric carcinoma, the mechanisms underlying the carcinogenic potential of *H. pylori* are still not completely understood.

Innovations and breakthroughs

The authors report our first results from a study of *H. pylori*-mediated TRAF1 cleavage. This study demonstrates for the first time that the cleavage of TRAF1 and the activation of caspase-8 were significantly inhibited by *H. pylori* infection in the presence of an apoptosis inducer. In addition, the virulence factor CagA was found to be mainly involved in the inhibition of TRAF1 cleavage.

Applications

This results suggest that, during its evolutionary adaptation to the host environment and to counteract detrimental conditions, *H. pylori* infection induced the up-regulation of TRAF1 as well as inhibited its cleavage. The up-regulation of TRAF1 inhibited cell apoptosis and increased the viability of infected cells, suggesting that TRAF1 may be a potential target for prevention and diagnosis during the pathogenesis of gastric cancer related to *H. pylori* infection.

Terminology

Cytotoxin-associated gene A (CagA), an important virulence factor and the only bacterial oncoprotein, is delivered into gastric epithelial cells *via* type Ⅳ secretion of *H. pylori*. Upon delivery, CagA perturbs multiple host signaling pathways by interacting with the host signaling molecules, resulting in cytopathic effects and subsequent cell transformation.

Peer-review

The following are comments and questions to authors. I hope to take

consideration for further improvement: (1) the detailed characteristics of AGS cell and the reason why this cell line was selected needs to be explained; (2) the authors revealed TRAF1 was cleaved by an apoptosis inducer and it was attenuated by a caspase-8 inhibitor. Do the authors have data that caspase-8 itself cleaved TRAF1? (3) for easy understanding, a correlation diagram in *H. pylori* and TRAF1 cleavage should be presented.

REFERENCES

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

2 **Marshall BJ**, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1**: 1311-1315 [PMID: 6145023]

3 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186 [PMID: 12374879]

4 **Bessède E**, Dubus P, Mégraud F, Varon C. Helicobacter pylori infection and stem cells at the origin of gastric cancer. *Oncogene* 2015; **34**: 2547-2555 [PMID: 25043305 DOI: 10.1038/onc.2014.187]

5 **Fischer W**. Assembly and molecular mode of action of the Helicobacter pylori Cag type IV secretion apparatus. *FEBS J* 2011; **278**: 1203-1212 [PMID: 21352490 DOI: 10.1111/j.1742-4658.2011.08036.x]

6 **Tohidpour A**. CagA-mediated pathogenesis of Helicobacter pylori. *Microb Pathog* 2016; **93**: 44-55 [PMID: 26796299 DOI: 10.1016/j.micpath.2016.01]

7 **Lee SY**, Choi Y. TRAF1 and its biological functions. *Adv Exp Med Biol* 2007; **597**: 25-31 [PMID: 17633014]

8 **Guo F**, Sun A, Wang W, He J, Hou J, Zhou P, Chen Z. TRAF1 is involved in the classical NF-kappaB activation and CD30-induced alternative activity in Hodgkin's lymphoma cells. *Mol Immunol* 2009; **46**: 2441-2448 [PMID: 19540595 DOI: 10.1016/j.molimm.2009.05.178]

9 **Xie P**, Hostager BS, Munroe ME, Moore CR, Bishop GA. Cooperation between TNF receptor-associated factors 1 and 2 in CD40 signaling. *J Immunol* 2006; **176**: 5388-5400 [PMID: 16622006]

10 **Sughra K**, Birbach A, de Martin R, Schmid JA. Interaction of the TNFR-receptor associated factor TRAF1 with I-kappa B kinase-2 and TRAF2 indicates a regulatory function for NF-kappa B signaling. *PLoS One* 2010; **5**: e12683 [PMID: 20856938 DOI: 10.1371/journal.pone.0012683]

11 **Wang F**, Luo LD, Pan JH, Huang LH, Lv HW, Guo Q, Xu CX, Shen SR. Comparative genomic study of gastric epithelial cells co-cultured with Helicobacter pylori. *World J Gastroenterol* 2012; **18**: 7212-7224 [PMID: 23326126 DOI: 10.3748/wjg.v18.i48.7212]

12 **Wan XK**, Yuan SL, Tao HX, Diao LP, Wang YC, Cao C, Liu CJ. The Upregulation of TRAF1 Induced by Helicobacter pylori Plays an Antiapoptotic Effect on the Infected Cells. *Helicobacter* 2016; Epub ahead of print [PMID: 27060717 DOI: 10.1111/hel]

13 **Jang HD**, Chung YM, Baik JH, Choi YG, Park IS, Jung YK, Lee SY. Caspase-cleaved TRAF1 negatively regulates the antiapoptotic signals of TRAF2 during TNF-induced cell death. *Biochem Biophys Res Commun* 2001; **281**: 499-505 [PMID: 11181075]

14 **Leo E**, Deveraux QL, Buchholtz C, Welsh K, Matsuzawa S, Stennicke HR, Salvesen GS, Reed JC. TRAF1 is a substrate of caspases activated during tumor necrosis factor receptor-alpha-induced apoptosis. *J Biol Chem* 2001; **276**: 8087-8093 [PMID: 11098060]

15 **Irmler M**, Steiner V, Ruegg C, Wajant H, Tschopp J. Caspase-induced inactivation of the anti-apoptotic TRAF1 during Fas ligand-mediated apoptosis. *FEBS Lett* 2000; **468**: 129-133 [PMID: 10692572]

16 **Schwenzer R**, Siemienski K, Liptay S, Schubert G, Peters N, Scheurich P, Schmid RM, Wajant H. The human tumor necrosis factor (TNF) receptor-associated factor 1 gene (TRAF1) is up-regulated by cytokines of the TNF ligand family and modulates TNF-induced activation of NF-kappaB and c-Jun N-terminal kinase. *J Biol Chem* 1999; **274**: 19368-19374 [PMID: 10383449]

17 **Zirlik A**, Bavendiek U, Libby P, MacFarlane L, Gerdes N, Jagielska J, Ernst S, Aikawa M, Nakano H, Tsitsikov E, Schönbeck U. TRAF-1, -2, -3, -5, and -6 are induced in atherosclerotic plaques and differentially mediate proinflammatory functions of CD40L in endothelial cells. *Arterioscler Thromb Vasc Biol* 2007; **27**: 1101-1107 [PMID: 17332487]

18 **Zapata JM**, Krajewska M, Krajewski S, Kitada S, Welsh K, Monks A, McCloskey N, Gordon J, Kipps TJ, Gascoyne RD, Shabaik A, Reed JC. TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J Immunol* 2000; **165**: 5084-5096 [PMID: 11046039]

19 **Rajandram R**, Bennett NC, Wang Z, Perry-Keene J, Vesey DA, Johnson DW, Gobe GC. Patient samples of renal cell carcinoma show reduced expression of TRAF1 compared with normal kidney and functional studies in vitro indicate TRAF1 promotes apoptosis: potential for targeted therapy. *Pathology* 2012; **44**: 453-459 [PMID: 22810054 DOI: 10.1097/PAT.0b013e3283557748]

20 **Wang CY**, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AS. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* 1998; **281**: 1680-1683 [PMID: 9733516]

21 **Micheau O**, Lens S, Gaide O, Alevizopoulos K, Tschopp J. NF-kappaB signals induce the expression of c-FLIP. *Mol Cell Biol* 2001; **21**: 5299-5305 [PMID: 11463813]

22 **Karin M**, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 2005; **5**: 749-759 [PMID: 16175180]

23 **Doger FK**, Meteoglu I, Ozkara E, Erkul ZK, Okyay P, Yükselen V. Expression of NF-kappaB in Helicobacter pylori infection. *Dig Dis Sci* 2006; **51**: 2306-2309 [PMID: 17103034]

24 **Backert S**, Naumann M. What a disorder: proinflammatory signaling pathways induced by Helicobacter pylori. *Trends Microbiol* 2010; **18**: 479-486 [PMID: 20863705 DOI: 10.1016/j.tim.2010.08.003]

25 **Brandt S**, Kwok T, Hartig R, König W, Backert S. NF-kappaB activation and potentiation of proinflammatory responses by the Helicobacter pylori CagA protein. *Proc Natl Acad Sci USA* 2005; **102**: 9300-9305 [PMID: 15972330]

26 **Suzuki M**, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Morikawa H, Nagai S, Koyasu S, Gilman RH, Kersulyte D, Berg DE, Sasakawa C. Helicobacter pylori CagA phosphorylation-independent function in epithelial proliferation and inflammation. *Cell Host Microbe* 2009; **5**: 23-34 [PMID: 19154985 DOI: 10.1016/j.chom.2008.11.010]

27 **Lim JW**, Kim KH, Kim H. alphaPix interacts with Helicobacter pylori CagA to induce IL-8 expression in gastric epithelial cells. *Scand J Gastroenterol* 2009; **44**: 1166-1172 [PMID: 19672789 DOI: 10.1080/00365520903144398]

28 **Lamb A**, Yang XD, Tsang YH, Li JD, Higashi H, Hatakeyama M, Peek RM, Blanke SR, Chen LF. Helicobacter pylori CagA activates NF-kappaB by targeting TAK1 for TRAF6-mediated Lys 63 ubiquitination. *EMBO Rep* 2009; **10**: 1242-1249 [PMID: 19820695 DOI: 10.1038/embor.2009.210]

P- Reviewer: Pellicano R, Suzuki H, Vaziri F, Yamaoka Y
S- Editor: Qi Y **L- Editor**: Filipodia **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.v22.i48.10575

World J Gastroenterol 2016 December 28; 22(48): 10575-10583
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Repair of a common bile duct defect with a decellularized ureteral graft

Yao Cheng, Xian-Ze Xiong, Rong-Xing Zhou, Yi-Lei Deng, Yan-Wen Jin, Jiong Lu, Fu-Yu Li, Nan-Sheng Cheng

Yao Cheng, Xian-Ze Xiong, Rong-Xing Zhou, Yi-Lei Deng, Yan-Wen Jin, Jiong Lu, Fu-Yu Li, Nan-Sheng Cheng, Department of Bile Duct Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Author contributions: Cheng Y and Xiong XZ contributed equally to this work; Cheng Y and Cheng NS designed the study; Zhou RX, Deng YL and Jin YW carried out the study; Xiong XZ and Lu J collected and analyzed the data; Cheng Y and Li FY wrote the paper.

Supported by National Natural Science Foundation of China, No. 30972923.

Institutional review board statement: The study was reviewed and approved by the Local Ethics Committee of West China Hospital (Sichuan, China).

Institutional animal care and use committee statement: All of the procedures were performed strictly according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Appropriate measures were taken to minimize the animals' pain and discomfort.

Conflict-of-interest statement: We declare that we have no financial or personal relationships with other people or organizations that could inappropriately influence our work.

Data sharing statement: The technical appendix, statistical code and dataset are available from the corresponding author at nanshengcheng@gmail.com.

Manuscript source: Unsolicited manuscript

Correspondence to: Nan-Sheng Cheng, MD, Professor, Department of Bile Duct Surgery, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang, Chengdu 610041, Sichuan Province, China. nanshengcheng@gmail.com
Telephone: +86-18980601366

Received: August 26, 2016
Peer-review started: August 29, 2016
First decision: September 20, 2016
Revised: October 4, 2016
Accepted: November 12, 2016
Article in press: November 22, 2016
Published online: December 28, 2016

Abstract

AIM

To evaluate the feasibility of repairing a common bile duct defect with a decellularized ureteral graft in a porcine model.

METHODS

Eighteen pigs were randomly divided into three groups. An approximately 1 cm segment of the common bile duct was excised from all the pigs. The defect was repaired using a 2 cm long decellularized ureteral graft over a T-tube (T-tube group, n = 6) or a silicone stent (stent group, n = 6). Six pigs underwent bile duct reconstruction with a graft alone (stentless group). The surviving animals were euthanized at 3 mo. Specimens of the common bile ducts were obtained for histological analysis.

RESULTS

The animals in the T-tube and stent groups survived until sacrifice. The blood test results were normal in both groups. The histology results showed a biliary epithelial layer covering the neo-bile duct. In contrast, all the animals in the stentless group died due to biliary peritonitis and cholangitis within two months post-

surgery. Neither biliary epithelial cells nor accessory glands were observed at the graft sites in the stentless group.

CONCLUSION

Repair of a common bile duct defect with a decellularized ureteral graft appears to be feasible. A T-tube or intraluminal stent was necessary to reduce postoperative complications.

Key words: Decellularization; Stent; Bile duct injury; Biliary reconstruction; Ureteral graft

Core tip: A common bile duct defect is a challenge for hepatobiliary surgeons. A decellularized ureteral graft was introduced in this experimental study to repair a common bile duct defect. If the biliary reconstruction was performed with a T-tube or stent insertion into the ureteral graft, all animals survived with normal liver function. The histology results showed a biliary epithelial layer regeneration over the graft. Thus, repair of a common bile duct defect with a decellularized ureteral graft appears to be feasible. In addition, a T-tube or stent was found to be necessary to reduce postoperative complications in this study.

Cheng Y, Xiong XZ, Zhou RX, Deng YL, Jin YW, Lu J, Li FY, Cheng NS. Repair of a common bile duct defect with a decellularized ureteral graft. *World J Gastroenterol* 2016; 22(48): 10575-10583 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10575.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10575

INTRODUCTION

Defects of the common bile duct may result from operations to treat a congenital choledochous cyst, extrahepatic cholangiocarcinoma or Mirizzi syndrome. However, most defects generally follow iatrogenic bile duct injury during cholecystectomy[1-4]. A small defect may be successfully treated by a plastic repair to enlarge the lumen or an end-to-end anastomosis over a T-tube if there is enough normal biliary tissue to repair the defect without tension[4-7]. A long defect from extensive loss of the common bile duct is a major challenge for hepatobiliary surgeons[1-3]. The most commonly used method for repair of a long common bile duct defect is a biliary-enteric anastomosis (*e.g.*, hepaticojejunostomy, choledochojejunostomy, choledochoduodenostomy)[1-3]. However, a biliary-enteric anastomosis may cause complications, such as retrograde biliary infection, anastomotic stricture, hepatolithiasis and biliary cirrhosis, because of the lack of the sphincter of Oddi[5-7]. An alternative method for repairing the defect is the use of autologous tubular tissue (*e.g.*, artery, vein, appendix), which may cause fibrosis in the free graft and stricture formation at the location of the anastomosis. These issues can impair long-term success[8-11].

In recent years, several new materials with fewer foreign body reactions have been successfully introduced as substitutes for normal bile ducts for biliary defect reconstruction[12-16]. To our knowledge, decellularized ureter tissue has not yet been used as a substitute in experimental biliary defect repair. The primary aim of the present study was to evaluate the feasibility of common bile duct defect repair with a decellularized ureteral graft in a porcine model. The secondary purpose was to determine the effect of a T-tube and intraluminal stent on the outcomes of the present study.

MATERIALS AND METHODS

Laboratory animals

The study was approved by the Local Ethics Committee of West China Hospital (Sichuan, China) (Permit Number: SYXK2013-119) and was in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. A total of 18 experimental pigs (Neijiang cross) were obtained from the Chengdu Ping'an Experimental Animal Reproduction Center (Sichuan, China). Male and female pigs were used and all weighed between 25 and 35 kg. Before starting this experiment, all animals were housed and fed for one week at the Experimental Animal Center at Sichuan University to allow time for adaptation to the new environment. All procedures were carried out under anesthesia. Analgesics were administered to minimize the suffering of the animals. According to the previous published studies[12-16], the pigs were subjected to euthanasia under anesthesia 3 mo post-surgery by an intravenous injection of 10% potassium chloride. In case of an unexpected death, the animal was subjected to autopsy to find the cause of death.

Decellularized ureteral grafts

Ureters of four experimental pigs (Neijiang cross) sacrificed by another team were harvested under sterile conditions. The peripheral connective tissues surrounding the ureters were mechanically removed. Before decellularization, ureters were cut into pieces of 5.0 cm in length. First, ureters were shaken and washed in phosphate-buffered saline (PBS, pH 7.4) for 2 h. Second, ureters were placed in a 1% sodium dodecyl sulfate (SDS; Demchem, Shanghai, China) solution and stirred (500 rpm) with a magnetic stirrer for 24 h. Third, ureters were washed in PBS for 2 h. Fourth, ureters were placed in 1% Triton-100 (Amresco, OH, United States) and stirred (500 rpm) with a magnetic stirrer for 24 h. Then, the decellularized ureteral grafts were washed in PBS for 24 h. SDS, Triton-100 and PBS solutions were changed every 12 h. Finally, the decellularized ureteral grafts were sterilized by gamma radiation and stored in PBS with

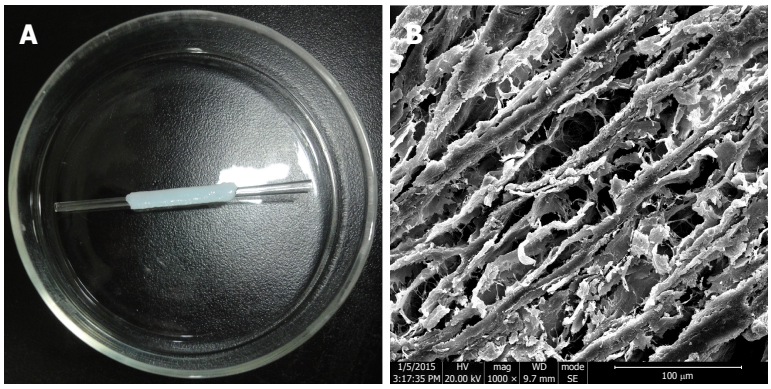

Figure 1 Appearance and structure of a graft used in common bile duct reconstruction. A: The decellularized ureteral graft is over a stent. B: Scanning electron microscope photogram shows porous extracellular matrix and collagen fibers. Scanning bar indicates 100 μm.

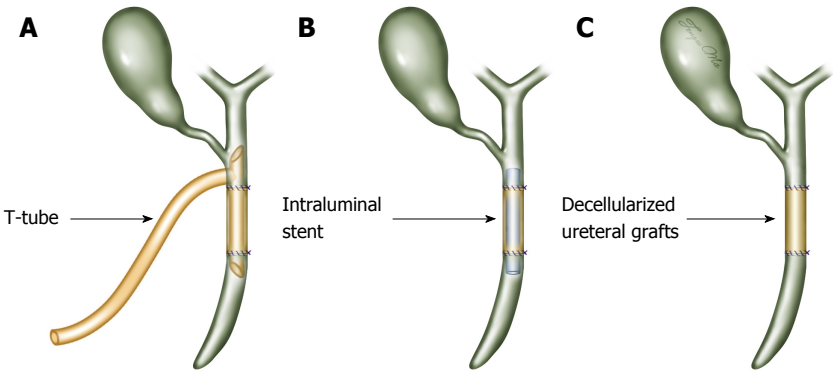

Figure 2 Surgical technique of common bile duct reconstruction. A: The common bile duct defect was reconstructed with a 2 cm long decellularized ureteral graft over the lower limb of the T-tube (T-tube group); B: The defect was reconstructed with a graft over an intraluminal stent (stent group); C: The defect was reconstructed with a graft alone without any T-tube or intraluminal stent insertion (stentless group).

1% antibiotic solution at 4 ℃. These grafts were cut to 2.0 cm in length (Figure 1A). A scanning electron microscopy (SEM) photogram was obtained to evaluate the graft surface (Figure 1B).

Animal model of a common bile duct defect

Preoperatively, all animals were fasted for 12 h but had free access to water. Thirty minutes before surgery, cefoxitin sodium (0.15 g/kg; Jiangsu Yangzijiang, Co., Ltd, China) was administered intravenously. Anesthesia was induced by an intravenous injection of diazepam (0.2 mg/kg) (Hubei Pharmacy, Co., Ltd, China), fentanyl (10 μg/kg) (Yichang Renhe, Co., Ltd, China), ketamine (2 mg/kg) (Zhejiang Jiuxu, Co., Ltd, China) and succinylcholine (2 mg/kg) (Jiangsu Yangzijiang, Co., Ltd, China). After endotracheal intubation, anesthesia was maintained by oxygen and isoflurane (NunanBeite, Co., Ltd, China). All operations were performed under sterile conditions. All animals were placed in the supine position. After a sterile skin preparation, a right subcostal incision was made. After gross inspection of the abdominal cavity, the hepatoduodenal ligament was dissected to identify the cystic duct and common bile duct. After mobilization of the entire common bile duct, a 1 cm long segment distal to the entrance of the cystic duct was resected to create a model of a common bile duct defect. The gallbladder was left *in situ*.

Study design and surgical technique

Eighteen pigs were randomly divided into three groups, namely, the T-tube (n = 6), stent (n = 6) and stentless (n = 6) groups. In the T-tube group, an 8- or 10-Fr latex T-tube was inserted through a separate incision in the proximal common bile duct approximately 0.5 cm away from the defect. The common bile duct defect was bridged by a 2 cm long decellularized ureteral graft and repaired without tension. An end-to-end anastomosis over the lower limb of the T-tube was performed in this group (Figure 2A). The anastomoses were completed using two continuous 7-0 prolene sutures (Ethicon, NY, United States). A pedicled omentum flap was brought up to wrap the interposed graft for vascularization. The abdomen was closed in two layers (peritoneum and skin) using interrupted 0# silk sutures (Ethicon, NY, United States). The T-tube was fixed on the abdomen and no abdominal drain was placed. The animal had free access to water on the day of the procedure and resumed a normal oral diet on the first postoperative day. Cefoxitin sodium (0.15 g/kg; Jiangsu Yangzijiang, Co., Ltd, China) was administered intramuscularly for the first 3 postoperative days. The T-tube was allowed to drain freely for 3 mo. In the stent group, an end-to-end anastomosis was performed with continuous 7-0

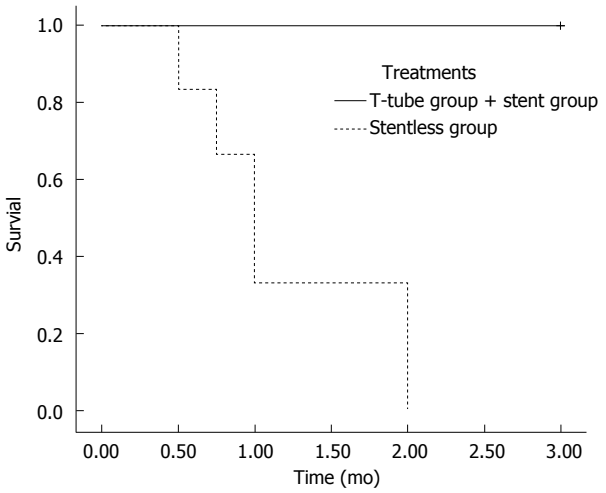

Figure 3 Survival rates. All animals in the T-tube group and stent group survived until their sacrifice after 3 mo. In stentless group, all of the animals died in 2 mo.

prolene sutures (Ethicon, NY, United States) over an 8-Fr silicon stent (Figure 2B). The endoluminal stent was approximately 4 cm in length and had a decellularized ureteral graft in the middle. The stent spread out 1 cm into the proximal and distal common bile duct stumps. Two transmural stitches using 7-0 prolene sutures were made to fix the position of the stent. In the stentless group, the end-to-end anastomosis was completed with continuous 7-0 prolene sutures (Ethicon, NY, United States) without any T-tube or intraluminal stent insertion (Figure 2C).

Biochemistry and cholangiography

Blood samples were drawn at postoperative week 1 and once a month. They were sent to the biochemical laboratory at West China Hospital, Sichuan University to evaluate each animal's liver function. The tests included total bilirubin (TB), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT). At 3 mo postoperatively, cholangiography was performed for the surviving animals under general anesthesia. During the cholangiography, 20 mL of contrast media (50% iohexol injection; Zhejiang Tianrui, Co., Ltd, China) was injected into the biliary tract through either the T-tube for animals in the T-tube group or percutaneous gallbladder puncture using guided ultrasonography for animals in the stent and stentless groups. Images were obtained and evaluated by a radiologist at West China Hospital.

Histopathology

Following the cholangiography, all surviving animals underwent an exploratory laparotomy. After a general inspection of the abdominal cavity for signs of biliary leakage and abdominal abscess, all animals were sacrificed by an intravenous injection of 10% potassium chloride. The entire extrahepatic biliary tree, including grafts and native bile ducts, were harvested from the animals. Specimens of the bile ducts were fixed in either 10% neutral buffered formalin or liquid nitrogen and sent to the Pathology Department of West China Hospital for histopathology evaluation. Conventional hematoxylin/eosin (H&E) staining was performed to observe the morphology of the bile duct. An anti-cytokeratin 19 antibody (CK19, 1:200, Sigma, United States) was used for immunofluorescent staining to label the bile duct epithelium.

Table 1 Post-operative complications in animals

Complications	Groups		
	T-tube	Stent	Stentless
Bile leakage	0/6	0/6	1/6
Biliary stricture	0/6	1/6	6/6
Hepatolithiasis	0/6	0/6	3/6

Statistical analysis

SPSS statistical software package (version 22.0, SPSS Inc., Chicago, IL, United States) was used for the statistical analyses. A Mann-Whitney *U* test or Kruskal-Wallis analysis of variance with multiple comparisons was applied to analyze various continuous variables. Data were considered statistically significant when the *P* value was less than 0.05.

RESULTS

Survival and complications

All 18 pigs survived the operation. The animals in the T-tube and stent groups gained weight and were in a good general condition after 3 mo at the time of sacrifice. However, the use of only a decellularized ureteral graft for the bile duct reconstruction (stentless group) led to the death of four animals in the first month and two animals in the second month. None of the animals survived until the mandatory sacrifice at 3 mo (Figure 3).

None of the animals in the T-tube group suffered from any post-operative complications. One biliary stricture occurred in the 6 pigs in the stent group. This animal appeared clinically well without a significantly decreased appetite, jaundice or weight loss at the 3-mo sacrifice. However, a mild narrowing at the proximal anastomosis and a mild intrahepatic and extrahepatic biliary dilatation were noted. It appeared that stent migration resulted in this biliary stricture because it was noted at autopsy that the stent was not present at the graft site. In the stentless group, repair of the common bile duct defect with a graft alone was not adequate. Within two weeks of the operation, 1 of the 6 pigs (16.7%) developed bile leakage and biliary stricture and subsequently died due to biliary peritonitis. The other five pigs in the stentless group suffered from severe biliary stricture and subsequent death due to acute obstructive suppurative cholangitis. Hepatolithiasis was observed in three pigs that suffered from severe biliary stricture in the stentless group (Table 1).

Table 2 Biochemical results of animals in each group

Parameters	Normal range	Groups	1 mo postoperative	2 mo postoperative	3 mo postoperative
Total bilirubin (μmol/L)	1.7-8.5	T-tube	3.0 ± 0.3	3.2 ± 0.3	3.3 ± 0.4
		Stent	3.2 ± 0.5	3.4 ± 0.5	3.6 ± 0.5
		Stentless	68.2 ± 15.1[a,c]	106.3 ± 29.2[a,c]	NA
Alanine aminotransferase (IU/L)	15.0-66.0	T-tube	35.2 ± 12.6	30.1 ± 5.3	36.1 ± 10.3
		Stent	34.3 ± 12.1	32.1 ± 6.2	33.2 ± 7.2
		Stentless	122.5 ± 35.1[a,c]	209.5 ± 35.4[a,c]	NA
Gamma-glutamyl transpeptidase (IU/L)	10.0-88.0	T-tube	51.2 ± 7.2	50.1 ± 5.3	48.8 ± 6.6
		Stent	47.1 ± 5.3	52.1 ± 6.2	52.0 ± 10.6
		Stentless	233.4 ± 61.5[a,c]	265.8 ± 82.6[a,c]	NA

[a]$P < 0.05$, stent group *vs* stentless group; [c]$P < 0.05$, T-tube group *vs* stentless group. NA: Not available.

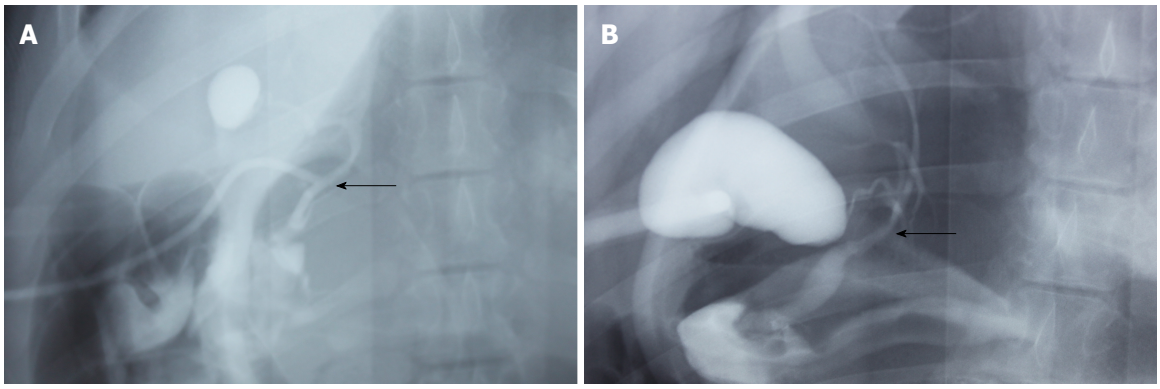

Figure 4 Postoperative cholangiography. A: Cholangiography of a survivor in the T-tube group performed at 3 mo; B: Cholangiography of a survivor in the stent group performed at 3 mo. Arrows indicate the area of graft interposition. There is no evidence of biliary stricture formation. There is no sign of proximal dilatation or biliary obstruction.

Biochemistry and cholangiography

The biochemical results of the animals in each group are shown in Table 2. In the T-tube and stent groups, all the biochemical parameters (TB, ALT and GGT) were within normal ranges throughout the study. There was no significant difference in any parameter at each time point between the T-tube and stent groups. In contrast, the levels of TB, ALT and GGT were significantly increased in the surviving animals in the stentless group. The mean levels of TB, ALT and GGT in the stentless group were significantly higher than those in the T-tube and stent groups at each time point.

The animals that survived in the T-tube and stent groups underwent cholangiography at 3 mo before sacrifice. Cholangiograms showed that the contrast passed freely from the bile duct into the duodenum. Neither biliary leakages nor filling defects were observed on any cholangiogram in the T-tube group (Figure 4A). However, 1 biliary stricture was detected among the 6 pigs in the intraluminal stent group due to stent migration. A minimal narrowing at the proximal biliary anastomosis and a mild dilation of the intrahepatic bile ducts were noted in this animal. There was no evidence of biliary stricture or proximal biliary dilatation in any other animals after 3 mo in the stent group (Figure 4B).

Histopathology

In the T-tube group, the autopsy at 3 mo revealed that the common bile ducts were patent in all the explanted specimens without any bile leakage (Figure 5A). There was no gross evidence of biliary stricture or proximal biliary dilatation. Remnants of the 7-0 prolene sutures were observed at the end-to-end anastomosis. The decellularized ureteral grafts appeared to be completely degraded. Neo-ducts were observed at 3 mo post-surgery. The lumen of each neo-bile duct was covered with a layer of smooth mucosa, which had an appearance similar to that of the native common bile duct. There was a progressive contraction of the graft area from an initial length of 2 cm to 1.1 (0.9-1.2) cm.

In the stent group, the intraluminal stents were observed *in situ* in all but one animal during the autopsy at 3 mo (Figure 5B). The macroscopic results of the five animals with the stent *in situ* were similar to those of the animals in the T-tube group (Figure 5B). However, the intraluminal stent in one animal disappeared due to stent migration. This animal developed mild biliary stricture and proximal biliary dilatation. The mean length of the graft area was 1.0 (0.8-1.1) cm. In the stentless group, marked biliary strictures and proximal biliary dilatations were observed in all the animals during the autopsy (Figure 5C). In most of the animals,

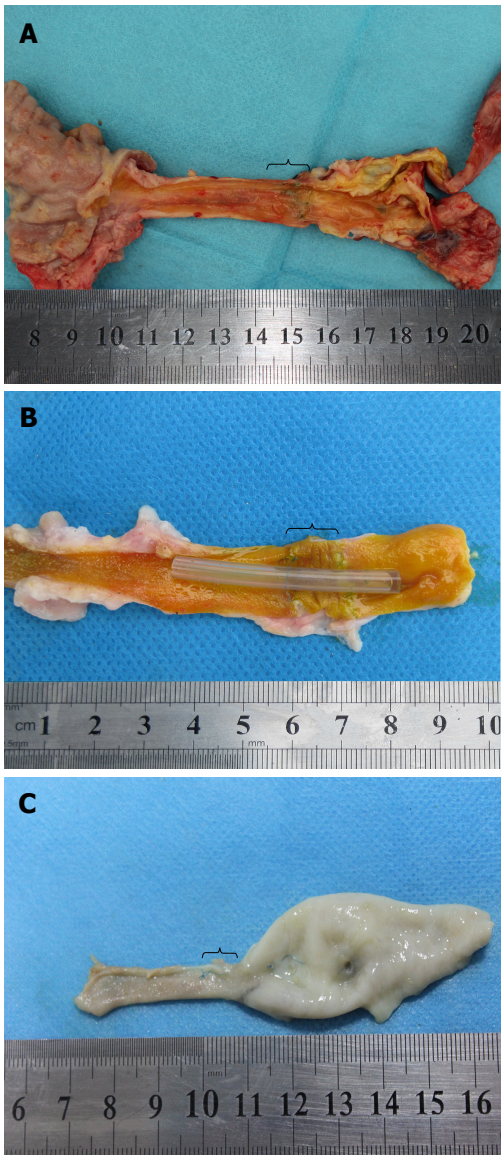

Figure 5 Macroscopic results. A: Explanted bile duct of a 3-mo survivor in the T-tube group; B: Explanted bile duct of a 3-mo survivor in the stent group; C: Explanted bile duct of an animal who died at 1 mo in the stentless group. Brackets indicate the area of graft interposition.

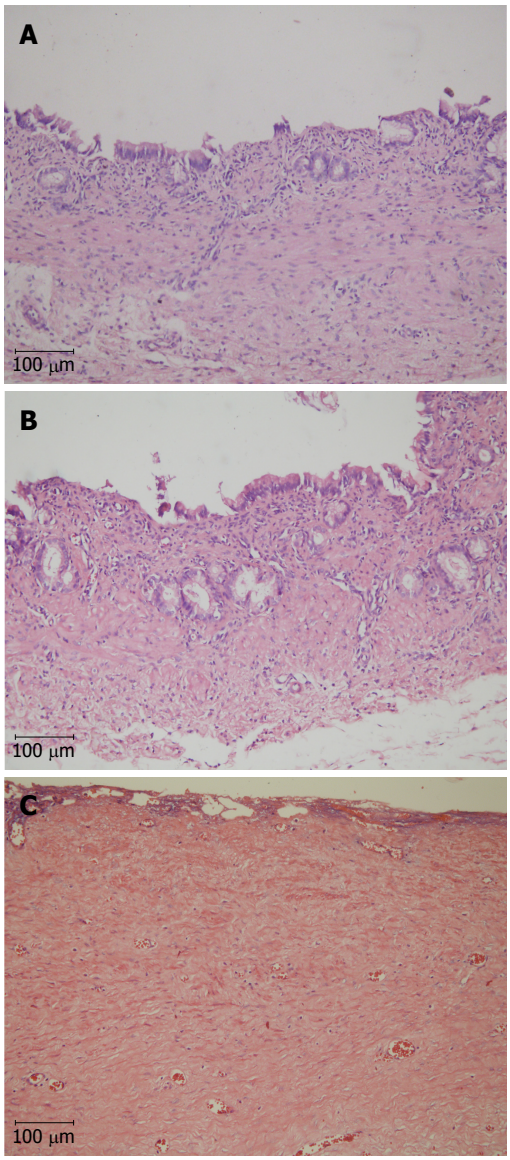

Figure 6 Hematoxylin/eosin staining of the graft site. A: H&E staining of a 3-mo survivor in the T-tube group; B: H&E staining of a 3-mo survivor in the stent group; C: H&E staining of an animal who died at 1 mo in the stentless group. Scale bar indicates 100 μm. H&E: Hematoxylin/eosin.

the lumen of the graft site had biliary sludge and degraded decellularized ureteral graft remnants. The mean length of the graft area was 0.6 (0.4-1.0) cm.

HE staining results

At 3 mo, there was no recognizable element of the graft and the decellularized ureteral graft appeared completely degraded in the T-tube and stent groups. Cellularization and collagen remodeling were observed at the graft sites. Only slight inflammation of the graft sites was noted in both groups. The neo-bile duct showed a native biliary histological structure. A smooth mucosa layer encircled the lumen of the neo-bile duct, which was similar to that of the native bile duct (T-tube group: Figure 6A; Stent group: Figure 6B). In contrast, bile duct reconstruction using a graft alone (stentless group) led to dense fibroplasia of the graft site. No mucosa was detected (Figure 6C).

CK19 immunofluorescent staining

CK19 immunofluorescent staining was performed to investigate the biliary epithelial layer. At 3 mo, both neo-epithelial cells and accessory glands were observed at the graft sites and stained positive for CK19 in the animals in the T-tube and stent groups (T-tube group: Figure 7A; Stent group: Figure 7B). Most of the graft sites, except for some central areas, were covered by a layer of irregular biliary epithelium. However, the CK19 staining at the graft site was negative in the animals in the stentless group. Neither epithelial cells nor accessory glands were observed at the graft sites (Figure 7C).

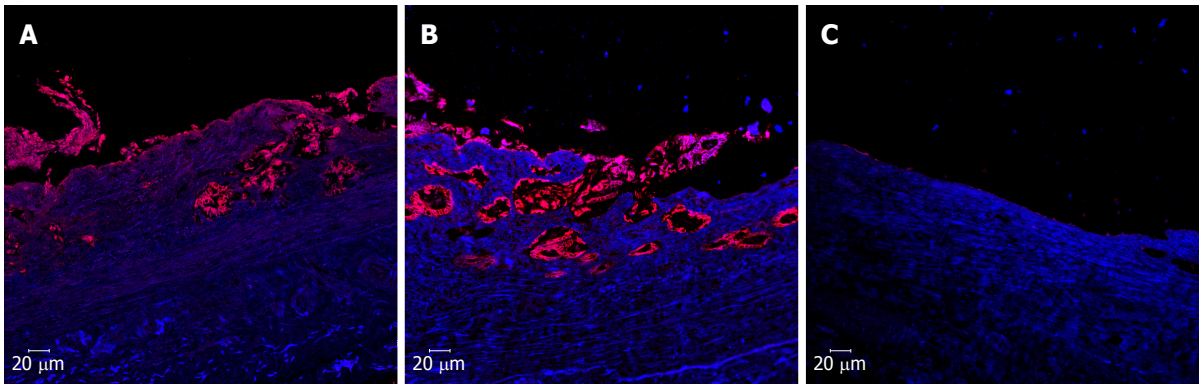

Figure 7 Cytokeratin 19 immunofluorescent staining of the graft site. A: CK19 staining of a 3-mo survivor in the T-tube group; B: CK19 staining of a 3-mo survivor in the stent group; C: CK19 staining of an animal who died at 1 mo in the stentless group. Scale bar indicates 20 μm. CK19: Cytokeratin 19.

DISCUSSION

This preliminary study suggests that in a porcine model, a common bile duct defect can be repaired by a decellularized ureteral graft if either a T-tube or intraluminal stent is used. The absence of either a T-tube or intraluminal stent resulted in bile leakage, biliary stricture and subsequent death. Regeneration of the biliary mucosa was observed in the surviving animals at 3 mo post-surgery.

The search for a suitable substitute to repair a long common bile duct defect caused by disease or trauma has attracted many researchers[9]. A wide variety of autografts (*e.g.*, artery, vein, ureter, appendix, jejunum) and synthetic grafts (*e.g.*, Vitallium, polyvinyl sponge, polytetrafluoroethylene, Teflon, Dacron) have been introduced as substitutes to repair common bile duct defects *in situ*[8-11,17-22]. Either a tubular graft or a patch graft was used in these experimental studies. However, the outcomes of various substitutes in large animals (*e.g.*, dogs, goats, pigs) are controversial. Based on studies performed in the past century, most of the substitutes are not degradable and have failed mostly because of varying degrees of cholangitis, biliary stricture and biliary peritonitis[8-11,17-22]. With the development of degradable materials, success has been reported by some researchers using various degradable substitutes (*e.g.*, small intestinal submucosa, polylactic acid and polycaprolactone, polyglycolic acid and trimethylene carbonate, collagen sponge and polypropylene) in this century[12-16]. Until recently, there were no satisfactory reliable substitutes with which to replace common bile duct defects and restore function in clinical practice. Only a few case reports have been published on repairs of the common bile duct defects using autografts[23-25].

The diameter of the ureter is similar to that of the common bile duct. Two research teams (Sedgwick *et al*[26] and Ulin *et al*[10]) evaluated the role of ureteral grafts in the repair of common bile duct defects in dogs. In general, free ureteral grafts were disappointing in both experimental studies. The entire free graft was replaced by fibrous tissue and biliary stricture occurred at the graft site due to immunological rejection and inflammatory reactions. Recently, various decellularization techniques have been introduced to remove cellular antigens and preserve the extracellular matrix (ECM)[27]. The ECM is a biomaterial composed of structural and functional proteins (*e.g.*, collagen, elastin), glycosaminoglycans and growth factors. ECM biomaterial is biodegradable, decellularized and non-immunogenic material derived from human or animal tissue processing[12] and has been shown to have regeneration and various tissue healing capabilities[12,27]. This material has been widely used in tissue engineering[12,27]. ECM biomaterial can not only prevent immunological rejection and inflammatory reactions but also facilitate cell adhesion and differentiation to repair injured tissue and prevent scar tissue formation[12,27]. Rosen *et al*[12] used small intestinal submucosa (a type of ECM biomaterial) as a bile duct substitute for common bile duct reconstruction in dogs. Neither a T-tube nor intraluminal stent was used in the study[12]. At 1 mo post-surgery, the graft material was completely covered by biliary epithelium and by 5 mo, the graft material was completely replaced by a neo-bile duct. The biliary epithelium of the neo-bile duct was grossly and microscopically indistinguishable from that of a normal canine common bile duct[12]. The authors concluded that small intestinal submucosa can be used to repair bile duct defects in a canine model without requiring stenting[12]. However, Gómez *et al*[28] were concerned about the safety of small intestinal submucosa for common bile duct reconstruction because 1 of the 5 dogs (20%) developed a biliary stricture at 2 mo post-surgery in Rosen's experimental study. They suggested that further studies are needed to establish the benefits and complications of small intestinal submucosa in common bile duct reconstruction[28]. In our experiment, a ureter instead of a common bile duct was chosen as the graft because the smooth muscle layer of the former is thicker than that of the latter. A simple decellularization method was adopted to remove cells and make ECM biomaterial. Regarding the ECM biomaterial for the common bile duct reconstruction, there were significant differences

between our findings and Rosen's observations. All the experimental animals in the stentless group died from various complications when a sole decellularized ureteral graft without stenting was used to repair the bile duct defect. We found at the autopsy that the decellularized ureteral graft collapsed and shrunk with severe biliary stricture formation. Possible reasons for this failure in the stentless group were that the decellularized ureteral graft had a soft texture and the pressure in the biliary system was too low to maintain the luminal structure of the graft. Consequently, it appeared that either a T-tube or intraluminal stent was necessary to improve the survival rate in this study.

T-tube drainage and biliary stenting have been introduced by some surgeons to reduce postoperative complications after bile duct reconstructions. However, the use of a T-tube and biliary stent during bile duct reconstruction is controversial. A T-tube and biliary stent may reduce the incidence of biliary stricture but may be associated with significant morbidity. T-tube-related complications include premature dislodgement, biliary leakage and biliary infection[29]. In addition, bile duct reconstruction over a T-tube requires several weeks of extra-abdominal drainage, which may cause loss of appetite and reduce quality of life. In contrast, placement of an intraluminal stent may reduce T-tube-related complications without extra-abdominal drainage. In this experiment, a silicone stent was inserted into the graft during reconstruction of the common bile duct but resulted in biliary stricture due to stent migration. In addition, the silicone stent was not biodegradable and an additional procedure (*e.g.*, endoscopy, laparotomy) was required to remove the stent. It appeared that T-tube drainage was superior to the silicone stent during the bile duct reconstruction in this experiment. Recently, biodegradable biliary stents have been developed and some experiments have reported encouraging results with them[30-32]. Future work is needed to compare the efficacy of T-tube drainage *vs* a biodegradable biliary stent for common bile duct reconstruction.

In summary, the use of decellularized ureteral grafts appears to be feasible for the repair of common bile duct defects. Either a T-tube or intraluminal stent is necessary to improve the survival rate and prevent bile leakage and biliary stricture formation. Additional studies are planned to investigate the optimal time for T-tube or intraluminal stent removal and the optimal method to promote regeneration of the biliary tract.

ACKNOWLEDGMENTS

We thank Jie Zhang and Yi-Xin Lin for assistance with the development of the article.

COMMENTS

Background

A common bile duct defect generally follows iatrogenic bile duct injury during cholecystectomy, a challenge for hepatobiliary surgeons.

Research frontiers

In recent years, several new materials with fewer foreign body reactions have been successfully introduced as substitutes to normal bile ducts for biliary defect reconstruction.

Innovations and breakthroughs

A decellularized ureteral graft was introduced in this experimental study to repair a common bile duct defect. If the biliary reconstruction was performed with a T-tube or stent insertion into the ureteral graft, all animals survived with normal liver function. The histology results showed a biliary epithelial layer regeneration over the graft.

Applications

Repair of a common bile duct defect with a decellularized ureteral graft appears to be feasible. In addition, a T-tube or stent was found to be necessary to reduce postoperative complications.

Terminology

Extracellular matrix is a biomaterial composed of structural and functional proteins (*e.g.*, collagen, elastin), carbohydrate polymers and growth factors. Decellularization is the process to remove cellular components from a tissue.

Peer-review

This is a novel and well designed experiment that provides a new approach to a complex and common surgical problem. The authors have provided excellent evidence that the decellularized ureteral graft might be an adequate replacement for a damaged common bile duct. Long term results with the new method need to be addressed in future.

REFERENCES

1 **Melton GB**, Lillemoe KD, Cameron JL, Sauter PA, Coleman J, Yeo CJ. Major bile duct injuries associated with laparoscopic cholecystectomy: effect of surgical repair on quality of life. *Ann Surg* 2002; **235**: 888-895 [PMID: 12035047 DOI: 10.1097/00000658-200206000-00018]

2 **Connor S**, Garden OJ. Bile duct injury in the era of laparoscopic cholecystectomy. *Br J Surg* 2006; **93**: 158-168 [PMID: 16432812 DOI: 10.1002/bjs.5266]

3 **de Reuver PR**, Busch OR, Rauws EA, Lameris JS, van Gulik TM, Gouma DJ. Long-term results of a primary end-to-end anastomosis in peroperative detected bile duct injury. *J Gastrointest Surg* 2007; **11**: 296-302 [PMID: 17458601 DOI: 10.1007/s11605-007-0087-1]

4 **Sicklick JK**, Camp MS, Lillemoe KD, Melton GB, Yeo CJ, Campbell KA, Talamini MA, Pitt HA, Coleman J, Sauter PA, Cameron JL. Surgical management of bile duct injuries sustained during laparoscopic cholecystectomy: perioperative results in 200 patients. *Ann Surg* 2005; **241**: 786-792; discussion 793-795 [PMID: 15849514 DOI: 10.1097/01.sla.0000161029.27410.71]

5 **Nealon WH,** Urrutia F. Long-term follow-up after bilioenteric anastomosis for benign bile duct stricture. *Ann Surg* 1996; **223**: 639-645 discussion 645-648 [PMID: 8645037 DOI: 10.1097/00000658-199606000-00002]

6 **Moraca RJ**, Lee FT, Ryan JA, Traverso LW. Long-term biliary function after reconstruction of major bile duct injuries with hepaticoduodenostomy or hepaticojejunostomy. *Arch Surg* 2002; **137**: 889-893; discussion 893-894 [PMID: 12146986 DOI: 10.1001/archsurg.137.8.889]

7 **Walsh RM**, Henderson JM, Vogt DP, Brown N. Long-term outcome of biliary reconstruction for bile duct injuries from laparoscopic cholecystectomies. *Surgery* 2007; **142**: 450-456; discussion 456-457 [PMID: 17950335 DOI: 10.1016/j.surg.2007.07.008]

8 **Belzer FO**, Watts JM, Ross HB, Dunphy JE. Auto-reconstruction of the common bile duct after venous patch graft. *Ann Surg* 1965; **162**: 346-355 [PMID: 5318669 DOI: 10.1097/00000658

-196509000-00004]
9 **Myers RT**, Meredith JH, Rhodes J, Gilbert JW. The fate of free grafts in the common bile duct. *Ann Surg* 1960; **151**: 776-782 [PMID: 14425624 DOI: 10.1097/00000658-196005000-00018]
10 **Ulin AW**, Van Ess L, Entine J, Pearce AE, Martin WL. Further experiences with the experimental reconstruction of the common bile duct: use of autogenous and homologous, fresh and preserved grafts of blood vessel, ureter and common duct. *Am Surg* 1953; **19**: 867-873 [PMID: 13080626]
11 **Grosfeld JL**, Weinberger M, Clatworthy HW. Vascularized appendiceal transplants in biliary and urinary tract replacement. *J Pediatr Surg* 1971; **6**: 630-638 [PMID: 5126283 DOI: 10.1016/0022-3468(71)90389-7]
12 **Rosen M**, Ponsky J, Petras R, Fanning A, Brody F, Duperier F. Small intestinal submucosa as a bioscaffold for biliary tract regeneration. *Surgery* 2002; **132**: 480-486 [PMID: 12324762 DOI: 10.1067/msy.2002.126505]
13 **Miyazawa M**, Torii T, Toshimitsu Y, Okada K, Koyama I, Ikada Y. A tissue-engineered artificial bile duct grown to resemble the native bile duct. *Am J Transplant* 2005; **5**: 1541-1547 [PMID: 15888066 DOI: 10.1111/j.1600-6143.2005.00845.x]
14 **Nau P**, Liu J, Ellison EC, Hazey JW, Henn M, Muscarella P, Narula VK, Melvin WS. Novel reconstruction of the extrahepatic biliary tree with a biosynthetic absorbable graft. *HPB* (Oxford) 2011; **13**: 573-578 [PMID: 21762301 DOI: 10.1111/j.1477-2574.2011.00337.x]
15 **Li Q**, Tao L, Chen B, Ren H, Hou X, Zhou S, Zhou J, Sun X, Dai J, Ding Y. Extrahepatic bile duct regeneration in pigs using collagen scaffolds loaded with human collagen-binding bFGF. *Biomaterials* 2012; **33**: 4298-4308 [PMID: 22429981 DOI: 10.1016/j.biomaterials.2012.03.003]
16 **Pérez Alonso AJ**, Del Olmo Rivas C, Romero IM, Cañizares Garcia FJ, Poyatos PT. Tissue-engineering repair of extrahepatic bile ducts. *J Surg Res* 2013; **179**: 18-21 [PMID: 23010513 DOI: 10.1016/j.jss.2012.08.035]
17 **Trentini EA**, Crema E, Llanos JC, Lerco MM, Magna LA, Leonardi LS. Biliary tract reconstruction using jejunal tube: an experimental study in dogs. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 179-185 [PMID: 19357033]
18 **Pearse HE**. Vitallium tubes in biliary surgery. *Ann Surg* 1942; **115**: 1031-1042 [PMID: 17858033 DOI: 10.1097/00000658-194206000-00015]
19 **Bergan JJ**, Anderson MC, Lounsbury BF. Vascularized polyvinyl sponge prosthesis. Its use to replace the canine common bile duct. *Arch Surg* 1962; **84**: 301-305 [PMID: 13867620 DOI: 10.1001/archsurg.1962.01300210035007]
20 **Mendelowitz DS**, Beal JM. Expanded polytetrafluoroethylene in reconstruction of the canine biliary system. *Am J Surg* 1982; **143**: 221-224 [PMID: 7058992 DOI: 10.1016/0002-9610(82)90073-3]
21 **Thomas JP**, Metropol HJ, Myers RT. Teflon patch graft for reconstruction of the extrahepatic bile ducts. *Ann Surg* 1964; **160**: 967-970 [PMID: 14246149 DOI: 10.1097/00000658-196412000-00004]
22 **Stephens FO**, Dunphy JE. Grafts of the common bile duct. An historical review. *Aust N Z J Surg* 1963; **33**: 53-57 [PMID: 14058876 DOI: 10.1111/j.1445-2197.1963.tb03291.x]
23 **Helmy AA**, Hamad MA, Aly AM, Sherif T, Hashem M, El-Sers DA, Semieka M. Novel technique for biliary reconstruction using an isolated gastric tube with a vascularized pedicle: a live animal experimental study and the first clinical case. *Ann Surg Innov Res* 2011; **5**: 8 [PMID: 21985492 DOI: 10.1186/1750-1164-5-8]
24 **Watanabe M**, Yamazaki K, Tsuchiya M, Otsuka Y, Tamura A, Shimokawa K, Kaneko H, Teramoto T. Use of an opened umbilical vein patch for the reconstruction of the injured biliary tract. *J Hepatobiliary Pancreat Surg* 2007; **14**: 270-275 [PMID: 17520202 DOI: 10.1007/s00534-006-1183-4]
25 **Kaushik R**, Sharma R. Jejunal serosal onlay flap for repair of large common bile duct defect in Mirizzi's syndrome. *Trop Gastroenterol* 2001; **22**: 169-170 [PMID: 11681115]
26 **Sedgwick CE**. Reconstruction of the common bile duct with a free ureteral graft; an experimental study. *Surg Gynecol Obstet* 1951; **92**: 571-573 [PMID: 14835212 DOI: 10.1146/annurev-immunol-030409-101225]
27 **Gilbert TW**, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials* 2006; **27**: 3675-3683 [PMID: 16519932 DOI: 10.1016/j.biomaterials.2006.02.014]
28 **Gómez NA**, Zapatier JA, Vargas PE. Re: "Small intestinal submucosa as a bioscaffold for biliary tract regeneration". *Surgery* 2004; **135**: 460 [PMID: 15041976]
29 **Wills VL**, Gibson K, Karihaloot C, Jorgensen JO. Complications of biliary T-tubes after choledochotomy. *ANZ J Surg* 2002; **72**: 177-180 [PMID: 12071447 DOI: 10.1046/j.1445-2197.2002.02308.x]
30 **Grolich T**, Crha M, Novotný L, Kala Z, Hep A, Nečas A, Hlavsa J, Mitáš L, Misík J. Self-expandable biodegradable biliary stents in porcine model. *J Surg Res* 2015; **193**: 606-612 [PMID: 25201575 DOI: 10.1016/j.jss.2014.08.006]
31 **Wang Y**, Liang Y, Wang W, Jin R, Cai X. Management of electrothermal injury of common bile duct with a degradable biliary stent: an experimental study in a porcine model. *J Gastrointest Surg* 2013; **17**: 1760-1765 [PMID: 23949424 DOI: 10.1007/s11605-013-2316-0]
32 **Tashiro H**, Ogawa T, Itamoto T, Ushitora Y, Tanimoto Y, Oshita A, Amano H, Asahara T. Synthetic bioabsorbable stent material for duct-to-duct biliary reconstruction. *J Surg Res* 2009; **151**: 85-88 [PMID: 18674784 DOI: 10.1016/j.jss.2008.02.056]

P- Reviewer: Leitman M, Shimizu Y **S- Editor**: Gong ZM
L- Editor: Roemmele A **E- Editor**: Liu WX

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.v22.i48.10584

World J Gastroenterol 2016 December 28; 22(48): 10584-10591
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Regulating effect of TongXie-YaoFang on colonic epithelial secretion *via* Cl^- and HCO_3^- channel

Cheng Yang, Ying Xiong, Sheng-Sheng Zhang, Fang-Mei An, Jing Sun, Qing-Lin Zhang, Qiang Zhan

Cheng Yang, Fang-Mei An, Jing Sun, Qing-Lin Zhang, Qiang Zhan, Department of Gastroenterology, Wuxi People's Hospital, Nanjing Medical University, Wuxi 214023, Jiangsu Province, China

Ying Xiong, Community Health Service Center of Jinkui Street, Wuxi 214023, Jiangsu Province, China

Sheng-Sheng Zhang, Digestive Disease Diagnosis and Treatment Center, Beijing Chinese Medicine Hospital, Capital Medical University, Beijing 100010, China

Author contributions: Yang C made contributions to the study design, data interpretation, and wrote the manuscript; Zhang SS made contributions to the study design; Xiong Y, An FM, Sun J and Zhan Q performed the majority of experiments and data analysis; Zhang QL conducted the majority of quality control for the herbs of the formula.

Supported by Medical and public health technology research and development projects of WuXi science and technology development fund, No. CSE31N1501.

Institutional review board statement: All experiments were reviewed and approved by the Ethical Committee and Institutional Animal Care and Use Committee of Dongzhimen Hospital, No. SYXK(Beijing)2009.0028, and the methods were performed in strict accordance with the Animal Management Rules of the Chinese Ministry of Health.

Institutional animal care and use committee statement: All animal care and experimental procedures were conducted according to the institutional ethical guidelines and conformed to the requirements of the Institutional Animal Care and Use Committee of Beijing University of Chinese Medicine and the Animal Ethics Committee of Dongzhimen Hospital, No. SYXK(Beijing)2009.0028.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Zhan Qiang, PhD, Associate Professor, Department of Gastroenterology, Wuxi People's Hospital, Nanjing Medical University, 299 Qing-Yang Road, Nan-Chang District, Wuxi 214023, Jiangsu Province, China. zhanq33@163.com
Telephone: +86-510-85351120
Fax: +86-510-85351120

Received: August 23, 2016
Peer-review started: August 24, 2016
First decision: September 28, 2016
Revised: October 10, 2016
Accepted: November 15, 2016
Article in press: November 16, 2016
Published online: December 28, 2016

Abstract

AIM

To investigate the pharmacological effect of TongXie-YaoFang (TXYF) formula, a Chinese herbal formula, on Diarrhea-predominant irritable bowel syndrome (D-IBS) rats.

METHODS

In a neonatal maternal separation plus restraint stress (NMS + RS) model of D-IBS, male Sprague Dawley rats were randomly divided into two groups (NMS + RS group and TXYF-formula group) with no handlings were used as controls (NH group). Starting from postnatal

day 60, rats in TXYF-formula group were administered TXYF-formula (4.92 g/100 g bodyweight) orally twice a day for 14 consecutive days while NH group and NMS + RS group were given distilled water. Using short-circuit current technology, we observed 5-HT-induced changes of current across ion channels, such as cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel, epithelial Na^+ channel (ENaC), Ca^{2+}-dependent Cl^- channel (CACC), Na^+-K^+-$2Cl^-$ co-transporter (NKCC), and Na^+-HCO_3^- co-transporter (NBC), in the colonic epithelium of three groups after exposure to drugs and specific blockers with a Power Lab System (AD Instruments International).

RESULTS

Under basal conditions, the changes of short-circuit current (ΔIsc, μA/cm^2) induced by 5-HT were similar in NH group and TXYF-formula group, and both higher than NMS + RS group (70.86 μA/cm^2 ± 12.32 μA/cm^2, 67.67 μA/cm^2 ± 11.68 μA/cm^2 *vs* 38.8 μA/cm^2 ± 7.25 μA/cm^2, $P < 0.01$, respectively). When CACC was blocked by 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid, 5-HT-induced ΔIsc was smaller in NMS + RS group than in NH group and TXYF-formula group, respectively (48.41 μA/cm^2 ± 13.15 μA/cm^2 *vs* 74.62 μA/cm^2 ± 10.73 μA/cm^2, 69.22 μA/cm^2 ± 11.7 μA/cm^2, $P < 0.05$, respectively). The similar result could be obtained when ENaC was blocked by Amiloride (44.69 μA/cm^2 ± 12.58 μA/cm^2 *vs* 62.05 μA/cm^2 ± 11.26 μA/cm^2, 62.11 μA/cm^2 ± 12.01 μA/cm^2, $P < 0.05$, respectively). However, when CFTR Cl^- channel was blocked by 1,1-dimethyl piperidinium chloride (DPC), 5-HT-induced ΔIsc did not significantly differ in three groups (42.28 μA/cm^2 ± 10.61 μA/cm^2 *vs* 51.48 μA/cm^2 ± 6.56 μA/cm^2 *vs* 47.75 μA/cm^2 ± 7.99 μA/cm^2, $P > 0.05$, respectively). The similar results could also be obtained in three groups when NBC and NKCC were respectively blocked by their blockers.

CONCLUSION

TXYF-formula can regulate the Cl^- and HCO_3^- secretion of colonic mucosa *via* CFTR Cl^- channel, Cl^-/HCO_3^- exchanger, NBC and NKCC co-transporters.

Key words: Ion channel; Diarrhea-predominant irritable bowel syndrome; Colonic mucosa; Short-circuit current; TongXie-YaoFang formula

Core tip: Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic functional gastrointestinal disease. Abnormal ion secretion of colonic mucosal epithelial cells is recognized as one of the pathophysiological factors. In this paper, through the observation of TongXie-YaoFang (TXYF) formula, a Chinese herbal formula, to D-IBS rats obtained by neonatal maternal separation plus restraint stress. The mucosal stripping under a microscope was used for tissue preparation. Short-circuit current technology was used for testing 5-HT-induced changes in the current across ion channels of colonic epithelium. The results indicated that TXYF-formula could regulate the secretion of Cl^- and HCO_3^- in colonic mucosa *via* cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel, Cl^-/HCO_3^- exchanger, Na^+-HCO_3^- co-transporter and Na^+-K^+-$2Cl^-$ co-transporter co-transporters.

Yang C, Xiong Y, Zhang SS, An FM, Sun J, Zhang QL, Zhan Q. Regulating effect of TongXie-YaoFang on colonic epithelial secretion *via* Cl^- and HCO_3^- channel. *World J Gastroenterol* 2016; 22(48): 10584-10591 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10584.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10584

INTRODUCTION

Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic functional gastrointestinal disease. Its clinical manifestations are characterized by diarrhea and abdominal pain or discomfort in the absence of a demonstrable pathology. The diagnosis of D-IBS is based on symptom assessment and the Rome Ⅲ Diagnostic Criteria[1,2]. According to an epidemiological study, D-IBS mainly affects young adults of 20-40 years old, and the quality of their lives is seriously affected[3].

The pathogenesis of D-IBS has not been fully clarified. Consequently, the usual treatment of the disease in Western medicine involves symptomatic therapy, which is unsatisfactory for patients while simultaneously increasing the use of health-care resources[4-6]. Because traditional Chinese medicine (TCM) can significantly improve patients' symptoms and quality of life, increasing numbers of patients have begun to seek treatment with TCM[7,8].

A series of randomized, double-blind, placebo-controlled trials had shown that TongXie-YaoFang (TXYF) formula can significantly improve the clinical symptoms, such as diarrhea and abdominal pain or discomfort, of patients with D-IBS and improve the quality of their lives[9,10]. However, the specific mechanism of it has not been completely elaborated. The purpose of this paper is to observe the regulating effects of TXYF-formula on colonic epithelial secretion *via* relevant ion channels.

MATERIALS AND METHODS

Animals

Neonatal Sprague Dawley rats, postnatal day 1, were purchased from Vital River Laboratories Animal Technology Co., Ltd. (Beijing, China; Number of qualitative qualification: 11400700015068, 11400700019786), and kept at Dongzhimen Hospital Affiliated with Beijing University of Chinese Medicine [Number of permit: SYXK(Beijing)2009.0028]. In this study, only male

litters were used to eliminate the impact of estrogen and hormones on the secretory and sensory responses of the intestine to excitants[11]. Then the pups were randomly assigned to one of the following two rearing conditions: (1) neonatal maternal separation plus restraint stress (NMS + RS); or (2) no handling or separation (NH).

Neonatal maternal separation plus restraint stress

On postnatal days 2-21, the NMS + RS litters were removed from their cages and separated from their dams for 180 min each day, whereas NH pups remained in their home cages[12,13]. Manipulation began at 0900 h ± 30 min each day to minimize the influence of circadian rhythms. During the 180 min period of separation, pups were removed from the nest to stand-alone compartments, where the temperature was maintained at 23 ℃ ± 0.5 ℃ in a thermally regulated facility. The compartments contained bedding of 2.5 cm wood chips and were adjacent to their home cages. The litters were returned to their home cages immediately after separation. All the rats were reared on a 12:12 h light-dark cycle (lights on at 0800 h) with access to food and water ad libitum. On day 22, sexes of the pups, including those in NH group, were distinguishable, so the females were removed and the males retained. On day 22, NMS + RS and NH rats were weaned and kept in individual cages with only 3-4 pups per cage. After weaning, pups were weighed once a week until the end of experiment. Manipulations were performed in the morning with the same measuring instrument and at the same location.

On days 50-59, NMS + RS rats were placed in transparent plastic restraint cylinders (4 cm × 4 cm × 18 cm), in which they could move forward and backward but could not turn around[14]. The rats remained in the restraint cylinders for 3 h, with access to food and water ad libitum, in the morning and in the afternoon of each day. Then NMS + RS rats were divided into two groups (NMS + RS group and TXYF-formula group).

All animal care and experimental procedures were conducted according to the institutional ethical guidelines and conformed to the requirements of the Institutional Animal Care and Use Committee of Beijing University of Chinese Medicine and the Animal Ethics Committee of Dongzhimen Hospital Affiliated with Beijing University of Chinese Medicine.

TXYF Composition and administration

TXYF-formula consisted of the following four Traditional Chinese Herbal Medicines: Bai Zhu (Atractylodesm macrocephala Koidz - Acta Horti Gothoburgensis 12(9): 310 1938)-93.75 g, Shao Yao (Paeonia lactiflora Pall. - Reise Russ. Reich. 3: 286. 1776)-62.5 g, Chen Pi (Citrus reticulata Blanco - Fl. Filip. 610 1837.)-46.875 g and Fang Feng (Saposhnikovia divaricata (Turcz.) Schischk. - Fl. URSS 17: 359 1951.)-31.25 g. It was manufactured by Preparation Room for TCM of Beijing Chinese Medicine Hospital. All formula raw materials were examined according to the quality control criteria in Chinese Pharmacopeia[15].

From postnatal day 60, rats in TXYF-formula group were daily given orally administered TXYF-formula (4.92 g/100 g bodyweight) while NH group and NMS + RS group were treated with distilled water. The delivery volume in three groups was 2 mL/100 g per day, for 14 consecutive days.

Tissue preparation

After treatment, the rats in three groups were first anesthetized abdominally with 7% chloral hydrate (35 mg/100 g bodyweight). The distal colon (6-7 cm from anus) was then quickly removed and incised longitudinally along the mesenteric border. The mucosa was fixed onto a Petri dish with silica gel in the bottom, with the lumen side down. The Petri dishes were filled with Krebs' solution under 95% oxygen and 5% carbon dioxide. The mucosal layer was carefully separated from the submucosa, muscularis, and serosal layer with fine tweezers under a microscope. The mucosal layer was then cut into small flat sheets, with areas of about 0.5 cm^2, for further analysis. Two sheets could be obtained from one segment of the distal colon.

Ussing chamber experiments

Six adjacent tissues from the distal colon of each rat were obtained and mounted in Ussing chambers. Krebs' solution (5 mL) was injected into two small adjacent compartments with circulating 95% oxygen and 5% carbon dioxide while pH was maintained at 7.35-7.45 and temperature at 37 ℃. The tissues were left to equilibrate for 60 min to allow the electrical parameters to stabilize. The voltage across tissues was then clamped to zero and the short-circuit current (Isc, μA/cm^2) was measured. When the Isc baseline was smooth, 10 μmol/L indomethacin was added to basolateral side of the tissue and maintained for 10 min to block the influence of endogenous prostaglandins[16,17]. The Isc value was recorded at this time. 5-HT (10 μmol/L) was then added to basolateral side and the maximum Isc recorded. The change in Isc (ΔIsc) after the addition of 5-HT was then calculated. To measure the transmembrane resistance (Rt, Ω/cm^2), electrical stimulation of 1 mV was applied to both sides of the epithelium. Tissue conductance was calculated according to Ohm's law and expressed in milliSiemens per square centimeter (mS/cm^2). The drugs or specific blockers were then applied to the apical or basolateral side of tissue and the ΔIsc of ion channels in colonic epithelium were calculated and recorded for further analysis.

Reagents

Krebs' solution of the following composition (in mol/L): 117 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 24.8 $NaHCO_3$, 1.2 KH_2PO_4, 2.56 $CaCl_2$, and 11.1 glucose; Krebs' solution without Cl^-: sodium gluconate instead of NaCl, potassium gluconate instead of KCl, calcium gluconate instead of $CaCl_2$,

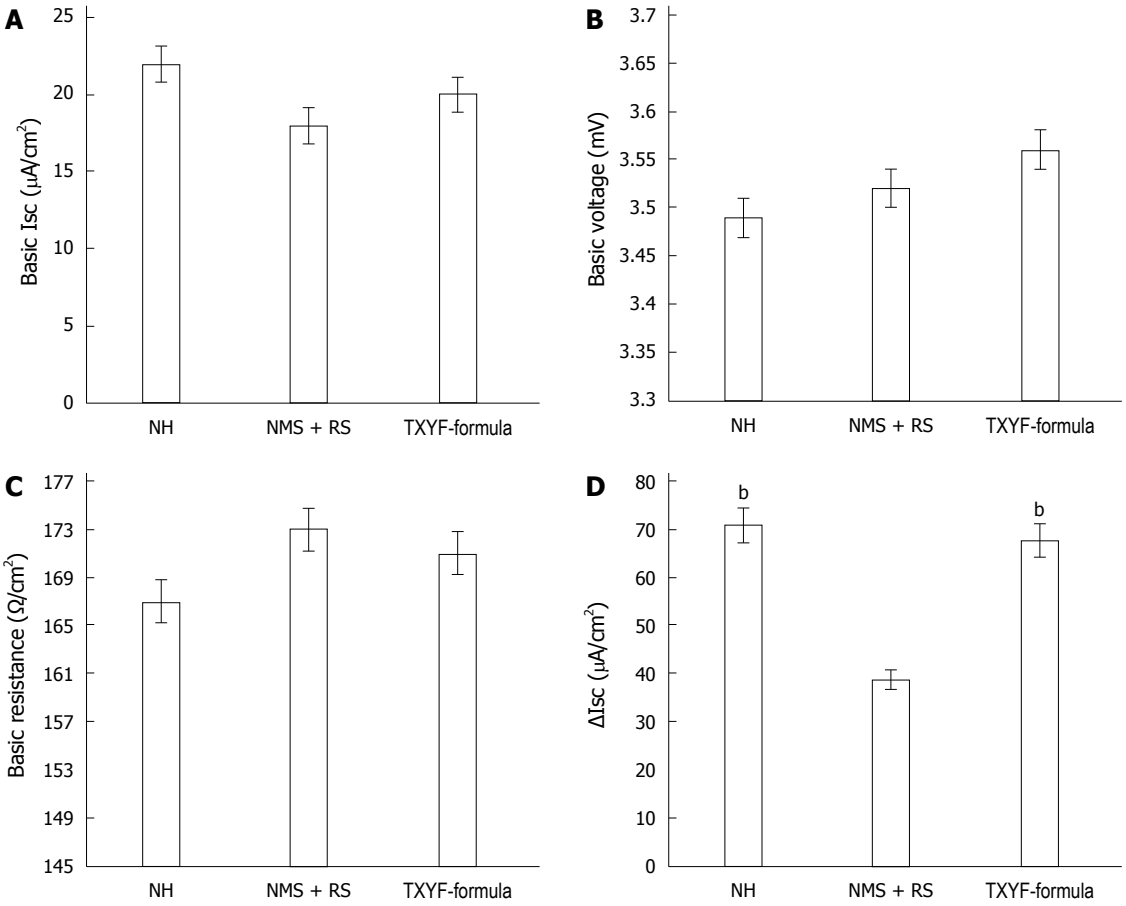

Figure 1 Comparison of basic electrophysiological properties of colonic mucosa in rats and 5-HT-induced the changes of short-circuit current among three groups. A-C: The basic Isc, basic voltage and basic resistance did not differ significantly between three groups, respectively ($P > 0.05$, respectively); D: TXYF-formula significantly increased 5-HT-induced ΔIsc in neonatal maternal separation plus restraint stress (NMS + RS) rats. $^bP < 0.01$ *vs* NMS + RS group. Isc: The short-circuit current; ΔIsc: The changes of Isc; TXYF: TongXie-YaoFang; NMS: Neonatal maternal separation; RS: Restraint stress; NH: No handlings as control.

and the remaining components were the same; Krebs' solution without HCO_3^-: 141.8 NaCl, 5.9 KCl, 1.2 $MgSO_4$·7 H_2O, 2.56 $CaCl_2$, 11.1 glucose, 10 HEPES free acid, and 5.6 Tris. Krebs' solution without Cl^- and HCO_3^-: sodium gluconate instead of NaCl and $NaHCO_3$, potassium gluconate instead of KCl, calcium gluconate instead of $CaCl_2$, and the remaining components were the same. 5-HT, batch number: 1001156278; Glibenclamide, batch number: 1001068037; Indomethacin, batch number: 1001087688; Bumetanide, batch number: 101016760; Amiloride, batch number: 101093389; SITS (4-acetamido-4'-isothio-cyanato-stilbene-2,2'-disulfonic), batch number: 1001208418; DPC (1,1-dimethyl piperidinium chloride), batch number: 101078880; $BaCl_2$, batch number: 1398; DIDS (4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid), batch number: 1001339605. cystic fibrosis transmembrane conductance regulator (CFTR)(inh)-172, batch number: 6311. All reagents were purchased from Sigma-Aldrich Co.

Apparatus

A multichannel voltage-current clamp (VCC MC6) was purchased from Physiologic Instruments Corporation; the Bridge amplifier (ML228), recording and analysis system for physiological data (Power Lab) was purchased from AD Instruments Corporation.

Statistical analysis

All experimental data were analyzed by SPSS 17.0 statistical software and expressed as means ± SE. The differences between groups were analyzed with a paired *t* test. $P < 0.05$ was considered statistically significant.

RESULTS

Basic electrophysiological properties of colonic mucosa in three groups were undifferentiated

The basic Isc did not differ significantly between three groups (21.02 μA/cm² ± 2.92 μA/cm² *vs* 20.29 μA/cm² ± 3.58 μA/cm² *vs* 20.71 μA/cm² ± 2.04 μA/cm², $n = 18$, $P > 0.05$, respectively; Figure 1A). The basic voltage in three groups had similar results (3.49 mV ± 0.54 mV *vs* 3.52 mV ± 0.69 mV *vs* 3.57 mV ± 0.62 mV, $n = 18$, $P > 0.05$, respectively; Figure 1B) as well as the basic resistance (166.8 Ω/cm² ± 20.11 Ω/cm² *vs* 173.66 Ω/cm² ± 16.39 Ω/cm² *vs* 171.94 Ω/cm² ± 19.03 Ω/cm², $n = 18$, $P > 0.05$, respectively; Figure 1C). The 5-HT-induced change of short-circuit current

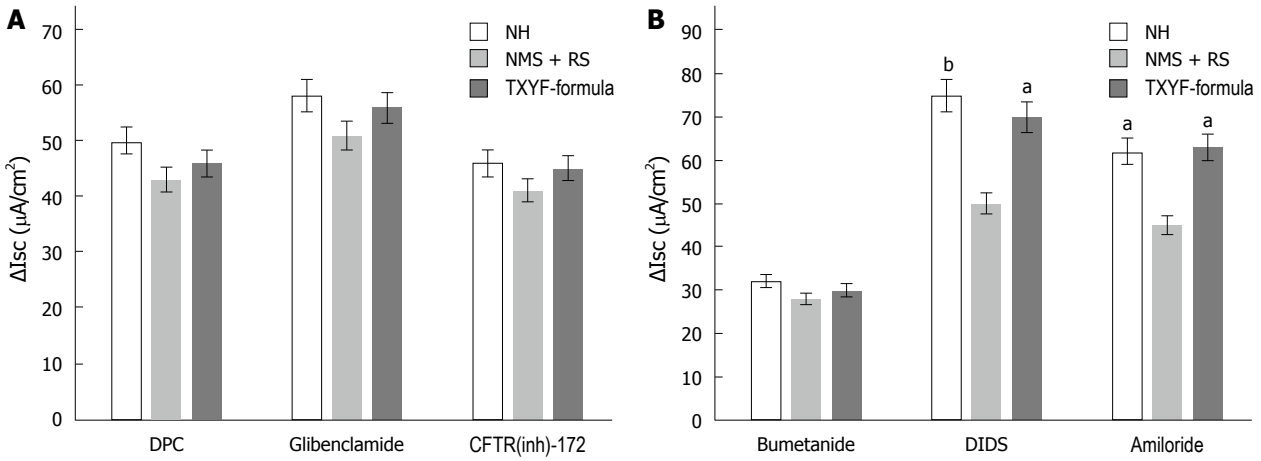

Figure 2 Effects of 1,1-dimethyl piperidinium chloride, Glibenclamide, cystic fibrosis transmembrane conductance regulator(inh)-172, 4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid, Amiloride and Bumetanide on 5-HT-induced the changes of short-circuit current in rats. A: After the intervention with DPC, Glibenclamide or CFTR(inh)-172, respectively, ΔIsc induced by 5-HT were similar in three groups ($P > 0.05$, respectively); B: 5-HT-induced ΔIsc was higher in TXYF-formula group than in NMS + RS group after the intervention with DIDS or Amiloride. $^aP < 0.05$ *vs* NMS + RS group; $^bP < 0.01$ *vs* NMS + RS group. DPC: 1,1-dimethyl piperidinium chloride; CFTR: Cystic fibrosis transmembrane conductance regulator; ΔIsc: the changes short-circuit current; DIDS: 4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid; TXYF: TongXie-YaoFang; NMS: Neonatal maternal separation; RS: Restraint stress; NH: No handlings as control.

(ΔIsc) in NMS + RS group was respectively smaller than that in NH group and TXYF-formula group (38.8 μA/cm² ± 7.25 μA/cm² *vs* 70.86 μA/cm² ± 12.32 μA/cm², 67.67 μA/cm² ± 11.68 μA/cm², $n = 18$, $P < 0.01$, respectively; Figure 1D).

5-HT-induced ΔIsc after the effects of CFTR Cl^- channel or Na^+-K^+-2Cl^- co-transporter blocker was similar in three groups. ΔIsc was higher in TXYF- formula group than in NMS + RS group after the effects of Ca^{2+}-dependent Cl^- channel or epithelial Na^+ channel blocker

DPC or Glibenclamide with final concentration 1 mmol/L were respectively added to apical side, namely left side of Ussing chamber, of colonic mucosa and equilibrated for 30 min. Then 10 μmol/L 5-HT was added to basolateral side (Equilibrated time and added concentration of 5-HT was same in the following experiment). ΔIsc induced by 5-HT were similar in TXYF-group and NMS + RS group, respectively (47.75 μA/cm² ± 7.99 μA/cm² *vs* 42.28 μA/cm² ± 10.61 μA/cm², 57.57 μA/cm² ± 14.25 μA/cm² *vs* 46.78 μA/cm² ± 11.68 μA/cm², $n = 8$, $P > 0.05$, respectively; Figure 2A). The similar result was obtained when CFTR(inh)-172 was added to apical side with final concentration 100 μmol/L (45.04 μA/cm² ± 9.18 μA/cm² *vs* 36.2 μA/cm² ± 9.64 μA/cm², $n = 8$, $P > 0.05$; Figure 2A).

5-HT-induced ΔIsc was higher in TXYF-formula group than in NMS + RS group after the effects of Ca^{2+}-dependent Cl^- channel (CACC) blocker DIDS (500 μmol/L, added to apical side) or epithelial Na^+ channel (ENaC) blocker Amiloride (100 μmol/L, added to apical side), respectively (69.22 μA/cm² ± 11.7 μA/cm² *vs* 48.41 μA/cm² ± 13.15 μA/cm², 62.11 μA/cm² ± 12.01 μA/cm² *vs* 44.69 μA/cm² ± 12.58 μA /cm², $n = 8$, $P < 0.05$, respectively; Figure 2B). There were no statistical differences in three groups when 100 μmol/L Na^+-K^+-2Cl^- co-transporter (NKCC) blocker Bumetanide was added to basolateral (37.64 μA/cm² ± 10.57 μA/cm² *vs* 27.55 μA/cm² ± 10.94 μA/cm² *vs* 29.43 μA/cm² ± 7.66 μA/cm², $n = 8$, $P > 0.05$; Figure 2B).

There were no significant differences in three groups after the effects of K^+ channel blocker, Na^+-HCO_3^- co-transporter blocker or Cl^-/HCO_3^- exchanger inhibitor on 5-HT-induced ΔIsc

ΔIsc induced by 5-HT were similar in three groups when 5 mmol/L K^+ channel blocker $BaCl_2$ or 100 μmol/L Cl^-/HCO_3^- exchanger inhibitor SITS was respectively added to basolateral side of colonic mucosa (25.63 μA/cm² ± 13.69 μA/cm² *vs* 13.92 μA/cm² ± 8.16 μA/cm² *vs* 17.03 μA/cm² ± 9.04 μA/cm², 49.92 μA/cm² ± 11.66 μA/cm² *vs* 40.41 μA/cm² ± 14.26 μA/cm² *vs* 47.7 μA/cm² ± 11.43 μA/cm², $n = 8$, $P > 0.05$, respectively; Figure 3). There was also no statistical difference in three groups after the effects of 200 μmol/L Na^+-HCO_3^- co-transporter (NBC) blocker DIDS (500 μmol/L, added to basolateral side) (12.27 μA/cm² ± 3.6 μA/cm² *vs* 10.74 μA/cm² ± 2.99 μA/cm² *vs* 11.88 μA/cm² ± 3.51 μA/cm², $n = 8$, $P > 0.05$; Figure 3).

5-HT-induced ΔIsc was higher in TXYF-formula group than in NMS + RS group when Na^+ in apical side of colonic mucosa was taken out. This phenomenon disappeared while Cl^- and HCO_3^- were taken out altogether or respectively

When Na^+ applied to apical side of colonic mucosa was substituted with sodium gluconate, 5-HT-induced ΔIsc was higher in TXYF-formula groups than in NMS + RS group (56.86 μA/cm² ± 11.2 μA/cm² *vs* 39.14 μA/cm² ± 10.83 μA/cm², $n = 8$, $P < 0.05$; Figure 4A). However, when basolateral side Na^+ was substituted with sodium gluconate, ΔIsc was similar in three groups (0.87 μA/cm² ± 0.33 μA/cm² *vs* 1.15 μA/cm² ± 0.5 μA/cm² *vs* 1.01 μA/cm² ± 0.49 μA/cm², $n = 8$, $P >$

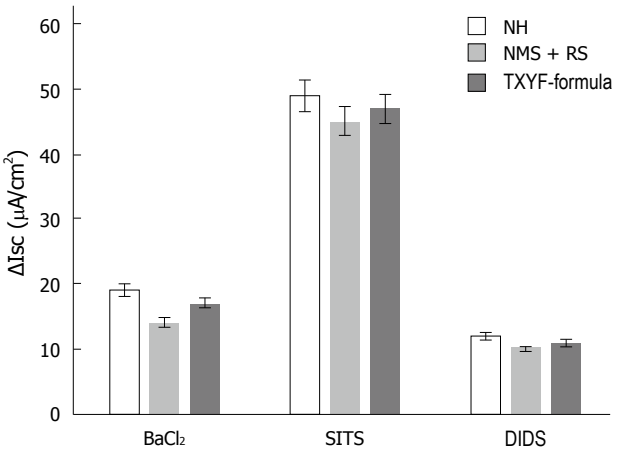

Figure 3 Effects of $BaCl_2$, 4-acetamido-4'-isothio-cyanato-stilbene-2,2'-disulfonic and 4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid on 5-HT-induced the changes of short-circuit current in rats. 5-HT-induced ΔIsc were similar in three groups after the intervention with $BaCl_2$, SITS or DIDS, respectively ($P > 0.05$, respectively). ΔIsc: the changes short-circuit current; SITS: 4-acetamido-4'-isothio-cyanato-stilbene-2,2'-disulfonic; DIDS: 4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid; TXYF: TongXie-YaoFang; NMS: Neonatal maternal separation; RS: Restraint stress; NH: No handlings as control.

0.05, respectively; Figure 4B).

5-HT-induced ΔIsc was similar in three groups when Cl^- and HCO_3^- was respectively removed from basolateral side of colonic mucosa (14.42 μA/cm² ± 6.07 μA/cm² *vs* 15.95 μA/cm² ± 5.64 μA/cm² *vs* 15.09 μA/cm² ± 4.04 μA/cm², 33.86 μA/cm² ± 7.47 μA/cm² *vs* 26.54 μA/cm² ± 8.9 μA/cm² *vs* 31.88 μA/cm² ± 6.07 μA/cm², $n = 8$, $P > 0.05$, respectively; Figure 4C). When both Cl^- and HCO_3^- were simultaneously removed from Krebs' solution applied to basolateral side, 5-HT-induced ΔIsc was similar in three groups (8.38 μA/cm² ± 1.15 μA/cm² *vs* 9.3 μA/cm² ± 2.16 μA/cm² *vs* 8.51 μA/cm² ± 1.2 μA/cm², $n = 8$, $P > 0.05$, respectively; Figure 4C).

DISCUSSION

The etiology and pathogenesis of D-IBS are complex, and its pathophysiological changes predominantly include dynamic gastrointestinal disorder and visceral sensory sensitivity. In TCM, D-IBS is classified as diarrhea or abdominal pain according to its clinical manifestations[18]. Previous studies had shown that TXYF-formula had an inhibitory effect on bowel movement and reduced intestinal peristalsis by regulating 5-HT[19,20]. Pharmacological study showed that Fang Feng could increase intestinal pressure threshold in rats so that to present its analgesic effect[21]. In this study, we used the method of neonatal maternal separation plus restraint stress to establish an animal mode of D-IBS, with the main simultaneous symptoms of diarrhea and high visceral sensitivity. This recapitulates the clinical symptoms of patients with D-IBS.

The secretion activities induced by 5-HT in colonic mucosae of NMS + RS rats were weaker than those in NH rats or TXYF-formula-treated rats in this study. This may be related to increased 5-HT and 5-HT-receptor levels in NMS + RS rats. When 5-HT was added to colonic mucosa from NMS + RS rats, the electrical activities across epithelium declined. The reaction to 5-HT in colonic mucosae of rats was restored by treatment with TXYF-formula, to a level almost the same as that observed in NH rats. This demonstrates that TXYF-formula has specific therapeutic effects on D-IBS.

We found that one of the therapeutic effects of TXYF-formula is achieved by regulating secretion of Cl^-. The specific results were as follows: 5-HT-induced ΔIsc in NMS + RS rats differed significantly from that in TXYF-formula group and NH group when extracellular Cl^- was not removed. However, when extracellular Cl^- was removed, the difference disappeared. When a CFTR Cl^- channel blocker was added to apical side or an NKCC co-transporter inhibitor was added to basolateral side of tissue, ΔIsc did not differ statistically in NMS + RS and TXYF-formula-treated rats. In presence of the non-selective K^+ channel blocker[22], 5-HT-induced ΔIsc did not differ significantly between NMS + RS group and TXYF-formula group. This indicates that Cl^- secretion by mucosal epithelium is dependent on electrochemical gradient across the serosal surface generated by K^+ transport.

We also found that TXYF-formula alters HCO_3^- secretion. When extracellular HCO_3^- was removed or NBC was inhibited, 5-HT-induced ΔIsc did not differ between NMS + RS group and TXYF-formula group. The same result was obtained when extracellular Cl^- and HCO_3^- were both removed or when Cl^-/HCO_3^- exchange was interrupted. Thus, the therapeutic effect of TXYF-formula is achieved by regulating the secretion of both Cl^- and HCO_3^-. Moreover, when Na^+ on apical side of membrane was removed or a Na^+ channel blocker was added, ΔIsc still differed between NMS + RS group and TXYF-formula group; and the differences between NMS + RS and NH rats did not disappear. Therefore, the relationship between therapeutic effects of TXYF-formula on Na^+ transport and D-IBS is not close. Nevertheless, after Na^+ in basilar membrane was removed, the electrical activity across epithelium did not differ between TXYF-formula group and NMS + RS group. Therefore, it can be seen that the regulatory effects of TXYF-formula on Cl^- and HCO_3^- secretion depends on Na^+ in basilar membrane.

There are two main types of Cl^- channels on apical side of epithelial cells: CFTR and CACC. CFTR is a cAMP-dependent, PKA-activated Cl^- channel, and is sensitive to DPC and Glibenclamide[23,24]. After CFTR Cl^- channel was blocked by DPC or Glibenclamide, the electrical activity across epithelium induced by 5-HT did not differ obviously between TXYF-formula group and NMS + RS group. However, the electrical activity in two groups differed markedly after the application of CACC blocker DIDS. Therefore, the regulatory effect

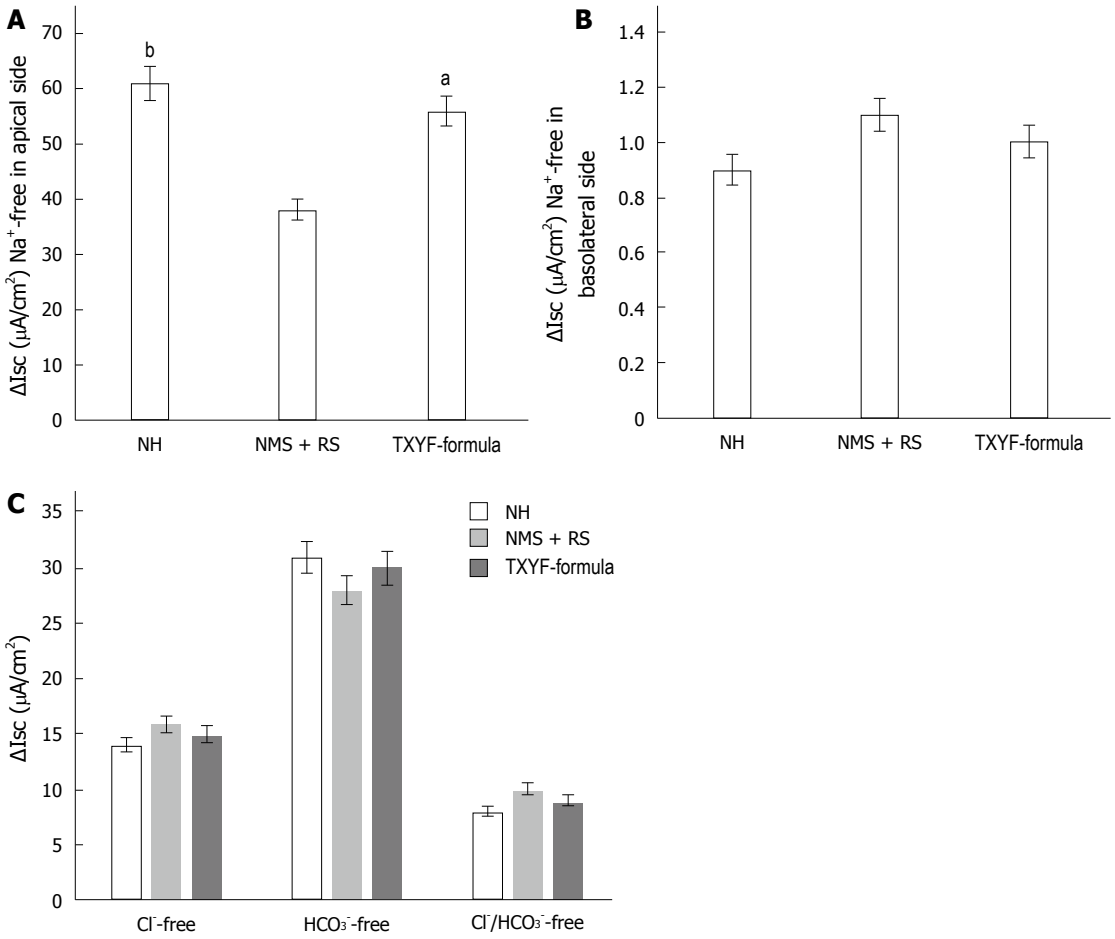

Figure 4 Effects of Na^+, Cl^- and HCO_3^- on 5-HT-induced the changes of short-circuit current in rats. A: 5-HT-induced ΔIsc was higher in TXYF-formula group than in NMS + RS group when Na^+ in apical side of colonic mucosa was taken out; B: When Na^+ in basolateral side was taken out, ΔIsc was similar in three groups; C: 5-HT-induced ΔIsc was similar in three groups when Cl^- and HCO_3^- was respectively or entirely removed from basolateral side of colonic mucosa. $^aP < 0.05$ *vs* NMS + RS group; $^bP < 0.01$ *vs* NMS + RS group. ΔIsc: the changes short-circuit current; TXYF: TongXie-YaoFang; NMS: Neonatal maternal separation; RS: Restraint stress; NH: No handlings as control.

of TXYF-formula on electrical activity across epithelia of D-IBS rats is associated with CFTR Cl^- channel, and has nothing to do with CACC channel.

Our results, combined with preliminary studies[25,26] about secretory mechanisms of epithelial anions in colonic mucosa, allow the following conclusions to be drawn. The regulating effects of TXYF-formula on D-IBS involves the secretion of Cl^- and HCO_3^- in colonic mucosa *via* CFTR Cl^- channel, Cl^-/HCO_3^- exchanger, and NBC and NKCC co-transporters.

COMMENTS

Background

Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic functional gastrointestinal disease. The pathogenesis of it has not been thoroughly elucidated while colonic abnormal secretory is recognized as one of the pathophysiological factors. The usual treatment in Western medicine, mainly involves symptomatic therapy, is unsatisfactory for patients while simultaneously increasing the use of health-care resources. Traditional Chinese medicine (TCM) can obviously alleviate patients' clinical symptoms, increasing numbers of them have begun to seek treatment with TCM. A lot of researches have shown that TongXie-YaoFang formula (TXYF-formula), a Chinese herbal formula, can significantly improve D-IBS patients' clinical symptoms and enhance their quality of lives.

Research frontiers

TXYF-formula is a Traditional Chinese classical prescription for clinical treatment on D-IBS. The research hotspot is its effect on colonic abnormal secretory *via* correlational ion channels, visceral sensitivity and colon movement.

Innovations and breakthroughs

Previous clinical and experimental studies had only shown that TXYF-formula can relieve diarrhea of patients with D-IBS. Its correlational effects might be realized by influencing secretion of colon. However, the specific mechanism and correlative ions are unclear. In this study, the mucosal stripping under a microscope was used for tissue preparation. And short-circuit current technology was applied to observe 5-HT-induced changes in current across ion channels of colonic epithelium so as to reveal the prescription effect of TXYF-formula ion transport in colon.

Applications

The key results of this study showed that TXYF-formula can regulate the secretion of Cl^- and HCO_3^- in colonic mucosa of D-IBS rats. And this may be related to cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel, Cl^-/HCO_3^- exchanger, and Na^+-HCO_3^- co-transporter (NBC) and Na^+-K^+-$2Cl^-$ co-transporter (NKCC) co-transporters.

Terminology

D-IBS, as a clinically common functional gastrointestinal disease, is closely related to early adverse life events. It seriously affects patient's quality of jobs and lives. TXYF-formula can regulate the Cl^- and HCO_3^- secretion of colonic mucosa *via* CFTR Cl^- channel, Cl^-/HCO_3^- exchanger, NBC and NKCC co-transporters.

Peer-review

Well written, quite meticulous methodology, nicely executed study.

REFERENCES

1 **Ford AC**, Bercik P, Morgan DG, Bolino C, Pintos-Sanchez MI, Moayyedi P. Validation of the Rome III criteria for the diagnosis of irritable bowel syndrome in secondary care. *Gastroenterology* 2013; **145**: 1262-70.e1 [PMID: 23994201 DOI: 10.1053/j.gastro.2013.08.048]

2 **Shalaby SA**, Sayed MM, Ibrahim WA, Abdelhakam SM, Rushdy M. The prevalence of coeliac disease in patients fulfilling Rome III criteria for irritable bowel syndrome. *Arab J Gastroenterol* 2016; **17**: 73-77 [PMID: 27349575 DOI: 10.1016/j.ajg.2016.05.003]

3 **Marquis P**, Lasch KE, Delgado-Herrera L, Kothari S, Lembo A, Lademacher C, Spears G, Nishida A, Tesler WL, Piault E, Rosa K, Zeiher B. Qualitative development of a patient-reported outcome symptom measure in diarrhea-predominant irritable bowel syndrome. *Clin Transl Gastroenterol* 2014; **5**: e59 [PMID: 24964994 DOI: 10.1038/ctg.2014.7]

4 **Nee J**, Zakari M, Lembo AJ. Current and emerging drug options in the treatment of diarrhea predominant irritable bowel syndrome. *Expert Opin Pharmacother* 2015; **16**: 2781-2792 [PMID: 26558923 DOI: 10.1517/14656566]

5 **Lacy BE**. Diagnosis and treatment of diarrhea-predominant irritable bowel syndrome. *Int J Gen Med* 2016; **9**: 7-17 [PMID: 26929659 DOI: 10.2147/IJGM]

6 **Chiba T**, Yamamoto K, Sato S, Suzuki K. Long-term efficacy and safety of ramosetron in the treatment of diarrhea-predominant irritable bowel syndrome. *Clin Exp Gastroenterol* 2013; **6**: 123-128 [PMID: 23922505 DOI: 10.2147/CEG.S32721]

7 **Teschke R**, Wolff A, Frenzel C, Eickhoff A, Schulze J. Herbal traditional Chinese medicine and its evidence base in gastrointestinal disorders. *World J Gastroenterol* 2015; **21**: 4466-4490 [PMID: 25914456 DOI: 10.3748/wjg.v21.i15.4446]

8 **Xiao Y**, Liu Y, Huang S, Sun X, Tang Y, Cheng J, Wang T, Li F, Kuang Y, Luo R, Zhao X. The efficacy of Shugan Jianpi Zhixie therapy for diarrhea-predominant irritable bowel syndrome: a meta-analysis of randomized, double-blind, placebo-controlled trials. *PLoS One* 2015; **10**: e0122397 [PMID: 25853241 DOI: 10.1371/journal.pone.0122397]

9 **Pan F**, Zhang T, Zhang YH, Xu JJ, Chen FM. Effect of Tongxie Yaofang Granule in treating diarrhea-predominate irritable bowel syndrome. *Chin J Integr Med* 2009; **15**: 216-219 [PMID: 19568715 DOI: 10.1007/s11655-009-0216-7]

10 **Lai LJ,** Han YC, Hui Zhang, Zhu XW, Han XM. The timeliness of Tongxie-Yaofang on patients with D-IBS about improving the clinical symptoms. *Shiyong Zhongxiyi Jiehe Linchuang* 2013; **13**: 27-28 [DOI: 10.3969/j.issn.1671-4040.2013.02.017]

11 **Ren TH**, Wu J, Yew D, Ziea E, Lao L, Leung WK, Berman B, Hu PJ, Sung JJ. Effects of neonatal maternal separation on neurochemical and sensory response to colonic distension in a rat model of irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G849-G856 [PMID: 17110521 DOI: 10.1152/ajpgi.00400.2006]

12 **Gareau MG**, Jury J, Perdue MH. Neonatal maternal separation of rat pups results in abnormal cholinergic regulation of epithelial permeability. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G198-G203 [PMID: 17510196 DOI: 10.1152/ajpgi.00392.2006]

13 **Oines E**, Murison R, Mrdalj J, Grønli J, Milde AM. Neonatal maternal separation in male rats increases intestinal permeability and affects behavior after chronic social stress. *Physiol Behav* 2012; **105**: 1058-1066 [PMID: 22155491 DOI: 10.1016/j.physbeh.2011.11.024]

14 **Irles C**, Nava-Kopp AT, Morán J, Zhang L. Neonatal maternal separation up-regulates protein signalling for cell survival in rat hypothalamus. *Stress* 2014; **17**: 275-284 [PMID: 24730533 DOI: 10.3109/10253890.2014.913017]

15 **Pharmacopoeia Commission of the Ministry of Public Health of PRC**. Chinese Pharmacopoeia: Chemical Industry Press, 2005

16 **Garsed K**, Chernova J, Hastings M, Lam C, Marciani L, Singh G, Henry A, Hall I, Whorwell P, Spiller R. A randomised trial of ondansetron for the treatment of irritable bowel syndrome with diarrhoea. *Gut* 2014; **63**: 1617-1625 [PMID: 24334242 DOI: 10.1136/gutjnl-2013-305989]

17 **Feng XY**, Li Y, Li LS, Li XF, Zheng LF, Zhang XL, Fan RF, Song J, Hong F, Zhang Y, Zhu JX. Dopamine D1 receptors mediate dopamine-induced duodenal epithelial ion transport in rats. *Transl Res* 2013; **161**: 486-494 [PMID: 23276732 DOI: 10.1016/j.trsl.2012.12.002]

18 **Li L,** Zhang SS. The mechanism research status in Irritable bowel syndrome with traditional Chinese medicine. *Zhongguo Zhongxiyi Jiehe Zazhi* 2012; **20**: 466-470

19 **Rahimi R**, Abdollahi M. Herbal medicines for the management of irritable bowel syndrome: a comprehensive review. *World J Gastroenterol* 2012; **18**: 589-600 [PMID: 22363129 DOI: 10.3748/wjg.v18.i7.589]

20 **Yang C**, Zhang SS, Li XL, Wang ZF, Zhao LQ. Inhibitory effect of TongXie-YaoFang formula on colonic contraction in rats. *World J Gastroenterol* 2015; **21**: 2912-2917 [PMID: 25780288 DOI: 10.3748/wjg.v21.i10.2912]

21 **Hu XG**, Liao SL, Wang YF, Gong MJ, Wang Man, Liu SS, Han Bin. Effect of Radix Saposhnikoviae in Tongxie Yaofang on PAR2 mRNA Expression and Inflammatory Mediators in the Colon of Rats with Postinfection Irritable Bowel Syndrome. *Zhongyao Linchuang Yaolixue* 2013; **24**: 5-9

22 **Fujita T**, Karaki S, Tateoka T, Kuwahara A. Desacetyl bisacodyl-induced epithelial Cl(-) secretion in rat colon and rectum. *Biomed Res* 2016; **37**: 13-20 [PMID: 26912136 DOI: 10.2220/biomedres.37.13]

23 **Ao M**, Sarathy J, Domingue J, Alrefai WA, Rao MC. Chenodeoxycholic acid stimulates Cl(-) secretion via cAMP signaling and increases cystic fibrosis transmembrane conductance regulator phosphorylation in T84 cells. *Am J Physiol Cell Physiol* 2013; **305**: C447-C456 [PMID: 23761628 DOI: 10.1152/ajpcell.00416.2012]

24 **Hou Y**, Guan X, Yang Z, Li C. Emerging role of cystic fibrosis transmembrane conductance regulator - an epithelial chloride channel in gastrointestinal cancers. *World J Gastrointest Oncol* 2016; **8**: 282-288 [PMID: 26989463 DOI: 10.4251/wjgo.v8.i3.282]

25 **Dürrnagel S**, Falkenburger BH, Gründer S. High Ca(2+) permeability of a peptide-gated DEG/ENaC from Hydra. *J Gen Physiol* 2012; **140**: 391-402 [PMID: 23008433 DOI: 10.1085/jgp.201210798]

26 **Cremon C**, Carini G, Wang B, Vasina V, Cogliandro RF, De Giorgio R, Stanghellini V, Grundy D, Tonini M, De Ponti F, Corinaldesi R, Barbara G. Intestinal serotonin release, sensory neuron activation, and abdominal pain in irritable bowel syndrome. *Am J Gastroenterol* 2011; **106**: 1290-1298 [PMID: 21427712 DOI: 10.1038/ajg.2011.86]

P- Reviewer: Garg P **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Liu WX

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.v22.i48.10592

World J Gastroenterol 2016 December 28; 22(48): 10592-10600
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Risk stratification for malignant progression in Barrett's esophagus: Gender, age, duration and year of surveillance

Piers Gatenby, Santanu Bhattacharjee, Christine Wall, Christine Caygill, Anthony Watson

Piers Gatenby, Santanu Bhattacharjee, Christine Wall, Christine Caygill, Anthony Watson, United Kingdom Barrett's Oesophagus Registry, UCL, NW32QG London, United Kingdom

Piers Gatenby, Regional Oesophagogastric Unit, Royal Surrey County Hospital, Guildford, GU2 7XH Surrey, United Kingdom

Piers Gatenby, Department of Clinical and Experimental Medicine, University of Surrey, Guildford, GU2 7XH Surrey, United Kingdom

Piers Gatenby, Division of Surgery and Interventional Science, UCL, Royal Free Campus, NW32QG London, United Kingdom

Author contributions: All authors contributed to this manuscript with writing, designing, and final approval.

Supported by The Barrett's Oesophagus Campaign; The Wexham Gastrointestinal Trust; The Childwick Trust, The R. L. St J. Harmsworth Memorial Research Fund; and The David and Frederick Barclay Foundation.

Conflict-of-interest statement: None declared, the authors confirm that we have no financial arrangements related to the research or manuscript preparation to disclose.

Manuscript source: Invited manuscript

Correspondence to: Piers Gatenby, MA MD FRCS, Division of Surgery and Interventional Science, UCL, Royal Free Campus, Pond Street, NW32QG London, United Kingdom. p.gatenby@ucl.ac.uk
Telephone: +44-20-76792000

Received: July 18, 2016
Peer-review started: July 20, 2016
First decision: September 20, 2016
Revised: October 17, 2016
Accepted: November 23, 2016
Article in press: November 28, 2016
Published online: December 28, 2016

Abstract

AIM

To clarify risk based upon segment length, diagnostic histological findings, patient age and year of surveillance, duration of surveillance and gender.

METHODS

Patients registered with the United Kingdom Barrett's Oesophagus Registry from 9 United Kingdom centers were included. The outcome measures were (1) development of all grades of dysplasia; (2) development of high-grade of dysplasia or adenocarcinoma; and (3) development of adenocarcinoma. Prevalent cases and subjects with < 1 year of follow-up were excluded. The covariates examined were segment length, previous biopsy findings, age at surveillance, duration of surveillance, year of surveillance and gender.

RESULTS

One thousand and one hundred thirty six patients were included (total 6474 patient-years). Fifty-four patients developed adenocarcinoma (0.83% per annum), 70 developed high-grade dysplasia/adenocarcinoma (1.1% per annum) and 190 developed any grade of dysplasia (3.5% per annum). High grade dysplasia and adenocarcinoma increased with age and duration of surveillance. The risk of low-grade dysplasia development was not dependent on age at surveillance. Segment length and previous biopsy findings were also significant factors for development of dysplasia and adenocarcinoma.

CONCLUSION
The risk of development of low-grade dysplasia is independent of age at surveillance, but high-grade dysplasia and adenocarcinoma were more commonly found at older age. Segment length and previous biopsy findings are also markers of risk. This study did not demonstrate stabilisation of the metaplastic segment with prolonged surveillance.

Key words: Dysplasia; Barrett's esophagus; Esophageal neoplasms; Public health; Epidemiology; Surveillance

Core tip: Current surveillance guidelines for Barrett's oesophagus base the enrolment into surveillance and surveillance interval on segment length, presence or absence of intestinal metaplasia and dysplasia. This study demonstrates the importance of age as an important risk factor for high-grade dysplasia and adenocarcinoma development and that stabilisation of the epithelium does not reliably occur at long-term follow-up.

Gatenby P, Bhattacharjee S, Wall C, Caygill C, Watson A. Risk stratification for malignant progression in Barrett's esophagus: Gender, age, duration and year of surveillance. *World J Gastroenterol* 2016; 22(48): 10592-10600 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10592.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10592

INTRODUCTION

The incidence of adenocarcinoma in Barrett's esophagus has been estimated to be around 0.5% per annum[1-3] and 0.32% in patients without dysplasia at index endoscopy[4]. This would suggest a lifetime risk of around 1:8 to 1:14 of developing adenocarcinoma and 1:5 to 1:6 of developing either high-grade dysplasia or adenocarcinoma[5]. A meta-analysis has shown a trend for a decrease in incidence of adenocarcinoma to be observed with time (which did not reach statistical significance, $P = 0.117$)[3] and in recent years large population-based cohort studies[6-8] have demonstrated lower adenocarcinoma incidence rates (0.22%, 0.43% and 0.12% per annum respectively). We have observed that the age at diagnosis of patients with Barrett's esophagus is falling[9] and that the life expectancy of those diagnosed with Barrett's esophagus is increasing[5]. The Danish pathology registry has demonstrated in their cohort that the adenocarcinoma incidence in Barrett's increases with older age[8].

The currently accepted principal risk factors for dysplasia and cancer development in Barrett's oesophagus are presence/absence of intestinal metaplasia, dysplasia and segment length[10-12]. These are the factors on which guidance for surveillance intervals are determined.

These observations prompt examination of what the time trends in cancer incidence in Barrett's esophagus are: Are the annual incidences of dysplasia and cancer changing in the population with Barrett's esophagus undergoing surveillance? Does an individual's risk change over time dependent on the patient's age at the time of surveillance? Does the Barrett's segment stabilize with prolonged follow-up such that patients might be reassured and discharged from further follow-up?

This study seeks to examine whether there is a demonstrable change in incidence of dysplasia and adenocarcinoma over time in patients undergoing surveillance of Barrett's esophagus who are registered with the United Kingdom Barrett's Oesophagus Registry and which are the most important demographic, histological and endoscopic features with regard to dysplasia and cancer risk.

MATERIALS AND METHODS

One thousand one hundred and thirty six patients who had been registered with the United Kingdom Barrett's Oesophagus Registry from 9 centers who did not have prevalent adenocarcinoma (diagnosed at index endoscopy or within one year of the index endoscopy) and who had a minimum of one year of follow-up were included in the study cohort. The three outcome measures were (1) development of any grade of dysplasia; (2) development of high-grade dysplasia or adenocarcinoma; and (3) development of adenocarcinoma. Follow-up time commenced at the diagnostic biopsy and was censored at the first biopsy reported as demonstrating the histological outcome or, when this was not attained, the final surveillance endoscopy and biopsy. The influence of 7 factors was then considered to provide further clarity as to an individual's risk of development of dysplasia and cancer. These were: (1) date (year) at which surveillance biopsies were undertaken; (2) age of the patient at which surveillance endoscopy and biopsy were undertaken; (3) length of time during which the patient had been undergoing surveillance; (4) patient gender; (5) segment length; (6) histological findings at the most recent (previous) endoscopy; and (7) histological findings at first and second endoscopies (in keeping with national guidelines on enrolment into surveillance programmes[10-12]).

Classification of histological results were: columnar-lined oesophagus without intestinal metaplasia, columnar-lined oesophagus with intestinal metaplasia, indefinite changes for dysplasia, low-grade dysplasia, high-grade dysplasia and adenocarcinoma.

The associations of dysplasia/adenocarcinoma risk with age at surveillance, year of surveillance and duration of surveillance were examined.

The associations between these factors and risk of development of dysplasia or cancer were examined

Table 1 Annual incidence of adenocarcinoma and dysplasia dependent on year of surveillance

Calendar years	Total patient-years follow-up	Adenocarcinoma		High-grade dysplasia and adenocarcinoma		All grades of dysplasia and adenocarcinoma	
		Number of cases	Annual incidence	Number of cases	Annual incidence	Number of cases	Annual incidence
1974-1989	237	0	0.00%	0	0.00%	1	0.46%
1990-1994	753	1	0.13%	1	0.13%	9	1.33%
1995-1999	1950	19	0.97%	21	1.09%	76	4.70%
2000-2004	2058	15	0.73%	23	1.12%	60	3.63%
2005-	1477	19	1.29%	25	1.17%	44	3.66%
Total	6474	54	0.83%	70	1.09%	190	3.54%

using binary logistic regression. The model was modified to exclude factors which did not reach statistical significance with removal of factors which showed no association with dysplasia or cancer risk. Due to the co-linearity of age, surveillance duration and year of surveillance, only the most closely-associated of these three variables was included in the final analyses.

Segment length data were available for 92.4% of cases and the analyses were repeated with the exclusion of segment length. Segment length was examined as a continuous variable and when separated into three similarly sized and clinically relevant groups: short segment (< 3 cm), 3-5 cm and > 5 cm.

Ethical permission

Approval for studies of this kind conducted by UKBOR was given by the London Multi-Centre Research Ethics Committee on 14th March 2002 number MREC/02/2/5.

Statistical analysis

Incidence calculations were undertaken using a patient-years at risk method and expressed as an annual percentage (cases per one hundred patient-years follow-up) and 95%CI were evaluated using an exact Poisson distribution. Logistic regression to ascertain the magnitude of the effect of the covariates was undertaken. *P* values < 0.05 were taken to be statistically significant.

RESULTS

The 1136 patients who fulfilled the criteria for inclusion in the study cohort comprised 783 males and 353 females diagnosed between 1974 and 2009. The mean age at diagnosis was 59.8 years, mean follow-up was 5.70 years and total follow-up was 6474 patient-years. The mean age at surveillance was 62.3 years, with a small trend for this to rise in males (60 in the 1990s, 61 in 2000-2004 and 62 from 2005 onwards), but no change in females (mean age at surveillance 65). During the follow-up period 54 patients developed adenocarcinoma, 70 developed either high-grade dysplasia or adenocarcinoma and 190 developed changes of any grade of dysplasia or cancer. The overall annual incidence of development of adenocarcinoma was 0.83% per annum, of high-grade dysplasia or adenocarcinoma, 1.1% per annum and of all grades of dysplasia and adenocarcinoma, 3.5% per annum.

Time trends for population during the cohort

During the first 15 years of the cohort from 1974-1989 only a small number of patients (76) were diagnosed and underwent surveillance, hence these patients have been grouped together; thereafter, dysplasia and adenocarcinoma incidence were examined over consecutive 5 year periods (Table 1).

Risk with year of surveillance

These data demonstrate that there was no clear trend for the incidence of either dysplasia or adenocarcinoma to fall appreciably during the study period (as examined by year of surveillance). The mean age at surveillance remained constant throughout the cohort; however there was an increased proportion of males in the latter portion of the cohort. When males and females were examined separately, there was no demonstrable change in the incidence of either adenocarcinoma or high-grade dysplasia for females with year of surveillance and a trend for a slight increase in adenocarcinoma incidence with later year of surveillance for males. The adenocarcinoma and high-grade dysplasia incidences were similar in males and females, but slightly lower in females. These results are depicted graphically in Figures 1 and 2.

Risk with age at surveillance

Table 2 and Figures 3 and 4 show the effect of age at surveillance on the detection of dysplasia and adenocarcinoma. This demonstrates a trend for an increasing risk of dysplasia detection as age at surveillance increases. When males and females were analysed separately, there was a trend for higher rates of dysplasia and adenocarcinoma incidence in males compared to females for specific decade of age at surveillance, but overall similar rates due to females being older than males at surveillance (40% of surveillance of females within the cohort occurring at age > 70, compared to 25% for males).

Risk with duration of surveillance

The results of the analysis which examined whether the

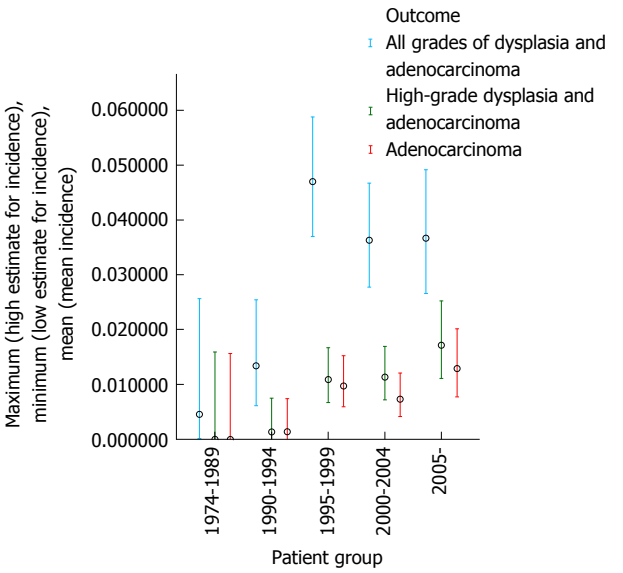

Figure 1 Annual incidence of adenocarcinoma and dysplasia dependent on calendar year of surveillance.

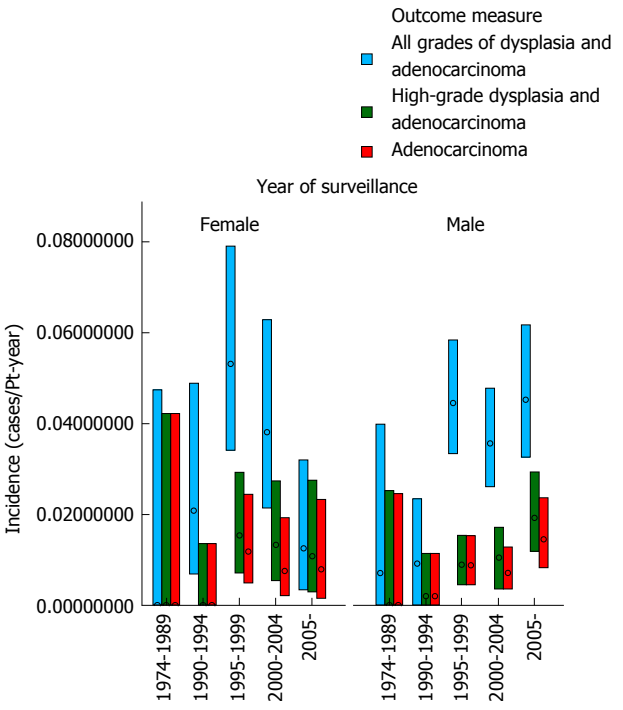

Figure 2 Annual incidence of adenocarcinoma and dysplasia dependent on calendar year of surveillance and gender.

risk of development of dysplasia and adenocarcinoma remains constant during the course of an individual patient's surveillance are shown in Table 3 and Figures 5 and 6. These demonstrate that there is a trend for increasing risk of development of high-grade dysplasia and adenocarcinoma with increasing duration of surveillance, but not of development of low-grade dysplasia. When males and females were analyzed separately, there did not appear to be a significant effect of gender on the results.

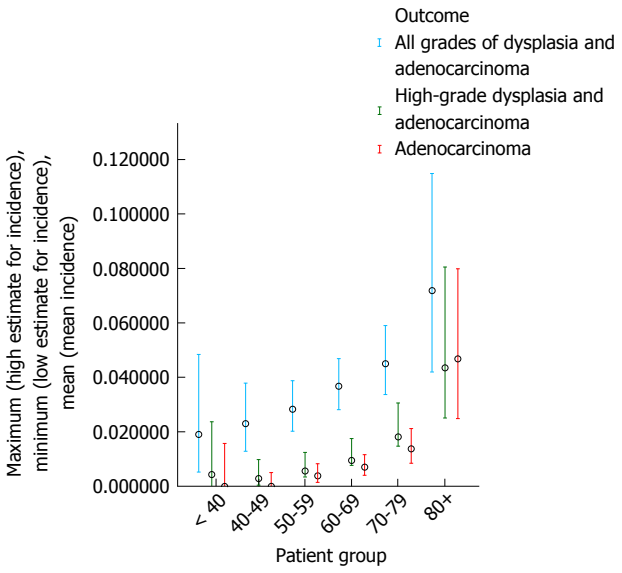

Figure 3 Annual incidence of adenocarcinoma and dysplasia dependent on age at surveillance.

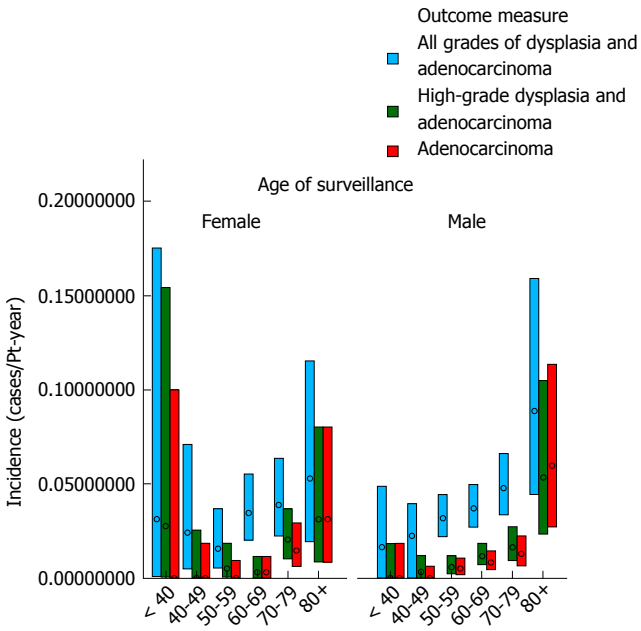

Figure 4 Annual incidence of adenocarcinoma and dysplasia dependent on age at surveillance and gender.

Logistic regression analyses

Initially all 7 variables were included in the logistic regression analyses. Gender was excluded as there was no association with the outcome measures and year at surveillance and duration of surveillance were also excluded as the association between the outcome variables was strongest with age at surveillance. For the outcome variables of high-grade dysplasia and adenocarcinoma: the final model showed that the statistically significantly associated factors were age at surveillance, segment length and previous histological biopsy grading. For the development of all grades of

Table 2 Annual incidence of adenocarcinoma and dysplasia dependent on age at surveillance

Age at surveillance	Total patient-years follow-up	Adenocarcinoma		High-grade dysplasia and adenocarcinoma		All grades of dysplasia and adenocarcinoma	
		Number of cases	Annual incidence	Number of cases	Annual incidence	Number of cases	Annual incidence
< 40	236	0	0.00%	1	0.42%	4	1.89%
40-49	740	0	0.00%	2	0.27%	15	2.30%
50-59	1602	6	0.37%	9	0.56%	39	2.82%
60-69	2154	15	0.70%	20	0.94%	63	3.66%
70-79	1464	20	1.37%	26	1.81%	52	4.49%
80+	278	13	4.67%	12	4.34%	17	7.18%
Total	6474	54	0.83%	70	1.09%	190	3.54%

Table 3 Annual incidence of adenocarcinoma and dysplasia dependent on duration of surveillance

Duration of surveillance (yr)	Total patient-years follow-up	Adenocarcinoma		High-grade dysplasia and adenocarcinoma		All grades of dysplasia and adenocarcinoma	
		Number of cases	Annual incidence	Number of cases	Annual incidence	Number of cases	Annual incidence
1-2	1848	14	0.76%	19	1.04%	72	4.47%
3-4	1235	9	0.73%	11	0.90%	41	4.02%
5-9	1587	20	1.26%	27	1.72%	54	4.47%
10+	668	11	1.65%	13	1.96%	23	4.52%

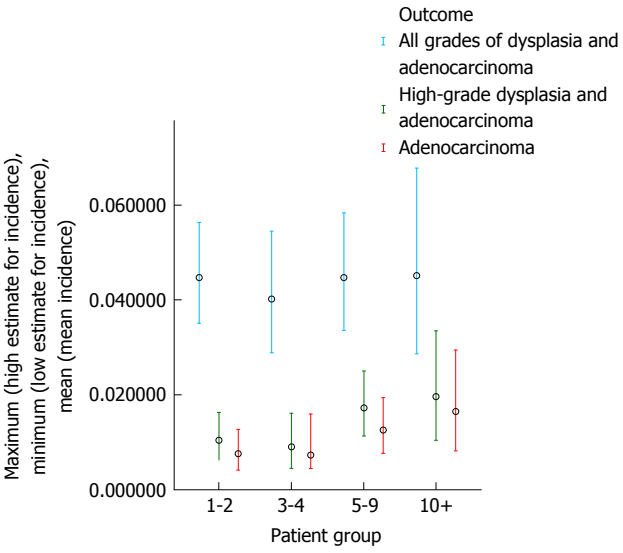

Figure 5 Annual incidence of adenocarcinoma and dysplasia dependent on duration of surveillance.

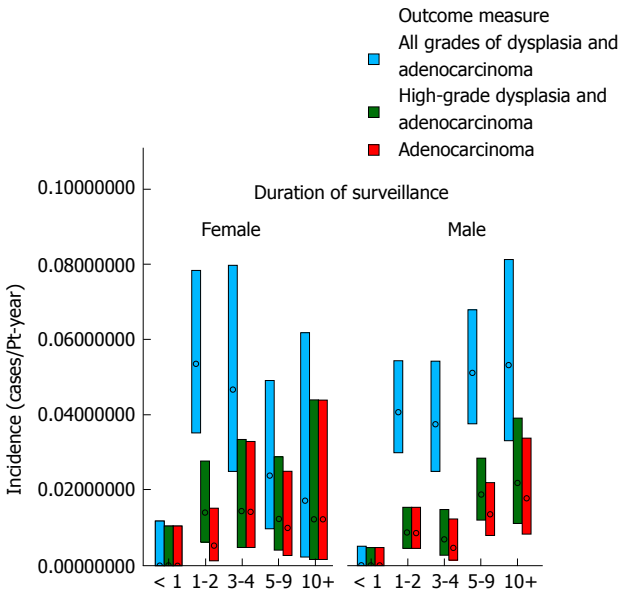

Figure 6 Annual incidence of adenocarcinoma and dysplasia dependent on duration of surveillance and gender.

dysplasia and cancer: segment length and the findings at the first two biopsies reached statistical significance. These results are shown in Table 4.

DISCUSSION

These results demonstrate that there was no clear evidence of any change in overall adenocarcinoma or dysplasia incidence throughout the cohort period nor for the risk of development of all grades of dysplasia to change with increasing duration of follow-up. The risk of development of high-grade dysplasia and adenocarcinoma tended to increase with increased duration of surveillance, but more strikingly: there was a relationship between older age at surveillance and higher rate of detection of high-grade dysplasia and adenocarcinoma.

These observations demonstrate that the metaplastic segment does not remain stable with prolonged follow-up: the rate of development of all grades of dysplasia remained around 3.5% per annum and there was a trend for increased risk of high-grade dysplasia and adenocarcinoma development at increased duration of follow-up in common with the Dutch cohort[7].

From the logistic regression, the most significant time-dependent factor is age at surveillance (with increasing risks of development of high-grade dysplasia or cancer at older age).

Subsequently, this analysis has not demonstrated

Table 4 Logistic regression results

Factor	Development of all grades of dysplasia and cancer	Development of high-grade dysplasia or cancer	Development of cancer
Age	$P = 0.187$	$P = 0.400$	$P = 0.034$
Segment length	$P = 0.043$	$P = 0.121$	$P = 0.129$
Most recent biopsy results		$P = 0.058$	$P = 0.047$
First two biopsy results	$P < 0.001$		

that after a prolonged period of stability there are patients who might be safely discharged from surveillance.

This observation would suggest that younger patients could undergo less intensive surveillance than older patients (with respect to development of high-grade dysplasia or adenocarcinoma), however younger patients potentially have longer periods of exposure to the risk of developing these changes and conceivably an increased lifetime risk[13]. These observations parallel those on esophageal cancer incidence in the United Kingdom population[14]. We have also observed that the average age at diagnosis of Barrett's esophagus is falling[9], which, whilst this may subsequently result in a reduction in the annual incidence of adenocarcinoma for the surveillance cohort overall, it will not result in any risk reduction for an individual patient[5].

Overall, females and males had similar risk of development of dysplasia and cancer in this cohort, however it is likely that this is in part due to the larger proportion of females undergoing surveillance at older age compared to males and that the risk for females when age-matched is lower than in males. Similar results were shown in the Dutch national cohort[7]. With higher life expectancy for females, this is an important consideration and this study does not provide data to support the suggestion that females might be safely discharged from surveillance. The Dutch cohort also confirmed the high cancer risk in older females[7]. Different proportions of older patients undergoing surveillance may explain some of the variability seen in the incidence of high-grade dysplasia and adenocarcinoma in published studies.

The risk for any individual patient will depend on a number of factors, some of which are modifiable and others which cannot be modified[15]. Caucasian ethnic origin[16], male gender[6,8,17], older age at diagnosis[8,18,19], longer Barrett's segment length[3,17,20,21] and the presence of dysplasia at diagnosis[6,8,20,22] have each been reported to be markers of higher malignant risk determined at the time of diagnosis. Longer duration of surveillance was also associated with increased cancer risk, but without separately examining the age at which surveillance was undertaken[20]. The role of intestinal metaplasia remains controversial[1,6,23-26]. Obesity[17,27,28], optimal reflux control[29-35], diet (poor intake of fruit, vegetables and anti-oxidants)[17,36,37], smoking[17,28,38,39] and medication use (particularly aspirin[40], non-steroidal anti-inflammatory drugs[41-43] and statins[40,44-46]) will influence an individual's risk at the time of diagnosis and subsequently. These modifiable factors should be addressed by public health programs and medical care. Additionally, absence of Helicobacter Pylori infection has been associated with higher prevalence of Barrett's esophagus and dysplasia than in subjects who have had *Helicobacter Pylori* infection[47]. It seems likely that much of the fate of the metaplastic segment is determined prior to the time of diagnosis. Once diagnosed, reflux control, smoking, medication use, diet and control of obesity may be managed, but unless the potentially malignant tissue is either completely resected or ablated, cancer risk in this tissue remains.

Encouraging data on the role of endoscopic ablation of the metaplastic segment have now been published, however long-term follow-up with respect to cancer development is awaited[48-50].

Unfortunately, the incidence of Barrett's esophagus[51-55] and esophageal adenocarcinoma[56-58] are increasing and without improved strategies to reduce the risk of cancer development within the population at risk and the size of this population, the number of cases of esophageal adenocarcinoma is likely to continue to rise[13].

The results of this study demonstrate that age at surveillance is an important factor for high-grade dysplasia and adenocarcinoma development and should be incorporated (with segment length and previous biopsy findings) into risk assessment in Barrett's oesophagus surveillance.

ACKNOWLEDGMENTS

We would like to thank the registrants for enabling the work to take place (see Supplementary Table).

COMMENTS

Background

The age at diagnosis of patients with Barrett's esophagus is falling and that the life expectancy of those diagnosed with Barrett's esophagus is increasing. The Danish pathology registry has demonstrated in their cohort that the adenocarcinoma incidence in Barrett's increases with older age.

Research frontiers

The currently accepted principal risk factors for dysplasia and cancer development in Barrett's oesophagus are presence/absence of intestinal metaplasia, dysplasia and segment length. These are the factors on which guidance for surveillance intervals are determined.

Innovations and breakthroughs

Seek to clarify risk based upon segment length, diagnostic histological findings, patient age and year of surveillance, duration of surveillance and gender.

Applications

This study demonstrates the importance of age as an important risk factor for high-grade dysplasia and adenocarcinoma development and that stabilisation of the epithelium does not reliably occur at long-term follow-up.

Peer-review

Interesting and practical paper, the relation between Barrett's esophagus and adenocarcinoma was very important.

REFERENCES

1 **Yousef F**, Cardwell C, Cantwell MM, Galway K, Johnston BT, Murray L. The incidence of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. *Am J Epidemiol* 2008; **168**: 237-249 [PMID: 18550563 DOI: 10.1093/aje/kwn121]

2 **Sikkema M**, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2010; **8**: 235-244; quiz e32 [PMID: 19850156 DOI: 10.1016/j.cgh.2009.10.010]

3 **Thomas T**, Abrams KR, De Caestecker JS, Robinson RJ. Meta analysis: Cancer risk in Barrett's oesophagus. *Aliment Pharmacol Ther* 2007; **26**: 1465-1477 [PMID: 17900269 DOI: 10.1111/j.1365-2036.2007.03528.x]

4 **Desai TK**, Krishnan K, Samala N, Singh J, Cluley J, Perla S, Howden CW. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut* 2012; **61**: 970-976 [PMID: 21997553 DOI: 10.1136/gutjnl-2011-300730]

5 **Gatenby P**, Caygill C, Wall C, Bhatacharjee S, Ramus J, Watson A, Winslet M. Lifetime risk of esophageal adenocarcinoma in patients with Barrett's esophagus. *World J Gastroenterol* 2014; **20**: 9611-9617 [PMID: 25071359 DOI: 10.3748/wjg.v20.i28.9611]

6 **Bhat S**, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, Murray LJ. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. *J Natl Cancer Inst* 2011; **103**: 1049-1057 [PMID: 21680910 DOI: 10.1093/jnci/djr203]

7 **de Jonge PJ**, van Blankenstein M, Looman CW, Casparie MK, Meijer GA, Kuipers EJ. Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. *Gut* 2010; **59**: 1030-1036 [PMID: 20639249 DOI: 10.1136/gut.2009.176701]

8 **Hvid-Jensen F**, Pedersen L, Drewes AM, Sørensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. *N Engl J Med* 2011; **365**: 1375-1383 [PMID: 21995385 DOI: 10.1056/NEJMoa1103042]

9 **Wall CM**, Charlett A, Caygill CP, Gatenby PA, Ramus JR, Winslet MC, Watson A. Are newly diagnosed columnar-lined oesophagus patients getting younger? *Eur J Gastroenterol Hepatol* 2009; **21**: 1127-1131 [PMID: 19295439 DOI: 10.1097/MEG.0b013e328329c414]

10 **Fitzgerald RC**, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, Trudgill N, Patel P, Kaye PV, Sanders S, O'Donovan M, Bird-Lieberman E, Bhandari P, Jankowski JA, Attwood S, Parsons SL, Loft D, Lagergren J, Moayyedi P, Lyratzopoulos G, de Caestecker J. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut* 2014; **63**: 7-42 [PMID: 24165758 DOI: 10.1136/gutjnl-2013-305372]

11 **Spechler SJ**, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. *Gastroenterology* 2011; **140**: 1084-1091 [PMID: 21376940 DOI: 10.1053/j.gastro.2011.01.031]

12 **Wang KK**, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 788-797 [PMID: 18341497 DOI: 10.1111/j.1572-0241.2008.01835.x]

13 **Gatenby PA**, Hainsworth A, Caygill C, Watson A, Winslet M. Projections for oesophageal cancer incidence in England to 2033. *Eur J Cancer Prev* 2011; **20**: 283-286 [PMID: 21422931 DOI: 10.1097/CEJ.0b013e32834572d2]

14 Office for National Statistics: Cancer Registrations in England, 2010. In: Office for National Statistics. Newport, Wales, UK: UK Statistics Authority, 2012

15 **Gatenby P**, Soon Y. Barrett's oesophagus: Evidence from the current meta-analyses. *World J Gastrointest Pathophysiol* 2014; **5**: 178-187 [PMID: 25133020 DOI: 10.4291/wjgp.v5.i3.178]

16 **Khoury JE**, Chisholm S, Jamal MM, Palacio C, Pudhota S, Vega KJ. African Americans with Barrett's esophagus are less likely to have dysplasia at biopsy. *Dig Dis Sci* 2012; **57**: 419-423 [PMID: 21909989 DOI: 10.1007/s10620-011-1900-y]

17 **Pohl H**, Wrobel K, Bojarski C, Voderholzer W, Sonnenberg A, Rösch T, Baumgart DC. Risk factors in the development of esophageal adenocarcinoma. *Am J Gastroenterol* 2013; **108**: 200-207 [PMID: 23247577 DOI: 10.1038/ajg.2012.387]

18 **den Hoed CM**, van Blankenstein M, Dees J, Kuipers EJ. The minimal incubation period from the onset of Barrett's oesophagus to symptomatic adenocarcinoma. *Br J Cancer* 2011; **105**: 200-205 [PMID: 21673678 DOI: 10.1038/bjc.2011.214]

19 **Gatenby PA**, Caygill CP, Ramus JR, Charlett A, Watson A. Barrett's columnar-lined oesophagus: demographic and lifestyle associations and adenocarcinoma risk. *Dig Dis Sci* 2008; **53**: 1175-1185 [PMID: 17939050 DOI: 10.1007/s10620-007-0023-y]

20 **Sikkema M**, Looman CW, Steyerberg EW, Kerkhof M, Kastelein F, van Dekken H, van Vuuren AJ, Bode WA, van der Valk H, Ouwendijk RJ, Giard R, Lesterhuis W, Heinhuis R, Klinkenberg EC, Meijer GA, ter Borg F, Arends JW, Kolkman JJ, van Baarlen J, de Vries RA, Mulder AH, van Tilburg AJ, Offerhaus GJ, ten Kate FJ, Kusters JG, Kuipers EJ, Siersema PD. Predictors for neoplastic progression in patients with Barrett's Esophagus: a prospective cohort study. *Am J Gastroenterol* 2011; **106**: 1231-1238 [PMID: 21577245 DOI: 10.1038/ajg.2011.153]

21 **Gatenby PA**, Caygill CP, Ramus JR, Charlett A, Fitzgerald RC, Watson A. Short segment columnar-lined oesophagus: an underestimated cancer risk? A large cohort study of the relationship between Barrett's columnar-lined oesophagus segment length and adenocarcinoma risk. *Eur J Gastroenterol Hepatol* 2007; **19**: 969-975 [PMID: 18049166 DOI: 10.1097/MEG.0b013e3282c3aa14]

22 **Gatenby P**, Ramus J, Caygill C, Shepherd N, Winslet M, Watson A. Routinely diagnosed low-grade dysplasia in Barrett's oesophagus: a population-based study of natural history. *Histopathology* 2009; **54**: 814-819 [PMID: 19635100 DOI: 10.1111/j.1365-2559.2009.03316.x]

23 **Kelty C**, Gough M, Van Wyck Q, Stephenson T, Ackroyd R: Barrett's oesophagus: Intestinal metaplasia is not essential for cancer risk. *Scand J Gastroenterol* 2007; **21**: 1-4 [PMID: 17852872 DOI: 10.1080/00365520701420735]

24 **Gatenby PA**, Ramus JR, Caygill CP, Shepherd NA, Watson A. Relevance of the detection of intestinal metaplasia in non-dysplastic columnar-lined oesophagus. *Scand J Gastroenterol* 2008; **43**: 524-530 [PMID: 18415743 DOI: 10.1080/00365520701879831]

25 **Dias Pereira A**, Chaves P. Columnar-lined oesophagus without intestinal metaplasia: results from a cohort with a mean follow-up of 7 years. *Aliment Pharmacol Ther* 2012; **36**: 282-289 [PMID: 22670705 DOI: 10.1111/j.1365-2036.2012.05170.x]

26 **Westerhoff M**, Hovan L, Lee C, Hart J. Effects of dropping the requirement for goblet cells from the diagnosis of Barrett's esophagus. *Clin Gastroenterol Hepatol* 2012; **10**: 1232-1236 [PMID: 22642957 DOI: 10.1016/j.cgh.2012.05.013]

27 **Duggan C**, Onstad L, Hardikar S, Blount PL, Reid BJ, Vaughan TL. Association between markers of obesity and progression from Barrett's esophagus to esophageal adenocarcinoma. *Clin Gastroenterol Hepatol* 2013; **11**: 934-943 [PMID: 23466711 DOI:

10.1016/j.cgh.2013.02.017]
28 **Hardikar S**, Onstad L, Blount PL, Odze RD, Reid BJ, Vaughan TL. The role of tobacco, alcohol, and obesity in neoplastic progression to esophageal adenocarcinoma: a prospective study of Barrett's esophagus. *PLoS One* 2013; **8**: e52192 [PMID: 23300966 DOI: 10.1371/journal.pone.0052192]
29 **Braghetto I**, Korn O, Valladares H, Debandi A, Díaz JC, Brunet L. Laparoscopic surgical treatment for patients with short- and long-segment Barrett's esophagus: which technique in which patient? *Int Surg* 2011; **96**: 95-103 [PMID: 22026298]
30 **Zaninotto G**, Parente P, Salvador R, Farinati F, Tieppo C, Passuello N, Zanatta L, Fassan M, Cavallin F, Costantini M, Mescoli C, Battaglia G, Ruol A, Ancona E, Rugge M. Long-term follow-up of Barrett's epithelium: medical versus antireflux surgical therapy. *J Gastrointest Surg* 2012; **16**: 7-14; discussion 14-15 [PMID: 22086718 DOI: 10.1007/s11605-011-1739-8]
31 **Li YM**, Li L, Yu CH, Liu YS, Xu CF. A systematic review and meta-analysis of the treatment for Barrett's esophagus. *Dig Dis Sci* 2008; **53**: 2837-2846 [PMID: 18427992 DOI: 10.1007/s10620-008-0257-3]
32 **Corey KE**, Schmitz SM, Shaheen NJ. Does a surgical antireflux procedure decrease the incidence of esophageal adenocarcinoma in Barrett's esophagus? A meta-analysis. *Am J Gastroenterol* 2003; **98**: 2390-2394 [PMID: 14638338 DOI: 10.1111/j.1572-0241.2003.08702.x]
33 **Rees JR**, Lao-Sirieix P, Wong A, Fitzgerald RC: Treatment for Barrett's oesophagus. *Cochrane Database Syst Rev* 2010; (**1**): CD004060 [PMID: 20091557 DOI: 10.1002/14651858.cd004060.pub2]
34 **Gatenby PA**, Ramus JR, Caygill CP, Charlett A, Winslet MC, Watson A. Treatment modality and risk of development of dysplasia and adenocarcinoma in columnar-lined esophagus. *Dis Esophagus* 2009; **22**: 133-142 [PMID: 19018855 DOI: 10.1111/j.1442-2050.2008.00886.x]
35 **Kastelein F**, Spaander MC, Steyerberg EW, Biermann K, Valkhoff VE, Kuipers EJ, Bruno MJ. Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2013; **11**: 382-388 [PMID: 23200977 DOI: 10.1016/j.cgh.2012.11.014]
36 **Lukić M**, Segec A, Segec I, Pinotić L, Pinotić K, Atalić B, Solić K, Vcev A. The impact of the vitamins A, C and E in the prevention of gastroesophageal reflux disease, Barrett's oesophagus and oesophageal adenocarcinoma. *Coll Antropol* 2012; **36**: 867-872 [PMID: 23213946]
37 **Ibiebele TI**, Hughes MC, Nagle CM, Bain CJ, Whiteman DC, Webb PM. Dietary antioxidants and risk of Barrett's esophagus and adenocarcinoma of the esophagus in an Australian population. *Int J Cancer* 2013; **133**: 214-224 [PMID: 23292980 DOI: 10.1002/ijc.28016]
38 **Coleman HG**, Bhat S, Johnston BT, McManus D, Gavin AT, Murray LJ. Tobacco smoking increases the risk of high-grade dysplasia and cancer among patients with Barrett's esophagus. *Gastroenterology* 2012; **142**: 233-240 [PMID: 22062359 DOI: 10.1053/j.gastro.2011.10.034]
39 **Ramus JR**, Gatenby PA, Caygill CP, Watson A, Winslet MC. The relationship between smoking and severe dysplastic disease in patients with Barrett's columnar-lined oesophagus. *Eur J Cancer Prev* 2012; **21**: 507-510 [PMID: 22433630 DOI: 10.1097/CEJ.0b013e328350b06f]
40 **Beales IL**, Vardi I, Dearman L. Regular statin and aspirin use in patients with Barrett's oesophagus is associated with a reduced incidence of oesophageal adenocarcinoma. *Eur J Gastroenterol Hepatol* 2012; **24**: 917-923 [PMID: 22569083 DOI: 10.1097/MEG.0b013e3283543f01]
41 **Wang F**, Lv ZS, Fu YK. Nonsteroidal anti-inflammatory drugs and esophageal inflammation - Barrett's esophagus - adenocarcinoma sequence: a meta-analysis. *Dis Esophagus* 2011; **24**: 318-324 [PMID: 21166737 DOI: 10.1111/j.1442-2050.2010.01153.x]
42 **Liao LM**, Vaughan TL, Corley DA, Cook MB, Casson AG, Kamangar F, Abnet CC, Risch HA, Giffen C, Freedman ND, Chow WH, Sadeghi S, Pandeya N, Whiteman DC, Murray LJ, Bernstein L, Gammon MD, Wu AH. Nonsteroidal anti-inflammatory drug use reduces risk of adenocarcinomas of the esophagus and esophagogastric junction in a pooled analysis. *Gastroenterology* 2012; **142**: 442-452.e5; quiz e22-23 [PMID: 22108196 DOI: 10.1053/j.gastro.2011.11.019]
43 **Thrift AP**, Pandeya N, Smith KJ, Green AC, Webb PM, Whiteman DC. The use of nonsteroidal anti-inflammatory drugs and the risk of Barrett's oesophagus. *Aliment Pharmacol Ther* 2011; **34**: 1235-1244 [PMID: 21967506 DOI: 10.1111/j.1365-2036.2011.04855.x]
44 **Kantor ED**, Onstad L, Blount PL, Reid BJ, Vaughan TL. Use of statin medications and risk of esophageal adenocarcinoma in persons with Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2012; **21**: 456-461 [PMID: 22241250 DOI: 10.1158/1055-9965.EPI-11-1014]
45 **Alexandre L**, Clark AB, Cheong E, Lewis MP, Hart AR. Systematic review: potential preventive effects of statins against oesophageal adenocarcinoma. *Aliment Pharmacol Ther* 2012; **36**: 301-311 [PMID: 22716127 DOI: 10.1111/j.1365-2036.2012.05194.x]
46 **Singh S**, Singh AG, Singh PP, Murad MH, Iyer PG. Statins are associated with reduced risk of esophageal cancer, particularly in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 620-629 [PMID: 23357487 DOI: 10.1016/j.cgh.2012.12.036]
47 **Thrift AP**, Pandeya N, Smith KJ, Green AC, Hayward NK, Webb PM, Whiteman DC. Helicobacter pylori infection and the risks of Barrett's oesophagus: a population-based case-control study. *Int J Cancer* 2012; **130**: 2407-2416 [PMID: 21681741 DOI: 10.1002/ijc.26242]
48 **Phoa KN**, van Vilsteren FG, Weusten BL, Bisschops R, Schoon EJ, Ragunath K, Fullarton G, Di Pietro M, Ravi N, Visser M, Offerhaus GJ, Seldenrijk CA, Meijer SL, ten Kate FJ, Tijssen JG, Bergman JJ. Radiofrequency ablation vs endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia: a randomized clinical trial. *JAMA* 2014; **311**: 1209-1217 [PMID: 24668102 DOI: 10.1001/jama.2014.2511]
49 **Shaheen NJ**, Sharma P, Overholt BF, Wolfsen HC, Sampliner RE, Wang KK, Galanko JA, Bronner MP, Goldblum JR, Bennett AE, Jobe BA, Eisen GM, Fennerty MB, Hunter JG, Fleischer DE, Sharma VK, Hawes RH, Hoffman BJ, Rothstein RI, Gordon SR, Mashimo H, Chang KJ, Muthusamy VR, Edmundowicz SA, Spechler SJ, Siddiqui AA, Souza RF, Infantolino A, Falk GW, Kimmey MB, Madanick RD, Chak A, Lightdale CJ. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N Engl J Med* 2009; **360**: 2277-2288 [PMID: 19474425 DOI: 10.1056/NEJMoa0808145]
50 **Fleischer DE**, Overholt BF, Sharma VK, Reymunde A, Kimmey MB, Chuttani R, Chang KJ, Muthasamy R, Lightdale CJ, Santiago N, Pleskow DK, Dean PJ, Wang KK. Endoscopic radiofrequency ablation for Barrett's esophagus: 5-year outcomes from a prospective multicenter trial. *Endoscopy* 2010; **42**: 781-789 [PMID: 20857372 DOI: 10.1055/s-0030-1255779]
51 **Corley DA**, Kubo A, Levin TR, Block G, Habel L, Rumore G, Quesenberry C, Buffler P. Race, ethnicity, sex and temporal differences in Barrett's oesophagus diagnosis: a large community-based study, 1994-2006. *Gut* 2009; **58**: 182-188 [PMID: 18978173 DOI: 10.1136/gut.2008.163360]
52 **Caygill CP**, Reed PI, Johnston BJ, Hill MJ, Ali MH, Levi S. A single centre's 20 years' experience of columnar-lined (Barrett's) oesophagus diagnosis. *Eur J Gastroenterol Hepatol* 1999; **11**: 1355-1358 [PMID: 10654794 DOI: 10.1097/00042737-199912000-00003]
53 **Coleman HG**, Bhat S, Murray LJ, McManus D, Gavin AT, Johnston BT. Increasing incidence of Barrett's oesophagus: a population-based study. *Eur J Epidemiol* 2011; **26**: 739-745 [PMID: 21671079 DOI: 10.1007/s10654-011-9596-z]
54 **van Soest EM**, Dieleman JP, Siersema PD, Sturkenboom MC, Kuipers EJ. Increasing incidence of Barrett's oesophagus in the general population. *Gut* 2005; **54**: 1062-1066 [PMID: 15857935

DOI: 10.1136/gut.2004.063685]

55 **Alexandropoulou K**, van Vlymen J, Reid F, Poullis A, Kang JY. Temporal trends of Barrett's oesophagus and gastro-oesophageal reflux and related oesophageal cancer over a 10-year period in England and Wales and associated proton pump inhibitor and H2RA prescriptions: a GPRD study. *Eur J Gastroenterol Hepatol* 2013; **25**: 15-21 [PMID: 23022985 DOI: 10.1097/MEG.0b013e3283595086]

56 **Lepage C**, Rachet B, Jooste V, Faivre J, Coleman MP. Continuing rapid increase in esophageal adenocarcinoma in England and Wales. *Am J Gastroenterol* 2008; **103**: 2694-2699 [PMID: 18853967 DOI: 10.1111/j.1572-0241.2008.02191.x]

57 **Pohl H**, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst* 2005; **97**: 142-146 [PMID: 15657344 DOI: 10.1093/jnci/dji181]

58 **Dikken JL**, Lemmens VE, Wouters MW, Wijnhoven BP, Siersema PD, Nieuwenhuijzen GA, van Sandick JW, Cats A, Verheij M, Coebergh JW, van de Velde CJ. Increased incidence and survival for oesophageal cancer but not for gastric cardia cancer in the Netherlands. *Eur J Cancer* 2012; **48**: 1624-1632 [PMID: 22317953 DOI: 10.1016/j.ejca.2012.01.009]

P- Reviewer: Ono T, Stasek M **S- Editor**: Yu J **L- Editor**: A
E- Editor: Zhang FF

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.v22.i48.10601

World J Gastroenterol 2016 December 28; 22(48): 10601-10608
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Clinical significance of mesenteric panniculitis-like abnormalities on abdominal computerized tomography in patients with malignant neoplasms

Eli D Ehrenpreis, Grigory Roginsky, Richard M Gore

Eli D Ehrenpreis, Grigory Roginsky, University of Chicago, Center for the Study of Complex Diseases, Gastroenterology, NorthShore University HealthSystem, Highland Park Hospital, Highland Park, IL 60035, United States

Richard M Gore, Department of Radiology, NorthShore University HealthSystem, Evanston, IL 60201, United States

Author contributions: Ehrenpreis ED contributed to conception and design of the study, data acquisition, analysis and interpretation of data, drafting the article, making critical revisions related to important intellectual content of the manuscript and final approval of the version of the article to be published; Roginsky G contributed to data acquisition, analysis and interpretation of data, drafting the article, making critical revisions and final approval of the version of the article to be published; Gore RM contributed to data acquisition, analysis and interpretation of data, drafting the article and final approval of the version of the article to be published.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest related to this study.

Supported by An unrestricted grant from the Keyser Family Fund (partly).

Institutional review board statement: This study was approved by the Institutional Review Board of NorthShore University HealthSystem.

Informed consent statement: Informed consent for this retrospective study was waived by the Institutional Review Board.

Data sharing statement: No additional data is available.

Manuscript source: Invited manuscript

Correspondence to: Eli D Ehrenpreis, MD, Associate Professor of Clinical Medicine, Director, Staff Physician, University of Chicago, Center for the Study of Complex Diseases, Gastroenterology, NorthShore University HealthSystem, Highland Park Hospital, 777 Park Avenue West, Highland Park, IL 60035, United States. ehrenpreis@gipharm.net
Telephone: +1-11-8476571900

Received: October 14, 2016
Peer-review started: October 16, 2016
First decision: October 28, 2016
Revised: November 14, 2016
Accepted: November 23, 2016
Article in press: November 28, 2016
Published online: December 28, 2016

Abstract

AIM

To clarify the association of malignancy with mesenteric panniculitis-like changes on computed tomography (CT).

METHODS

All abdominal CT scans performed at NorthShore University HealthSystem showing mesenteric panniculitis from January 2005 to August 2010 were identified in the Radnet (RadNet Corporation, Los Angeles, CA) database. Patients with a new or known diagnosis of a malignancy were included for this analysis. Longitudinal clinical histories were obtained from electronic medical records.

RESULTS

In total, 147794 abdominal CT scans were performed during the study period. Three hundred and fifty-nine patients had mesenteric panniculitis (MP)-like abnormalities on their abdominal CT. Of these patients, 81 patients (22.6%) had a known history of cancer at the time of their CT scan. Nineteen (5.3%) had a new diagnosis of cancer in concurrence with their CT, but the majority of these (14/19, 74%) were undergoing CT as part of a malignancy evaluation. Lymphomas were the most common cancers associated with MP-like findings on CT (36 cases, 36%), with follicular lymphoma being the most frequent subtype (17/36). A variety of solid tumors, most commonly prostate (7) and renal cell cancers (6) also were seen. CT follow up was obtained in 56 patients. Findings in the mesentery were unchanged in 45 (80%), worsened in 6 (11%), and improved in 5 patients (9%). Positron emission tomography (PET) scans performed in 44 patients only showed a positive uptake in the mesenteric mass in 2 patients (5%).

CONCLUSION

A new diagnosis of cancer is uncommon in patients with CT findings suggestive of MP. MP-like mesenteric abnormalities on CT generally remain stable in patients with associated malignancies. PET scanning is not recommended in the evaluation of patients with mesenteric panniculitis-like findings on CT.

Key words: Panniculitis; Peritoneal; X ray; Neoplasms; Computed tomography; Small intestine; Misty mesentery; Lymphoma; Tomography; Positron emission tomography

Core tip: The most important question when a patient has new mesenteric panniculitis - like findings on abdominal computed tomography (CT) scan is the likelihood that a malignancy is present. Based on our study, a first diagnosis of cancer occurs in approximately 5% and is unexpected diagnosis in 1.4% of these patients. Lymphomas are the most likely malignancies. A careful clinical evaluation for lymphoma as well as solid tumors is advised. Follow up is also important, as an additional 5% of patients will eventually be diagnosed with a malignancy. In patients with a malignancy, CT findings are likely to remain stable, and positron emission tomography scanning is not advised.

Ehrenpreis ED, Roginsky G, Gore RM. Clinical significance of mesenteric panniculitis-like abnormalities on abdominal computerized tomography in patients with malignant neoplasms. *World J Gastroenterol* 2016; 22(48): 10601-10608 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10601.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10601

INTRODUCTION

The computed tomography (CT) finding of mesenteric panniculitis (MP) (defined as a "misty" or "hazy" mesentery and/or an unexplained mesenteric mass), is uncommon. When truly defined by mesenteric biopsy, MP is a rare disease characterized by unexplained inflammation of the mesentery[1-3]. A variety of conditions are associated with MP including abdominal trauma[4], lymphoma and other neoplasms[5,6] and autoimmune diseases[7-9]. Infectious associations with MP include mycobacterial[10] and cryptococcal[11] infections and cholera[12]. In some patients, especially those having an acute presentation of the disease, a viral mesenteritis is likely. Fever of unknown origin[13] and chylous ascites have also been described in patients with MP. CT imaging of the abdomen in these patients demonstrates a mass of the mesentery, characterized by fat enhancement and multiple soft tissue nodules along the root of the small bowel without vascular compromise; these are distinguishing radiographic features of MP. Histologically, MP is characterized by chronic inflammation, fat necrosis and fibrosis within the connective tissue of the mesentery[14-16].

CT findings suggestive of MP can be concurrent with a previously unidentified underlying malignancy[3]. Other patients with a known diagnosis of cancer will develop new mesenteric abnormalities on CT, raising the specter of mesenteric metastasis. The development of MP on CT imaging currently has an unclear influence on the natural history of cancer in these patients. The types of cancer likely to occur in the setting of MP also requires further exploration. We herein describe a group of patients with the CT finding of MP and the diagnosis of malignancy, in an attempt to clarify the clinical significance of the simultaneous presence of these two conditions in the same patient.

MATERIALS AND METHODS

A query of the, Radnet database (RadNet Corporation, Los Angeles, CA, United States) of the NorthShore University HealthSystem, (comprised of 4 hospitals in the Northern suburbs of Chicago, Illinois, United States) was performed. The terms used in the query were "mesenteric panniculitis" or "misty mesentery". All charts of patients with these radiographic findings between January 1st 2005 and April 30th 2010 were reviewed. Some of these patients have been previously described[3]. Only patients that were undergoing a CT for follow up of a known cancer and patients with a new cancer diagnosed after the finding of the mesenteric abnormality were included in this analysis. Findings of follow up CT scans were evaluated. Results of mesenteric biopsies and positron emission tomography (PET) scanning were also noted.

Table 1 Cancer types in patients with computed tomography findings of mesenteric panniculitis

Total patients	*n* = 100
Lymphomas	*n* = 36
Follicular	17
Diffuse large B cell	9
Small lymphocytic	3
Mantle cell	2
T cell	2
Other (Burkitt, Hodgkin, Marginal zone B cell)	3
Solid tumors	*n* = 64
Prostate	7
Renal cell	6
Lung	6
Chronic lymphocytic leukemia	5
Bladder	5
Endometrial	5
Breast	4
Colon	3
Rectal	3
Other	20

Table 2 Cancer subtypes in 19 patients with new malignancy and mesenteric panniculitis

6	Follicular lymphoma
2	T cell lymphoma
2	Endometrial cancer
1	Acute myeloid leukemia
1	Diffuse large B cell lymphoma
1	Waldenstrom's macroglobulinemia
1	Esophageal cancer
1	Small cell lung cancer
1	Lung adenocarcinoma, with diffuse metastases
1	Intraductal papillary mucinous neoplasm
1	Bladder cancer
1	Melanoma, metastases to small bowel

RESULTS

In total, 147794 CT scans reviewed. Within this group, there were 359 patients with MP-like CT abnormalities. Of these patients 19 (5.3%) had a new diagnosis of cancer. Eighty-one patients (22.6%) had a known previous history of cancer. Lymphomas were the most common cancers associated with MP (36 patients, 36%). Follicular lymphoma was the most common lymphoma subtype and was found in 17 patients. A variety of solid tumors were found, the most common being prostate (7), lung, and renal cell cancer (6). For additional details of these findings, see Table 1. Tables 2 and 3 show the distribution of cancers in patients with a new diagnosis and a known diagnosis of cancer at the time of their CT scan, respectively. Figures 1-3 show representative examples of CT imaging in patients with lymphoma and MP-like findings.

Fourteen of the 19 patients with newly diagnosed cancers were undergoing abdominal CT scanning as part of a malignancy workup. The remaining five patients with newly diagnosed cancers underwent abdominal CT scanning for unexplained abdominal pain or other systemic symptoms.

Table 3 Cancer subtypes in 81 patients with known malignancy and mesenteric panniculitis-like findings on computed tomography *n* (%)

Cancer subtype	Number of patients
Lymphomas	27 (33)
Follicular	11 (41)
Diffuse large B cell	6 (30)
Small lymphocytic lymphoma	3 (11)
Mantle cell	2 (7)
Burkitt's, Hodgkin's, marginal zone B cell	3 (11)
Solid tumors	45 (67)
Prostate	7 (9)
Renal cell	6 (7)
Chronic lymphocytic leukemia	5 (6)
Lung	5 (6)
Bladder	4 (5)
Breast	4 (5)
Colon	3 (4)
Endometrial	3 (4)
Rectal	3 (4)
Pancreatic	2 (3)
Myelodysplasia	2 (3)
Other - gallbladder, ampullary, chronic myeloid leukemia, gastric, glioma, IPMN, salivary gland adenocarcinoma, small bowel carcinoid, neuroendocrine tumor	10 (12) total

IPMN: Intraductal papillary mucinous neoplasm.

The majority of the patients with a known history of malignancy underwent CT scanning for staging and tumor follow up (67/81, 83%).

Follow up CT scans were performed in 56 patients. No change in mesenteric abnormalities was seen in the majority of these, (45 patients, 80%). An increase in size of the mesenteric abnormality was seen in 6 patients (11%), and a decrease in size of the mesenteric abnormality was seen in 5 patients on follow up (9%). Figures 4 and 5 show follow up CT scans in patients with non-Hodgkins lymphoma.

Forty-four patients underwent PET scans. Only 2 (5%) of these patients, both with stage IV-B diffuse large B cell lymphoma, had positive uptake in the mesenteric mass. Both of these patients had resolution of this finding on repeat PET scan following treatment. CT and PET findings are shown in two patients with non-Hodgkins lymphoma in Figures 6 and 7.

Five patients underwent a mesenteric biopsy. Four patients had biopsies of mesenteric lymph nodes, all showing lymphoma (two with diffuse large B-cell lymphoma and two with follicular lymphoma). One patient had a biopsy of a mesenteric mass that showed fibrosis and benign mesothelial cells, consistent with mesenteric panniculitis.

DISCUSSION

Biopsy-proven MP is an autoimmune disease, characterized by the histologic findings of fat necrosis and chronic inflammatory infiltrates of the mesentery.

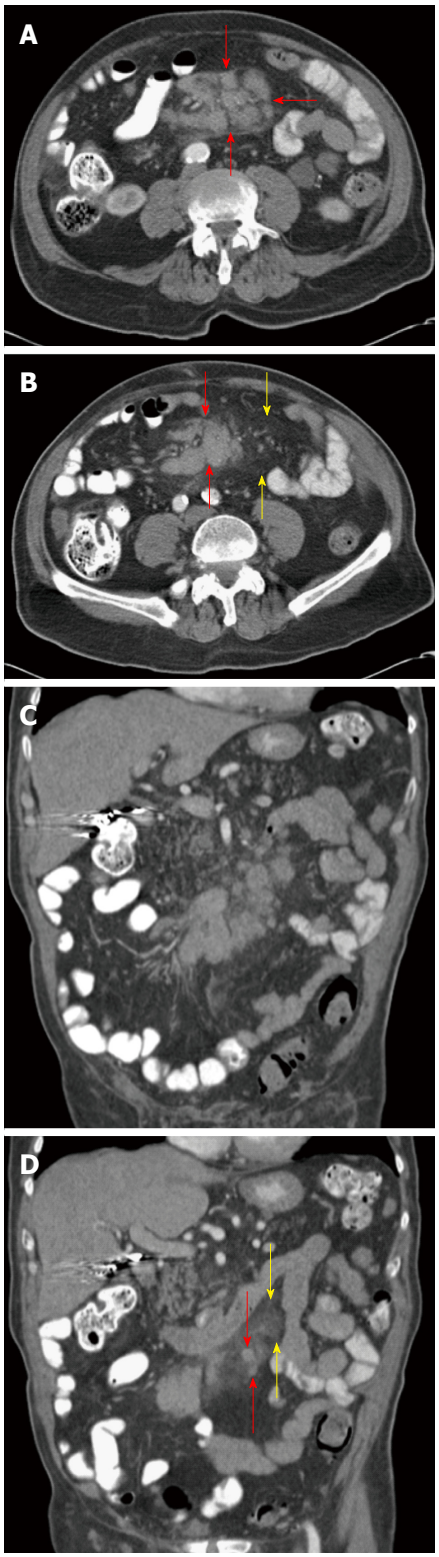

Figure 1 Mesenteric panniculitis in a patient with non-Hodgkin lymphoma. A: Axial computed tomography (CT) scan at the level of the inferior aspect of the kidneys shows extensive mesenteric adenopathy (red arrows); B: Axial CT image obtained more caudally, at the level of the iliac crest reveals mesenteric adenopathy (red arrows) and synchronous mesenteric panniculitis (yellow arrows) characterized by hazy mesenteric fat and separation of the mesenteric vessels; C: Coronal reformatted CT image demonstrates the extensive mesenteric adenopathy (red arrows); D: Coronal reformatted CT image, take dorsal to (C) depicts synchronous mesenteric adenopathy (red arrows) and mesenteric panniculitis (yellow arrows).

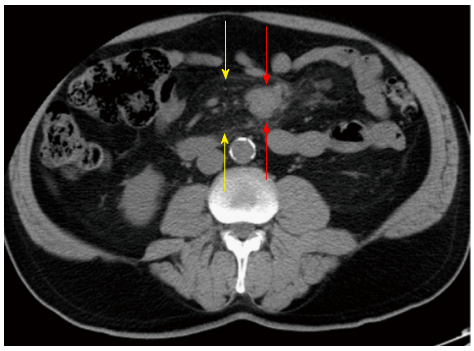

Figure 2 Mesenteric panniculitis in a patient with non-Hodgkin lymphoma. Axial computed tomography scan shows synchronous mesenteric panniculitis (yellow arrows), manifested by fat separating the mesenteric vessels, and mesenteric adenopathy (red arrows).

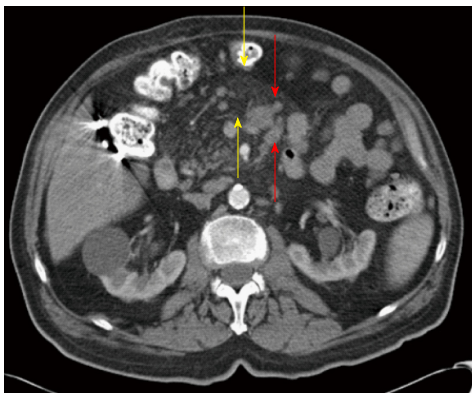

Figure 3 Mesenteric panniculitis in a patient with non-Hodgkin lymphoma. Axial computed tomography image shows synchronous mesenteric adenopathy (red arrows) and mesenteric adenopathy (yellow arrows).

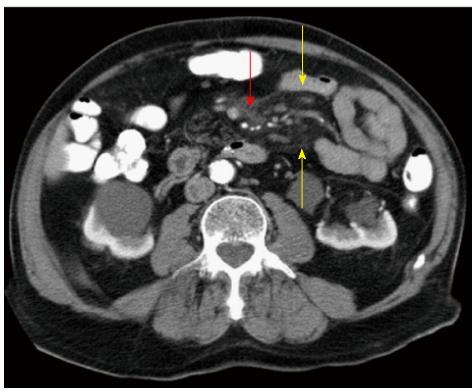

Figure 4 Mesenteric panniculitis in a patient with non-Hodgkin lymphoma (same patient as Figures 3 and 7) following chemotherapy. Axial computed tomography image shows almost complete resolution of the mesenteric adenopathy (red arrow) but persistent mesenteric panniculitis (yellow arrows).

However, the diagnosis of MP is often made empirically based on characteristic radiographic findings, and not all patients with these findings will require a biopsy. It is imperative to fully characterize the clinical significance of MP-like findings on abdominal CT. The risk for the presence of malignancy, in patients

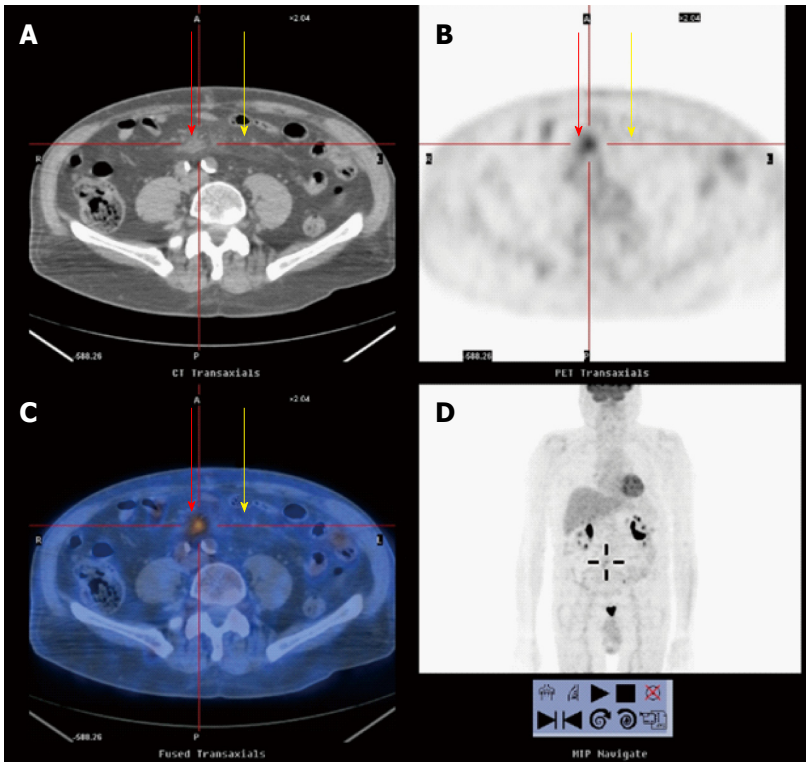

Figure 5 Mesenteric panniculitis in a patient with non-Hodgkin lymphoma. Positron emission tomography-computed tomography (PET-CT) scan following chemotherapy (same patient as Figures 3, 4 and 7). Computed tomography (CT) image (A), PET image (B), fused PET-CT image (C), and whole body PET image (D) demonstrates almost complete resolution of the mesenteric adenopathy (red arrow) with no fludeoxyglucose uptake in the portion of mesentery involved by mesenteric panniculitis (yellow arrows). The disseminated adenopathy seen in Figure 5D has almost completely resolved.

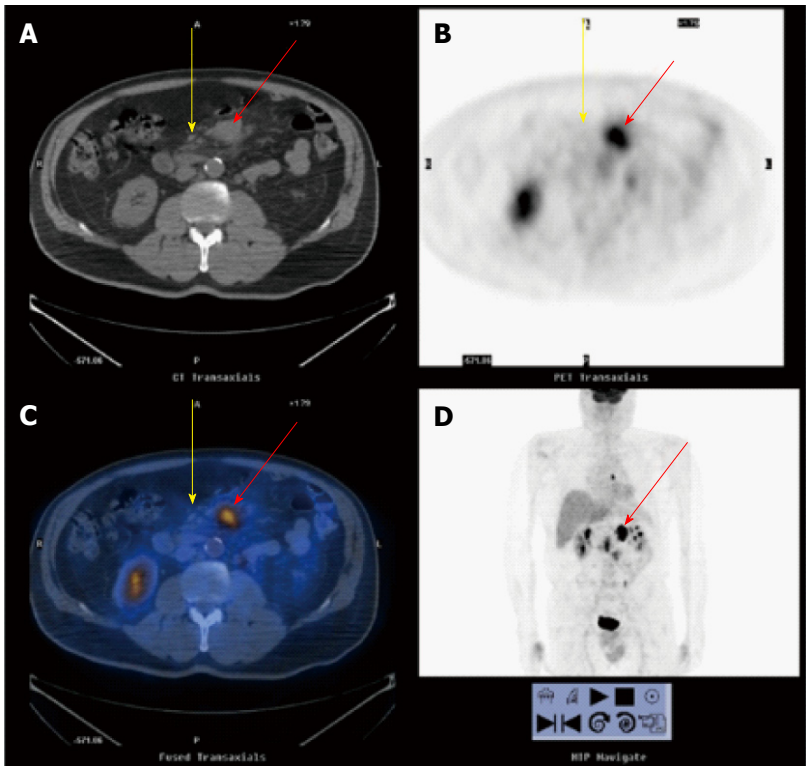

Figure 6 Mesenteric panniculitis in a patient with non-Hodgkin lymphoma (same patient as Figure 2). Positron emission tomography-computed tomography (PET-CT) scan. CT image (A), PET image (B), fused PET-CT image (C), and whole body PET image (D) shows abnormal fludeoxyglucose uptake in the mesenteric lymph nodes containing tumor (red arrows) but not in the portion of mesentery involved by mesenteric panniculitis (yellow arrows).

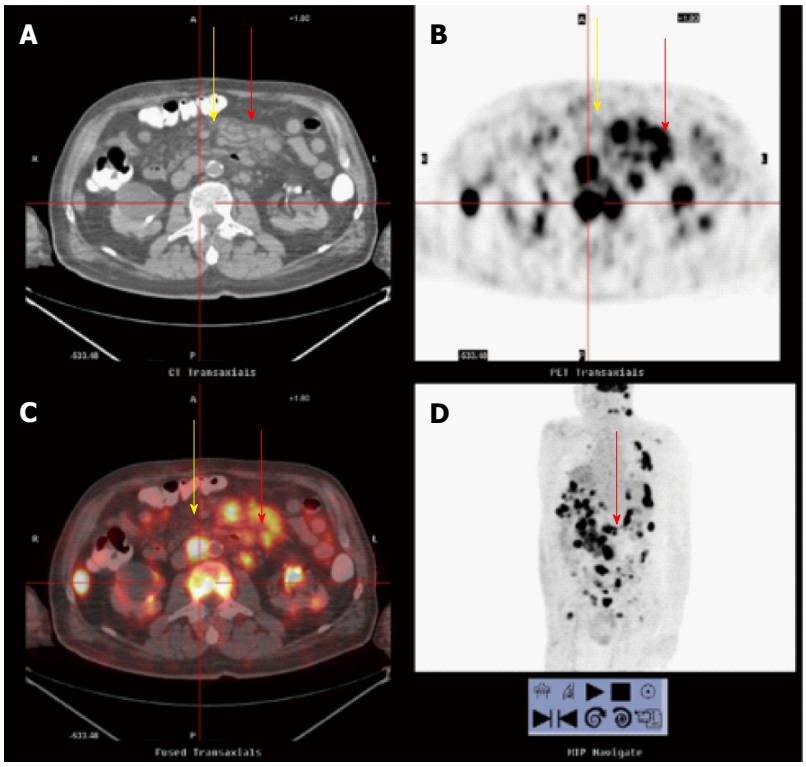

Figure 7 Mesenteric panniculitis in a patient with non-Hodgkin lymphoma (same patient as Figure 3). Positron emission tomography-computed tomography (PET-CT) scan. Computed tomography image (A), PET image (B), fused PET-CT image (C), and whole body PET image (D) shows abnormal fludeoxyglucose (FDG) uptake in the mesenteric lymph nodes containing tumor (red arrows) but not in the portion of mesentery involved by mesenteric panniculitis (yellow arrows). Notice the innumerable regions of abnormal FDG uptake (black regions in D) corresponding to the patient's disseminated adenopathy.

having MP-like abnormalities on abdominal CT creates great concern for patients and clinicians alike. Prior studies that have suggested the finding of MP-like abnormalities on abdominal CT are associated with a very high risk of malignancy that ranges between 30 and 63%. Our study on the other hand shows a much lower rate of 5.3% of new cancers associated with the currents of the CT findings. In fact, our data suggests that only five of the total of 359 patients having MP -like abnormalities on abdominal CT were not suspected of having cancer at the time performance of the examination. This would suggest that the rate of completely new cancers found in the setting of MP-like mesenteric abnormalities on abdominal CT scan is only 1.4%. The discrepancy between the high rate of cancer associated with MP-like abnormalities in previous studies and the very low rate in our study may be explained in part by the inclusion of patients with known malignancy in prior studies that calculated the rate of association of these two conditions. In our study, if all patients with known and newly suspected cancers are included, 100 of the 359 number of patients with MP-like abnormalities on abdominal CT (or 28% of the total group) had an associated cancer. In our view, the most important question to the clinician having a patient with new MP -like findings on abdominal CT is the likelihood that a further evaluation will reveal the presence of malignancy. Based on these data, the likelihood that a cancer is initially present is approximately 5% and is a new diagnosis in 1.4%. Newly diagnosed malignancies in association with the CT findings are likely to be lymphomas, and careful clinical evaluation for lymphoma and some of the associated solid tumors found in our study is advised. Follow up is also important as an additional 5% of patients will eventually be diagnosed with a malignancy[17].

Most studies and reviews on MP recommend follow-up evaluation with abdominal imaging[18-21]. Our data also suggests that at least in patients with a malignancy, mesenteric findings are likely to remain stable.

Mesenteric uptake of fludeoxyglucose (FDG) during PET scanning within the thickened area of mesentery is rare, and was only seen in 5% of patients undergoing PET in our study. These findings are consistent with our prior observation in a case report[22]. On the other hand, Zissin *et al*[23] suggested that the addition of PET to CT scanning may help to differentiate between inflammatory and neoplastic forms of mesenteric thickening. Our data suggest that PET will not be useful to distinguish an inflammatory mesenteric mass due to MP from a malignant mesenteric mass, or rule out the possibility that a patient has a neoplasm. In fact, our data appears to demonstrate that the MP-like mesenteric abnormality that occurs in patients with malignancy may in fact be an epiphenomenon of the underlying neoplasm. Because most patients with malignancy demonstrate little change in this

mesenteric abnormality on follow up CT, MP-like findings appear to have little influence on the natural history of neoplastic disease. As noted by prior authors, MP may in fact represent a paraneoplastic process[6].

Since this was a retrospective study, evaluation including the performance of follow up CT was variable and not controlled. In addition, although our radiology practice has agreed to use the terms "misty mesentery" and "mesenteric panniculitis" when describing an MP-like finding on abdominal CT scanning, it is feasible but some cases in which the aforementioned terms were not used in the radiology reports could have been missed. Since the vast majority of the patients in our practice with MP did not receive a biopsy of the mesenteric mass, this study does not reveal whether these abnormalities represent inflammation or neoplastic extension.

In conclusion, our study shows that a new and unexpected diagnosis of malignancy is rare in patients with MP-like abnormalities on CT scan. These CT findings generally remain stable in patients with known malignancies, and appear to represent a paraneoplastic process. PET scanning does not appear to have a role in assisting with the further characterization of MP-like findings in the mesentery.

COMMENTS

Background

Mesenteric panniculitis (MP) is a rare disease characterized by unexplained inflammation of the mesentery. Computed tomography (CT) scan imaging of the abdomen demonstrates thickening of mesenteric tissue and lymphadenopathy at the root of the small bowel mesentery without vascular compromise. Chronic inflammation, fat necrosis and fibrosis are a variety of conditions are associated seen on biopsy. MP is associated with neoplastic disease, abdominal trauma, autoimmune diseases and infections. MP-like findings on abdominal CT can occur in patients with previously unidentified cancers and in those with a known diagnosis of cancer. The development of MP on CT imaging currently has an unclear influence on the natural history of cancer in these patients. The authors herein describe a group of patients with the CT finding of MP and the diagnosis of malignancy, in an attempt to clarify the relationship between these conditions.

Research frontiers

Currently, little is known about MP, including its etiology, pathophysiology and clinical outcome in individual patients. Few studies have addressed appropriate evaluation and treatment with MP. Appropriate algorithms for diagnosis and treatment of MP are lacking in the medical literature.

Innovations and breakthroughs

Although prior studies have described the association of MP and malignancy, the current study shows that only 1.4% of patients with a CT finding of MP will be found to have a previously undiagnosed or suspected cancer. The higher rate of association of MP and cancer described in prior studies likely represents inclusion of patients with a known history of cancer. Additionally, this study shows that follow up abdominal CT in patients with cancer shows stability and not worsening of MP. Finally, the study shows that in patients with cancer, PET scanning rarely shows uptake within the mesenteric abnormality.

Applications

This study demonstrates reassurance to the clinician and patient that findings suggestive of MP on abdominal CT rarely represent the occurrence of a new cancer. Initial standard evaluation to rule out cancer and appropriate follow up is advised by the authors. Furthermore, these findings do not appear to represent a poorer prognosis in patients with existing cancer. Frequent monitoring with abdominal CT does not appear to be of benefit in these patients. PET scanning does not appear to have a role for follow up in these patients.

Terminology

Mesenteric panniculitis is a rare idiopathic inflammatory disorder of the mesentery characterized by the biopsy findings of fat necrosis, mesenteric infiltration with chronic inflammatory cells and fibrosis. Its natural history is unclear and diagnostic and treatment algorithms are incomplete.

Peer-review

This study represented an interesting advance toward the understanding of this condition.

REFERENCES

1 **Coulier B**. Mesenteric panniculitis. Part 2: prevalence and natural course: MDCT prospective study. *JBR-BTR* 2011; **94**: 241-246 [PMID: 22191288 DOI: 10.5334/jbr-btr.659]

2 **Nabzdyk CS**, Dermody M, Rhee J, Bankoff M, Weinstein B, Orkin BA. Progression of sclerosing mesenteritis into intra-abdominal abscesses. *Am Surg* 2013; **79**: E25-E27 [PMID: 23317596]

3 **Smith ZL**, Sifuentes H, Deepak P, Ecanow DB, Ehrenpreis ED. Relationship between mesenteric abnormalities on computed tomography and malignancy: clinical findings and outcomes of 359 patients. *J Clin Gastroenterol* 2013; **47**: 409-414 [PMID: 23188076 DOI: 10.1097/MCG.0b013e3182703148]

4 **Emory TS**, Monihan JM, Carr NJ, Sobin LH. Sclerosing mesenteritis, mesenteric panniculitis and mesenteric lipodystrophy: a single entity? *Am J Surg Pathol* 1997; **21**: 392-398 [PMID: 9130985 DOI: 10.1097/00000478-199704000-00004]

5 **Okamoto M**, Ohkubo N, Yamanaka M, Kimura A, Takagi T, Iwao A, Kawaguchi M, Iwasaki N, Takagi T, Sho KE, Kawamura S, Hibi T. [A case of mesenteric panniculitis complicated with malignant lymphoma]. *Nihon Shokakibyo Gakkai Zasshi* 1998; **95**: 884-889 [PMID: 9752698]

6 **Wilkes A**, Griffin N, Dixon L, Dobbs B, Frizelle FA. Mesenteric panniculitis: a paraneoplastic phenomenon? *Dis Colon Rectum* 2012; **55**: 806-809 [PMID: 22706134 DOI: 10.1097/DCR.0b013e318252e286]

7 **Sugihara T**, Koike R, Nosaka Y, Ogawa J, Hagiyama H, Nagasaka K, Nonomura Y, Nishio J, Nanki T, Kohsaka H, Kubota T, Miyasaka N. Case of subcutaneous and mesenteric acute panniculitis with Sjögren's syndrome. *Nihon Rinsho Meneki Gakkai Kaishi* 2002; **25**: 277-284 [PMID: 16578972 DOI: 10.2177/jsci.25.277]

8 **Tejón Menéndez P**, Alonso S, Alperi M, Ballina J. Mesenteric panniculitis in a patient with ankylosing spondylitis. *Reumatol Clin* 2013; **9**: 197 [PMID: 23523461 DOI: 10.1016/j.reumae.2013.04.003]

9 **Watanabe I**, Taneichi K, Baba Y, Sakai I, Chimoto T, Shibaki H. A case of mixed connective tissue disease with mesenteric panniculitis. *Nihon Naika Gakkai Zasshi* 1989; **78**: 93-94 [PMID: 2732584 DOI: 10.2169/naika.78.93]

10 **Borde JP**, Offensperger WB, Kern WV, Wagner D. Mycobacterium genavense specific mesenteritic syndrome in HIV-infected patients: a new entity of retractile mesenteritis? *AIDS* 2013; **27**: 2819-2822 [PMID: 24231409 DOI: 10.1097/01.aids.0000433820.25415.17]

11 **Alonso Socas MM**, Valls RA, Gómez Sirvent JL, López Lirola A, Aix SP, Higuera AC, Santolaria F. Mesenteric panniculitis by cryptococcal infection in an HIV-infected man without severe immunosuppression. *AIDS* 2006; **20**: 1089-1090 [PMID: 16603873 DOI: 10.1097/01.aids.0000222093.05400.bb]

12 **Roginsky G**, Mazulis A, Ecanow JS, Ehrenpreis ED. Mesenteric Panniculitis Associated With Vibrio cholerae Infection. *ACG Case Rep J* 2015; **3**: 39-41 [PMID: 26504876]

13 **Avelino-Silva VI**, Leal FE, Coelho-Netto C, Cotti GC, Souza RA, Azambuja RL, Rocha Mde S, Kallas EG. Sclerosing mesenteritis

as an unusual cause of fever of unknown origin: a case report and review. *Clinics* (Sao Paulo) 2012; **67**: 293-295 [PMID: 22473414 DOI: 10.6061/clinics/2012(03)16]

14 **Guettrot-Imbert G**, Boyer L, Piette JC, Delèvaux I, André M, Aumaître O. [Mesenteric panniculitis]. *Rev Med Interne* 2012; **33**: 621-627 [PMID: 22658529 DOI: 10.1016/j.revmed.2012.04.011]

15 **Nicholson JA**, Smith D, Diab M, Scott MH. Mesenteric panniculitis in Merseyside: a case series and a review of the literature. *Ann R Coll Surg Engl* 2010; **92**: W31-W34 [PMID: 20615306 DOI: 10.1308/147870810X12699662981393]

16 **Shin NY**, Kim MJ, Chung JJ, Chung YE, Choi JY, Park YN. The differential imaging features of fat-containing tumors in the peritoneal cavity and retroperitoneum: the radiologic-pathologic correlation. *Korean J Radiol* 2010; **11**: 333-345 [PMID: 20461188 DOI: 10.3348/kjr.2010.11.3.333]

17 **Badet N**, Sailley N, Briquez C, Paquette B, Vuitton L, Delabrousse É. Mesenteric panniculitis: still an ambiguous condition. *Diagn Interv Imaging* 2015; **96**: 251-257 [PMID: 25701479 DOI: 10.1016/j.diii.2014.12.002]

18 **Hussein MR**, Abdelwahed SR. Mesenteric panniculitis: an update. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 67-78 [PMID: 25220299 DOI: 10.1586/17474124.2014.939632]

19 **Robbrecht DG**, Alidjan F, Eikemans B, Haans DA, van Guldener C, van Wijngaarden P. [Mesenteric panniculitis: variable presentations]. *Ned Tijdschr Geneeskd* 2012; **155**: A4555 [PMID: 22748362]

20 **van Putte-Katier N**, van Bommel EF, Elgersma OE, Hendriksz TR. Mesenteric panniculitis: prevalence, clinicoradiological presentation and 5-year follow-up. *Br J Radiol* 2014; **87**: 20140451 [PMID: 25271412 DOI: 10.1259/bjr.20140451]

21 **Orcajo Rincón J**, Rotger Regi A, Mari Hualde A, Reguera Berenguer L, Hernandez Moreno L, Alonso Farto JC. A prospective study to determine the real value of mesenteric ^{18}F-FDG uptake in cancer patients. inflammatory or tumoral mesenteric paniculitis?. *Rev Esp Med Nucl Imagen Mol* 2014; **33**: 352-357 [PMID: 25241217 DOI: 10.1016/j.remn.2014.03.006]

22 **Ehrenpreis ED**, Rao AS, Aki R, Brown H, Pae T, Boiskin I. Normal Positron Emission Tomography-Computerized Tomogram in a Patient with Apparent Mesenteric Panniculitis: Biopsy Is Still the Answer. *Case Rep Gastroenterol* 2009; **3**: 131-137 [PMID: 20651979 DOI: 10.1159/000213653]

23 **Zissin R**, Metser U, Hain D, Even-Sapir E. Mesenteric panniculitis in oncologic patients: PET-CT findings. *Br J Radiol* 2006; **79**: 37-43 [PMID: 16421403 DOI: 10.1259/bjr/29320216]

P- Reviewer: Aosasa S, Sayegh AI, Tarnawski AS **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.v22.i48.10609

World J Gastroenterol 2016 December 28; 22(48): 10609-10616
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Non-variceal upper gastrointestinal bleeding: Rescue treatment with a modified cyanoacrylate

Roberto Grassia, Pietro Capone, Elena Iiritano, Katerina Vjero, Fabrizio Cereatti, Mario Martinotti, Gabriele Rozzi, Federico Buffoli

Roberto Grassia, Pietro Capone, Elena Iiritano, Katerina Vjero, Fabrizio Cereatti, Federico Buffoli, Digestive Endoscopy and Gastroenterology Unit, "ASST di Cremona", 26100 Cremona, Italy

Mario Martinotti, Department of Surgery, "ASST di Cremona", 26100 Cremona, Italy

Gabriele Rozzi, Department of Radiology, "ASST di Cremona", 26100 Cremona, Italy

Author contributions: Grassia R and Buffoli F designed the research and analyzed the data; Grassia R, Capone P, Iiritano E, Vjero K, Cereatti F, Martinotti M, Rozzi G and Buffoli F performed the research; Grassia R wrote the paper.

Institutional review board statement: For retrospective studies, Italian law (Official Gazette n. 76 of 31/03/2008) only requires notification to the Local Ethical Committee.

Informed consent statement: According to Italian law (01/03/2012, published on Official Gazette n.72 of March 26, 2012), there is no need to request informed consent for retrospective studies using anonymized data and not including genetic data.

Conflict-of-interest statement: There are no conflicts of interest to be disclosed.

Data sharing statement: No additional data available.

Manuscript source: Unsolicited manuscript

Correspondence to: Roberto Grassia, MD, Dirigente Medico I livello, Digestive Endoscopy and Gastroenterology Unit, "ASST di Cremona", Viale Concordia n 1, 26100 Cremona, Italy. robertograssia@yahoo.it
Telephone: +39-372-405255
Fax: +39-372-405254

Received: July 25, 2016
Peer-review started: July 27, 2016
First decision: September 28, 2016
Revised: October 13, 2016
Accepted: November 14, 2016
Article in press: November 16, 2016
Published online: December 28, 2016

Abstract

AIM

To evaluate the safety and efficacy of a modified cyanoacrylate [N-butyl-2-cyanoacrylate associated with methacryloxysulfolane (NBCA + MS)] to treat non-variceal upper gastrointestinal bleeding (NV-UGIB).

METHODS

In our retrospective study we took into account 579 out of 1177 patients receiving endoscopic treatment for NV-UGIB admitted to our institution from 2008 to 2015; the remaining 598 patients were treated with other treatments. Initial hemostasis was not achieved in 45 of 579 patients; early rebleeding occurred in 12 of 579 patients. Thirty-three patients were treated with modified cyanoacrylate: 27 patients had duodenal, gastric or anastomotic ulcers, 3 had post-mucosectomy bleeding, 2 had Dieulafoy's lesions, and 1 had duodenal diverticular bleeding.

RESULTS

Of the 45 patients treated endoscopically without initial

hemostasis or with early rebleeding, 33 (76.7%) were treated with modified cyanoacrylate glue, 16 (37.2%) underwent surgery, and 3 (7.0%) were treated with selective transarterial embolization. The mean age of patients treated with NBCA + MS (23 males and 10 females) was 74.5 years. Modified cyanoacrylate was used in 24 patients during the first endoscopy and in 9 patients experiencing rebleeding. Overall, hemostasis was achieved in 26 of 33 patients (78.8%): 19 out of 24 (79.2%) during the first endoscopy and in 7 out of 9 (77.8%) among early rebleeders. Two patients (22.2%) not responding to cyanoacrylate treatment were treated with surgery or transarterial embolization. One patient had early rebleeding after treatment with cyanoacrylate. No late rebleeding during the follow-up or complications related to the glue injection were recorded.

CONCLUSION

Modified cyanoacrylate solved definitively NV-UGIB after failure of conventional treatment. Some reported life-threatening adverse events with other formulations, advise to use it as last option.

Key words: Rescue treatment; Glubran; Non-variceal upper gastrointestinal bleeding; Endoscopic treatment; Cyanoacrylate

Core tip: Endoscopic hemostasis methods are very effective for managing non-variceal upper gastrointestinal bleeding (NV-UGIB), but an early rebleeding rate of approximately 10% reduces the success of initial hemostasis. A modified cyanoacrylate (NBCA + MS) glue used for variceal bleeding has occasionally also been used to treat NV-UGIB. In our 7 years of experience, 33 patients were treated with NBCA + MS after conventional treatment modalities failed. Hemostasis was achieved in approximately 80% of these patients. Modified cyanoacrylate effectively treated NV-UGIB after the failure of conventional treatment modalities.

Grassia R, Capone P, Iiritano E, Vjero K, Cereatti F, Martinotti M, Rozzi G, Buffoli F. Non-variceal upper gastrointestinal bleeding: Rescue treatment with a modified cyanoacrylate. *World J Gastroenterol* 2016; 22(48): 10609-10616 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10609.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10609

INTRODUCTION

Acute upper gastrointestinal bleeding is the most common, potentially life-threatening emergency occurring in gastroenterology departments[1]. The condition has an incidence ranging from approximately 50 to 150 per 100000 of the population each year, and the incidence is the highest in areas of the lowest socioeconomic status[1].

In the United States, acute upper gastrointestinal bleeding causes more than 300000 hospital admissions with an annual incidence of hospitalization equal to 1 per 1000 people[2] and a mortality rate of approximately 10%[3]. From a socioeconomic point of view, treating and preventing upper gastrointestinal bleeding costs many billions of dollars per year[4]. Despite the introduction of endoscopic therapies that reduce the rebleeding rate, the mortality rate has only slightly decreased over the last 30 years. This phenomenon is attributed to the increasing occurrence of upper gastrointestinal bleeding in the elderly. This group has a worse prognosis than others because of their greater use of antiplatelet medications or anticoagulants and their frequent comorbidities[5,6].

Mortality has been reported to be lower in specialist units[7]. This difference is more likely to be due to adherence to protocols and guidelines than to technical developments.

Upper gastrointestinal bleeding can be caused by a wide variety of medical conditions. Peptic ulcers have been reported to be the cause of approximately 50% of upper gastrointestinal bleeding cases, whereas Mallory-Weiss tears account for 5%-15% of cases[8]. Esophageal varices are a common source of upper gastrointestinal bleeding, especially in patients with liver dysfunction and chronic alcoholism. Less frequent causes of upper gastrointestinal bleeding include erosive duodenitis, neoplasms, aortoenteric fistulas, vascular lesions, Dieulafoy's ulcers and prolapse gastropathy[9].

In our country, the large "Prometeostudy"[10] of patients with upper gastrointestinal bleeding recently showed that peptic lesions were the main cause of bleeding (duodenal ulcer 36.2%, gastric ulcer 29.6%, gastric/duodenal erosion 10.9%). Comorbidities were present in 83% and 52.4% of patients treated with acetyl salicylic acid or other non-steroidal anti-inflammatory drugs (NSAIDs), respectively, and 13.3% of patients had experienced previous episodes of upper gastrointestinal bleeding. Early rebleeding was observed in 5.4% of patients, and surgery was required in 14.3%. Bleeding-related death occurred in 4.0% of patients.

Endoscopic therapy is typically considered based on the characteristics and the classification of a bleeding ulcer. The Forrest classification is commonly used in Europe and Asia to describe bleeding lesions[11]. Stigmata can be used to predict the risk of further bleeding and the need for therapeutic intervention[12,13].

Approximately 80% of upper gastrointestinal bleeding episodes appear to stop bleeding spontaneously[11]; the approximately 20% of episodes remaining either continue to bleed or will rebleed[14]. The recurrence of gastrointestinal hemorrhage is associated with an increased mortality rate, a greater need for surgery and blood transfusions, a prolonged hospital stay, and increased overall healthcare costs[15].

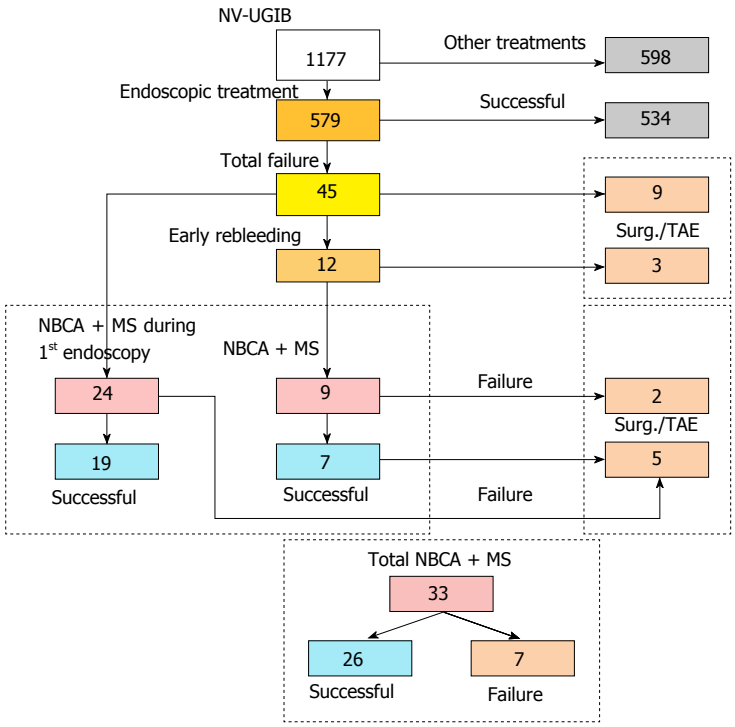

Figure 1 Treated patient results: Successes and failures. NV-UGIB: Non-variceal upper gastrointestinal bleeding; NBCA + MS: N-butyl-2-cyanoacrylate associated with methacryloxysulfolane; TAE: Transarterial embolization.

Endoscopy within the first 24 h is considered the standard of care for the management of upper gastrointestinal bleeding[15].

Endoscopic therapy can be performed by a variety of methods, such as thermal coagulation, sclerotherapy, laser excision, and clip placement. All types of endoscopic therapy are generally considered equivalent, and a combination of methods is superior to an individual therapy[15].

Among the treatments that can be used during endoscopic treatment, cyanoacrylate glues have several advantages: they can be easily and rapidly applied, they are relatively painless, and they eliminate the need for suture removal[16].

Cyanoacrylate is a liquid tissue adhesive that has a well-established utility in the endoscopic management of gastrointestinal variceal bleeding[17-19], but its role in non-variceal bleeding is less clear[19-21]. This limitation is probably related to the limited experience in this area due to the availability of alternative modes of hemostasis, *e.g.,* transarterial embolization, and to the potential side effects of endoscopic cyanoacrylate use in peptic ulcer disease[22]. In fact, despite the relatively safe use of cyanoacrylate glues for treating gastroesophageal variceal bleeding[23], there are concerns about potential serious complications, particularly distant embolization.

Given the possibility of this life-threatening adverse event[21], cyanoacrylate is considered a last resort for achieving endoscopic hemostasis in high surgical risk patients after conventional treatment methods have failed[19].

During the last 20 years, several cyanoacrylate formulations have been developed. In our clinical practice, we have chosen N-butyl-2-cyanoacrylate associated with methacryloxysulfolane (NBCA + MS: Glubran® 2) because of its peculiar characteristics. This formulation has hemostatic, sealing, bacteriostatic, adhesive and sclerosing properties. Polymerization begins 1-2 s after application and completes within 60-90 s. The polymerization reaction generates a temperature of approximately 45 ℃[24,25], which is lower than that of pure cyanoacrylates[16,26], and this formulation is the only cyanoacrylate glue approved for embolization therapy, as stated in the product's instructions for use.

In this paper, we retrospectively describe our personal experience using NBCA + MS injections for the management of (NV-UGIB) after the failure of conventional endoscopic modalities.

MATERIALS AND METHODS

Between April 2008 and May 2015, 1177 patients were referred to our center for NV-UGIB; 579 (49.2%) of these patients received endoscopic treatment.

Patients in whom initial hemostasis was not achieved or who had early rebleeding were treated with other measures, including surgery, selective transarterial embolization and/or cyanoacrylate glue injections (Figure 1).

NBCA + MS (Glubran® 2, GEM S.r.l.; Italy) were used according to manufacturer's indications, and all

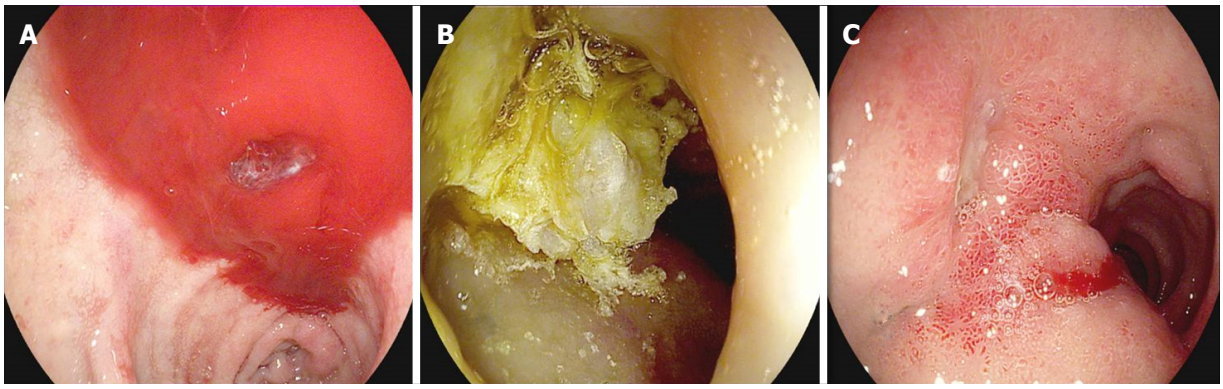

Figure 2 A duodenal bleeding ulcer (Forrest type Ⅰb) (A); endoscopic hemostasis achieved with cyanoacrylate (B); and injection site evaluation after 3 d (C).

possible precautions were taken to avoid intravasal penetration.

The technique involves injection through a sclerotherapy catheter with a 21- to 25-gauge retractable needle in or around actively bleeding points or non-bleeding vessels (Figure 2).

NBCA + MS is applied deep in the submucosa with an injection needle in 0.5-mL to 1.5-mL boluses around the relevant vessel to "compress" from the outside of the bleeding vessel. The technique is then repeated for each bleeding vessel.

We have used this approach in 24 patients during their first endoscopy and in 9 patients who experienced rebleeding after the initial success of a conventional treatment. Bleeding recurrence was considered in the event of any one of the following: the vomiting of fresh blood, hypotension and melena, or the requirement for more than four units of blood in the 72-h period after endoscopic treatment[23].

All 33 patients have received follow-up examinations to detect eventual rebleeding. Currently, the average follow-up period is 37.6 mo (median, 40; range, 3-57).

RESULTS

Endoscopic hemostasis could not be achieved in 45 of 579 patients (7.8%) with conventional treatments, and early rebleeding occurred in 12 patients (2.1%). Of the 45 patients who did not exhibit initial hemostasis or who had early rebleeding, 33 (76.7%) were treated with cyanoacrylate glue, 16 (37.2%) underwent surgery (including 5 patients in whom cyanoacrylate therapy failed), and 3 (7.0%) were treated with selective transarterial embolization (including 2 patients in whom cyanoacrylate therapy failed).

The group of patients treated with NBCA + MS consisted of 23 males and 10 females; the mean patient age was 74.5 years (median, 78; range, 38-94) (Tables 1 and 2).

The physical status of each patient was classified according to guidelines of the American Society of Anesthesiologists. Upon admission, the patients were categorized as follows: 1 patient, (3.0%) class Ⅰ; 9 patients, (27.3%) class Ⅱ; 20 patients, (60.6%) class Ⅲ; and 3 patients, (9.1%) class Ⅳ.

The presenting symptomatology was melena in 16 patients (48.5%), hematemesis in 16 patients (48.5%), and shock + hematemesis in 1 patient (3.0%). The average hemoglobin value at admission was 8.81 g/dL (range, 5.5-11.2). Seventeen (51.5%) patients had a duodenal ulcer, 6 (18.2%) had a gastric ulcer, 4 (12.1%) had an anastomotic ulcer, 2 (6.1%) had gastric post-mucosectomy bleeding, 2 (6.1%) had a gastric Dieulafoy's ulcer, 1 (3.0%) had duodenal diverticular bleeding, and 1 (3.0%) had esophageal post-mucosectomy bleeding.

Regarding the Forrest classification, 16 (48.5%) patients were grade Ⅰa, 6 were grade Ⅰb (18.2%), and 5 were grade Ⅱa (15.2%).

The initial endoscopic treatment consisted of epinephrine (Epi) + clip in 16 patients (48.5%), clip alone in 12 (36.4%), APC (Argon Plasma Coagulation) alone in 2 (6.1%), APC + Epi + clip in 1 (3.0%), Epi + APC in 1 (3.0%), and APC + clip in 1 (3.0%).

Glubran® 2 was used in 24 patients during the first endoscopy and in 9 patients experiencing rebleeding after an initially successful treatment.

Overall, hemostasis was achieved with NBCA + MS in 26 of 33 patients (78.8%). Hemostasis was successfully achieved with NBCA + MS during the first endoscopy in 19 of 24 patients (79.2%). Four patients (16.6%) who did not stop bleeding after the first endoscopy underwent surgery, and 1 (4.2%) was treated with selective transarterial embolization. One patient (4.1%) experienced early rebleeding after being treated with cyanoacrylate.

Of the 9 patients with early rebleeding, 7 (77.8%) achieved hemostasis with NBCA + MS usage, whereas 2 (22.2%) did not and were treated with surgery (1 patient) or transarterial embolization (1 patient).

No late rebleeding occurred during the follow-up period, and no complications related to the glue injections were recorded.

Table 1 Patients treated with cyanoacrylate injection during first endoscopy

Pat. No.	Age	Gender	Lesion	ASA	Forrest	Presentation	Hb (at entry)	Initial endoscopic treatment	Hemostasis with cyanoacrylate (first endoscopy)	Failure therapy	Follow-up (mo)
1	81	M	DU	Ⅲ	I a	Hematemesis	10.6	Epi + clip	Yes	No	45
2	74	M	GU	Ⅱ	I a	Melena	10.9	Epi + clip	Yes	No	51
3	86	F	G-EMR	Ⅲ	NA	Hematemesis	7.6	Apc	Yes	No	49
4	74	M	An-U	Ⅲ	I a	Melena	5.5	Epi + clip	Yes	No	40
5	80	M	DU	Ⅳ	I b	Melena	8.2	Clip	Yes	No	40
6	63	M	E-EMR	Ⅲ	NA	Hematemesis	9.6	Clip	Yes	No	47
7	71	M	GU	Ⅱ	2°	Melena	10.0	Apc + clip	Yes	No	57
8	75	M	DU	Ⅱ	2°	Melena	7.6	Epi + clip	Yes	No	49
9	82	F	DU	Ⅲ	2°	Melena	10.3	Epi + clip	Yes	No	40
10	43	F	DU	Ⅰ	I a	Melena	7.6	Epi + clip	No	Surgery	49
11	61	M	DU	Ⅱ	I a	Melena	8.6	Epi + clip	No	Surgery	39
12	85	F	DU	Ⅳ	I a	Hematemesis	6.5	Clip	Yes	No	35
13	71	M	DU	Ⅲ	I b	Melena	11.0	Epi + clip	Yes	No	39
14	70	M	GU	Ⅱ	I a	Melena	10.2	Apc + Epi + clip	Yes	No	47
15	85	M	G-Dieulafoy	Ⅲ	NA	Hematemesis	9.2	Clip	Yes	No	40
16	84	F	DU	Ⅲ	I a	Shock, Hematemesis	8.0	Clip	No	Surgery	39
17	89	M	GU	Ⅲ	I b	Melena	9.7	Epi + clip	Yes	No	42
18	82	F	DU	Ⅱ	I a	Hematemesis	8.9	Epi + clip	No	TAE	45
19	88	F	DU	Ⅲ	I a	Hematemesis	11.2	Epi + clip	Yes	No	34
20	61	M	An-U	Ⅱ	2°	Hematemesis	7.6	Epi + clip	No	Surgery	30
21	41	M	DU	Ⅱ	2°	Hematemesis	10.7	Clip	Yes	No	28
22	63	M	GU	Ⅲ	I b	Hematemesis	8.1	Epi + clip	Yes	No	11
23	81	M	DU	Ⅲ	I a	Melena	6.8	Clip	Yes	No	3
24	74	M	G-Dieulafoy	Ⅲ	NA	Hematemesis	7.2	Clip	Yes	No	3

ASA: American Society of Anesthesiologists; DU: Duodenal ulcer; GU: Gastric ulcer; An-U: Anastomotic ulcer; DD: Duodenal diverticulum; G-EMR: Gastric mucosectomy; E-EMR: Esophageal mucosectomy; Epi: Epinephrine; TAE: Transarterial embolization; NA: Not applicable.

Table 2 Patients who underwent cyanoacrylate injection for rebleeding

Pat. No.	Age	Gender	Lesion	ASA	Forrest	Presentation	Hb (at entry)	Initial endoscopic treatment	Hemostasis with cyanoacrylate (second endoscopy)	Failure therapy	Follow-up (mo)
r1	87	M	DU	Ⅲ	I a	Hematemesis	8.6	Clip	Yes	No	38
r2	38	F	An-U	Ⅱ	I b	Hematemesis	7.4	Clip	No	Surgery	43
r3	76	M	DD	Ⅲ	NA	Melena	7.2	Clip	Yes	No	47
r4	73	F	An-U	Ⅲ	I a	Hematemesis	9.7	Epi + clip	Yes	No	49
r5	82	M	DU	Ⅲ	I a	Hematemesis	9.3	Epi + clip	Yes	No	42
r6	94	M	G-EMR	Ⅳ	NA	Melena	8.6	Apc	Yes	No	36
r7	87	F	GU	Ⅲ	I b	Melena	8.8	Epi + apc	No	TAE	32
r8	78	M	DU	Ⅲ	I a	Melena	10.4	Clip	Yes	No	27
r9	80	M	DU	Ⅲ	I a	Hematemesis	9.0	Epi + clip	Yes	No	26

ASA: American Society of Anesthesiologists; DU: Duodenal ulcer; An-U: Anastomotic ulcer; DD: Duodenal diverticulum; G-EMR: Gastric mucosectomy; Epi: Epinephrine; NA: Not applicable.

DISCUSSION

Despite significant positive changes in recent years, NV-UGIB remains a common, challenging and often life threatening emergency for gastroenterologists and endoscopists. Although there have been significant improvements in endoscopic and supportive therapies, the overall mortality rate remains approximately 10% and may even reach 35% in the elderly and in hospitalized patients with serious comorbidities[27].

In most cases, peptic ulcers spontaneously stop bleeding, and high-dose intravenous proton pump inhibitors and endoscopic therapies for bleeding ulcers reduce recurrent bleeding risk and the need for surgery[28].

In approximately 4/5th of all upper gastrointestinal episodes, bleeding stops spontaneously[11], whereas in the remaining 1/5th, the bleeding either continues or will recur, causing a rebleeding episode[14]. Thus, the main open question seems to involve the management of rebleeding.

Because the recurrence of gastrointestinal hemorrhaging increases morbidity, mortality and cost[15], the timely identification and aggressive management of patients at high risk for continued bleeding or rebleeding has become the major focus of upper gastrointestinal bleeding therapy[8].

Rebleeding after initial endotherapy can be con-

trolled in approximately 75% of patients with a second endoscopic treatment, which is safer than undergoing surgery[29].

While cyanoacrylate glue injections effectively control variceal bleeding, the role of the material in NV-UGIB is less defined[30].

When administered by a suitably experienced endoscopist for hemostasis, cyanoacrylate glue is considered a safe, inexpensive and effective salvage alternative to surgery when other measures have failed or if selective transarterial embolization is unavailable[19-21,31]. It is important to note that several different formulations of cyanoacrylate glue are available; these different formulations merit investigation as they may lead significantly different results and safety profiles.

In variceal bleeding, the use of cyanoacrylate is very common and is included in several guidelines. However, few papers have report the results of its use in NV-UGIB, and some are simple case descriptions or short case series[19-20,22]. No direct comparisons of the cyanoacrylate formulations are available.

To the best of our knowledge, the largest (126 patients) and only randomized study was performed by Lee *et al*[21], who demonstrated significantly lower rebleeding rates in patients with Forrest type Ia lesions treated with a pure N-butyl-cyanoacrylate glue (Histoacryl®) compared with a hypertonic saline-adrenaline (HSE) injection; no overall benefits regarding hemostasis rates were observed in the other patients. However, although no complications followed HSE therapy, arterial embolization with infarction occurred in 2 patients treated with cyanoacrylate, and one of these patients died. Arterial embolization is considered the most dangerous complication of this treatment; therefore, this therapy is typically recommended as a final measure due to the potentially fatal adverse effects[21]. In contrast, in other published papers, complication rates are typically negligible[19,20,22], mirroring the low rates of complications recently reported with the use of cyanoacrylate for varices[23,30].

Our retrospective series of 33 cases supports the efficacy (78.8% success rate) and safety (no side effects) of the modified cyanoacrylate formulation (NBCA + MS) used in our Digestive Endoscopy and Gastroenterology Unit. Compared with other similar, but shorter, case series[19,20,22], the success rate observed in our patients might be due to the fact that we used the glue exclusively as a second-line therapy; in addition, we used a different methodology, *i.e.*, injection only, and our patients had different baseline diseases.

Although our results were obtained from a retrospective series, in our opinion, it would be possible to hypothesize that the lack of complications might be at least partly derived from our long experience in treating variceal bleeding with this product that has been largely documented in digestive endoscopy[29,32-34].

In addition, the differences among the cyanoacrylate formulations might be crucial. In particular, polymerization time and temperature may play a role in this respect. The polymerization time depends on the amount of injected liquid. However, NBCA + MS generally begins to polymerize 1 s to 2 s after application, and the polymerization is complete within 60 s to 90 s. In contrast, other cyanoacrylates take longer (150-180 s) to polymerize[16,26], and these glues only reach maximum mechanical strength upon complete polymerization. The differences regarding the temperature generated by the polymerization reaction appear to be more important than polymerization time. NBCA + MS generates a temperature of approximately 45 ℃ and thus causes very limited damage to the surrounding tissue[24,25]. In contrast, other cyanoacrylates generate temperatures of 80-90 ℃, causing more inflammation and, rarely, tissue necrosis and deep ulcers or fistulas[35].

Some authors consider surgery or selective transarterial embolization to be preferred methods for controlling rebleeding, even though it is accepted that treatments should be largely based on patient comorbidities and surgical risk[22]. In our opinion, NBCA + MS should be implemented before surgery because it is cheaper, is associated with fewer complications, and is very effective (77.8% in our early rebleeding patients), as previously reported by others[19].

Our retrospective observational study indicates that the formulation of cyanoacrylic glue associated with methacryloxysulfolane used in our department was safe and effective for treating NV-UGIB after the failure of conventional treatment modalities. As our results were obtained from a medium size retrospective series, no definitive conclusions can be drawn. In our experience, the glue has been safe and has not caused any side effects. Therefore, in agreement with the literature[21], we suggest its use in high surgical risk patients for endoscopic hemostasis as a last resort, given the possibility of life-threatening adverse events. Finally we consider important to underline that our results are derived from a retrospective observational study: it is well known that retrospective studies have a limited validity compared to randomized clinical trials because the characteristics of the subjects included, the data collected and measured outcomes are defined after the end of the recruitment. Our data have been collected in order to obtain a good level of quality, but, in retrospective studies this cannot be guaranteed. Furthermore, observational studies tend both to overestimate the effects of treatment and to have greater variability in effect estimates because of residual confounding. Hence our results should be read taking into account these possible biases. In our opinion a randomized clinical trial comparing NBCA + MS with the current standard rescue treatments in selected populations is therefore highly advisable.

In the case of positive outcomes, further comparisons with other cyanoacrylate formulations might be useful for establishing the role of NBCA + MS in patients with NV-UGIB after conventional treatments have failed and for clarifying the long-term differences

in safety and efficacy.

ACKNOWLEDGEMENTS

The authors would like to thank GEM Italy and IBIS Informatica for the manuscript revisions and statistical support.

COMMENTS

Background

Endoscopy within the first 24 h is considered the standard of care for the management of upper gastrointestinal bleeding. Cyanoacrylate has a well-established utility in the endoscopic management of gastrointestinal variceal bleeding, but its role in non-variceal bleeding is less clear.

Research frontiers

Several cyanoacrylate formulations are available. The authors use N-butyl-2-cyanoacrylate associated with methacryloxysulfolane (NBCA + MS: Glubran® 2) because of its peculiar characteristics: polymerization begins and completes in few second and the polymerization reaction generates a temperature of approximately 45 ℃, which is lower than that of other cyanoacrylates. Furthermore this formulation is the only cyanoacrylate glue approved for embolization therapy.

Innovations and breakthroughs

In this paper, the authors retrospectively describe their personal 5-year experience using NBCA + MS for the management of non-variceal upper gastrointestinal bleeding after the failure of conventional endoscopic modalities.

Applications

In their experience, the glue has been safe and has not caused any side effects. The authors suggest its use in high surgical risk patients for endoscopic hemostasis. Some reported life-threatening adverse events with other formulations, advise to use it as last option. Their results are derived from a retrospective observational study and therefore our results should be read taking into account these possible biases.

Peer-review

The authors conducted a retrospective study regarding the usefulness of modified cyanoacrylates a rescue method for failed hemostasis in NV-UGIB patients. The topic is interesting and the study showed some promising results, although the patient number is small.

REFERENCES

1 **Palmer K.** Non-variceal upper gastrointestinal haemorrhage: guidelines. *Gut* 2002; **51** Suppl 4: iv1-iv6 [PMID: 12208839 DOI: 10.1136/gut.51.suppl_4.iv1]
2 **Boonpongmanee S**, Fleischer DE, Pezzullo JC, Collier K, Mayoral W, Al-Kawas F, Chutkan R, Lewis JH, Tio TL, Benjamin SB. The frequency of peptic ulcer as a cause of upper-GI bleeding is exaggerated. *Gastrointest Endosc* 2004; **59**: 788-794 [PMID: 15173790 DOI: 10.1016/S0016-5107(04)00181-6]
3 **Palmer K**. Acute upper gastrointestinal haemorrhage. *Br Med Bull* 2007; **83**: 307-324 [PMID: 17942452 DOI: 10.1093/bmb/ldm023]
4 **Jiranek GC**, Kozarek RA. A cost-effective approach to the patient with peptic ulcer bleeding. *Surg Clin North Am* 1996; **76**: 83-103 [PMID: 8629205 DOI: 10.1016/S0039-6109(05)70424-2]
5 **Kaplan RC**, Heckbert SR, Koepsell TD, Furberg CD, Polak JF, Schoen RE, Psaty BM. Risk factors for hospitalized gastrointestinal bleeding among older persons. Cardiovascular Health Study Investigators. *J Am Geriatr Soc* 2001; **49**: 126-133 [PMID: 11207865 DOI: 10.1046/j.1532-5415.2001.49032.x]
6 **Peter DJ**, Dougherty JM. Evaluation of the patient with gastrointestinal bleeding: an evidence based approach. *Emerg Med Clin North Am* 1999; **17**: 239-261, x [PMID: 10101349 DOI: 10.1016/S0733-8627(05)70055-9]
7 **Sanderson JD**, Taylor RF, Pugh S, Vicary FR. Specialized gastrointestinal units for the management of upper gastrointestinal haemorrhage. *Postgrad Med J* 1990; **66**: 654-656 [PMID: 2217034 DOI: 10.1136/pgmj.66.778.654]
8 **Rivkin K**, Lyakhovetskiy A. Treatment of nonvariceal upper gastrointestinal bleeding. *Am J Health Syst Pharm* 2005; **62**: 1159-1170 [PMID: 15914876]
9 **Laine L.** Gastrointestinal bleeding. In: Braunwald E, Fauci AS, Kasper DL, McGraw-Hill, Hauser SL, Stone RM, Longo DL, Jameson JL, editors. Harrison's Principles of Internal Medicine: Textbook, Self-Assessment and Board Review. New York: McGraw-Hill Professional Publishing, 2001: 252
10 **Del Piano M**, Bianco MA, Cipolletta L, Zambelli A, Chilovi F, Di Matteo G, Pagliarulo M, Ballarè M, Rotondano G. The "Prometeo" study: online collection of clinical data and outcome of Italian patients with acute nonvariceal upper gastrointestinal bleeding. *J Clin Gastroenterol* 2013; **47**: e33-e37 [PMID: 22914349 DOI: 10.1097/MCG.0b013e3182617dcc]
11 **Kim SY**, Hyun JJ, Jung SW, Lee SW. Management of non-variceal upper gastrointestinal bleeding. *Clin Endosc* 2012; **45**: 220-223 [PMID: 22977806 DOI: 10.5946/ce.2012.45.3.220]
12 **Enestvedt BK**, Gralnek IM, Mattek N, Lieberman DA, Eisen G. An evaluation of endoscopic indications and findings related to nonvariceal upper-GI hemorrhage in a large multicenter consortium. *Gastrointest Endosc* 2008; **67**: 422-429 [PMID: 18206878 DOI: 10.1016/j.gie.2007.09.024]
13 **Laine L**, Peterson WL. Bleeding peptic ulcer. *N Engl J Med* 1994; **331**: 717-727 [PMID: 8058080 DOI: 10.1056/nejm199409153311107]
14 **Messmann H**, Schaller P, Andus T, Lock G, Vogt W, Gross V, Zirngibl H, Wiedmann KH, Lingenfelser T, Bauch K, Leser HG, Schölmerich J, Holstege A. Effect of programmed endoscopic follow-up examinations on the rebleeding rate of gastric or duodenal peptic ulcers treated by injection therapy: a prospective, randomized controlled trial. *Endoscopy* 1998; **30**: 583-589 [PMID: 9826134 DOI: 10.1055/s-2007-1001360]
15 **Barkun A**, Bardou M, Marshall JK. Consensus recommendations for managing patients with nonvariceal upper gastrointestinal bleeding. *Ann Intern Med* 2003; **139**: 843-857 [PMID: 14623622 DOI: 10.7326/0003-4819-139-10-200311180-00012]
16 **Montanaro L**, Arciola CR, Cenni E, Ciapetti G, Savioli F, Filippini F, Barsanti LA. Cytotoxicity, blood compatibility and antimicrobial activity of two cyanoacrylate glues for surgical use. *Biomaterials* 2001; **22**: 59-66 [PMID: 11085384 DOI: 10.1016/S0142-9612(00)00163-0]
17 **Choudhuri G**, Chetri K, Bhat G, Alexander G, Das K, Ghoshal UC, Das K, Chandra P. Long-term efficacy and safety of N-butylcyanoacrylate in endoscopic treatment of gastric varices. *Trop Gastroenterol* 2010; **31**: 155-164 [PMID: 21560518]
18 **Cheng LF**, Wang ZQ, Li CZ, Lin W, Yeo AE, Jin B. Low incidence of complications from endoscopic gastric variceal obturation with butyl cyanoacrylate. *Clin Gastroenterol Hepatol* 2010; **8**: 760-766 [PMID: 20621678 DOI: 10.1016/j.cgh.2010.05.019]
19 **Repici A**, Ferrari A, De Angelis C, Caronna S, Barletti C, Paganin S, Musso A, Carucci P, Debernardi-Venon W, Rizzetto M, Saracco G. Adrenaline plus cyanoacrylate injection for treatment of bleeding peptic ulcers after failure of conventional endoscopic haemostasis. *Dig Liver Dis* 2002; **34**: 349-355 [PMID: 12118953 DOI: 10.1016/S1590-8658(02)80129-3]
20 **Kurek K**, Baniukiewicz A, Swidnicka-Siergiejko A, Dąbrowski A. Application of cyanoacrylate in difficult-to-arrest acute non-variceal gastrointestinal bleeding. *Wideochir Inne Tech Maloinwazyjne* 2014; **9**: 489-493 [PMID: 25337181 DOI: 10.5114/wiitm.2014.44169]
21 **Lee KJ**, Kim JH, Hahm KB, Cho SW, Park YS. Randomized trial of N-butyl-2-cyanoacrylate compared with injection of hypertonic saline-epinephrine in the endoscopic treatment of bleeding peptic ulcers. *Endoscopy* 2000; **32**: 505-511 [PMID: 10917181 DOI: 10.1055/s-2000-3816]

22 **Loh DC**, Wilson RB. Endoscopic management of refractory gastrointestinal non-variceal bleeding using Histoacryl (N-butyl-2-cyanoacrylate) glue. *Gastroenterol Rep* (Oxf) 2016; **4**: 232-236 [PMID: 25991813 DOI: 10.1093/gastro/gov019]

23 **Cipolletta L**, Zambelli A, Bianco MA, De Grazia F, Meucci C, Lupinacci G, Salerno R, Piscopo R, Marmo R, Orsini L, Rotondano G. Acrylate glue injection for acutely bleeding oesophageal varices: A prospective cohort study. *Dig Liver Dis* 2009; **41**: 729-734 [PMID: 19362522 DOI: 10.1016/j.dld.2009.02.006]

24 **Schug-Pass C**, Jacob DA, Rittinghausen J, Lippert H, Köckerling F. Biomechanical properties of (semi-) synthetic glues for mesh fixation in endoscopic inguinal hernia repair. *Hernia* 2013; **17**: 773-777 [PMID: 23064973 DOI: 10.1007/s10029-012-1000-8]

25 **Kull S**, Martinelli I, Briganti E, Losi P, Spiller D, Tonlorenzi S, Soldani G. Glubran2 surgical glue: in vitro evaluation of adhesive and mechanical properties. *J Surg Res* 2009; **157**: e15-e21 [PMID: 19439320 DOI: 10.1016/j.jss.2009.01.034]

26 **Testini M**, Lissidini G, Poli E, Gurrado A, Lardo D, Piccinni G. A single-surgeon randomized trial comparing sutures, N-butyl-2-cyanoacrylate and human fibrin glue for mesh fixation during primary inguinal hernia repair. *Can J Surg* 2010; **53**: 155-160 [PMID: 20507786]

27 **Ferguson CB**, Mitchell RM. Non-variceal upper gastrointestinal bleeding. *Ulster Med J* 2006; **75**: 32-39 [PMID: 16457402]

28 **Gralnek IM**, Dumonceau JM, Kuipers EJ, Lanas A, Sanders DS, Kurien M, Rotondano G, Hucl T, Dinis-Ribeiro M, Marmo R, Racz I, Arezzo A, Hoffmann RT, Lesur G, de Franchis R, Aabakken L, Veitch A, Radaelli F, Salgueiro P, Cardoso R, Maia L, Zullo A, Cipolletta L, Hassan C. Diagnosis and management of non-variceal upper gastrointestinal hemorrhage: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* 2015; **47**: a1-a46 [PMID: 26417980 DOI: 10.1055/s-0034-1393172]

29 **Sáenz de Miera Rodríguez A**, Baltar Arias R, Vázquez Rodríguez S, Díaz Saa W, Ulla Rocha JL, Vázquez-Sanluis J, Vázquez Astray E. N-Butyl-2-cyanoacrylate plug on fundal varix: persistence 3 years after sclerosis. *Rev Esp Enferm Dig* 2009; **101**: 212-214 [PMID: 19388802 DOI: 10.4321/S1130-01082009000300008]

30 **Binmoeller KF**, Soehendra N. "Superglue": the answer to variceal bleeding and fundal varices? *Endoscopy* 1995; **27**: 392-396 [PMID: 7588355 DOI: 10.1055/s-2007-1005720]

31 **Cameron R**, Binmoeller KF. Cyanoacrylate applications in the GI tract. *Gastrointest Endosc* 2013; **77**: 846-857 [PMID: 23540441 DOI: 10.1016/j.gie.2013.01.028]

32 **Battaglia G**, MorbinT, Patarnello E, Carta A, Coppa F, Ancona A. Endoscopic diagnosis and treatment of gastric varices. *Acta Endoscopica* 1999; **29**: 97-114 [DOI: 10.1007/bf03020277]

33 **Seewald S**, Sriram PV, Naga M, Fennerty MB, Boyer J, Oberti F, Soehendra N. Cyanoacrylate glue in gastric variceal bleeding. *Endoscopy* 2002; **34**: 926-932 [PMID: 12430080 DOI: 10.1055/s-2002-35312]

34 **López J**, Rodriguez K, Targarona EM, Guzman H, Corral I, Gameros R, Reyes A. Systematic review of cyanoacrylate embolization for refractory gastrointestinal fistulae: a promising therapy. *Surg Innov* 2015; **22**: 88-96 [PMID: 24902686 DOI: 10.1177/1553350614535860]

35 **Levrier O**, Mekkaoui C, Rolland PH, Murphy K, Cabrol P, Moulin G, Bartoli JM, Raybaud C. Efficacy and low vascular toxicity of embolization with radical versus anionic polymerization of n-butyl-2-cyanoacrylate (NBCA). An experimental study in the swine. *J Neuroradiol* 2003; **30**: 95-102 [PMID: 12717295]

P- Reviewer: Stanley AJ, Yan SL **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Liu WX

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.v22.i48.10617

World J Gastroenterol 2016 December 28; 22(48): 10617-10624
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Assessment of disease activity by fecal immunochemical test in ulcerative colitis

Dae Gon Ryu, Hyung Wook Kim, Su Bum Park, Dae Hwan Kang, Cheol Woong Choi, Su Jin Kim, Hyeong Seok Nam

Dae Gon Ryu, Hyung Wook Kim, Su Bum Park, Dae Hwan Kang, Cheol Woong Choi, Su Jin Kim, Hyeong Seok Nam, Department of Internal Medicine, Pusan National University School of Medicine and Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan 626-770, South Korea

Author contributions: Ryu DG wrote the manuscript; Kim HW organized the manuscript; Park SB, Kang DH, Choi CW, Kim SJ and Nam HS contributed to the design, organization, and draft of the manuscript; all authors read and approved the final manuscript.

Supported by a 2-Year Research Grant of Pusan National University.

Conflict-of-interest statement: No potential conflict of interest relevant to this article was reported.

Manuscript source: Unsolicited manuscript

Correspondence to: Hyung Wook Kim, PhD, MD, Department of Internal Medicine, Pusan National University School of Medicine and Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, 20, Geumo-ro, Mulgeum-eup, Yangsan 626-770, Gyeongsangnam-do,
South Korea. mdkhwook@gamil.com
Telephone: +82-55-3601535
Fax: +82-55-3601536

Received: July 18, 2016
Peer-review started: July 21, 2016
First decision: October 11, 2016
Revised: October 15, 2016
Accepted: November 15, 2016
Article in press: November 16, 2016
Published online: December 28, 2016

Abstract

AIM

To evaluate the efficacy of quantitative fecal immunochemical test (FIT) as biomarker of disease activity in ulcerative colitis (UC).

METHODS

Between February 2013 and November 2014, a total of 82 FIT results, obtained in conjunction with colonoscopies, were retrospectivelyevaluated for 63 patients with UC. The efficacy of FIT for evaluation of disease activity was compared to colonoscopic findings. Quantitative fecal blood with automated equipment examined from collected feces. Endoscopic disease severity were assessed using the Mayo endoscopic subscore (MES) classification. The extent of disease were classified by proctitis (E1), left sided colitis (E2), and extensive colitis (E3). Clinical activity were subgrouped by remission or active.

RESULTS

All of 21 patients with MES 0 had negative FIT (< 7 ng/mL), but 22 patients with MES 2 or 3 had a mean FIT of > 134.89 ng/mL**.** The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of negative FIT about mucosal healing were 73.33%, 81.82%, 91.49%, 51.43% and 73.17%, respectively. The sensitivity, specificity, PPV, NPV and accuracy of predictive value of positive FIT (cutoff value > 100 ng/mL) about active disease status were 45.45%, 93.33%, 71.43%, 82.35%

and 26.83%, respectively. Among patients with clinical remission, FIT was negative in 31 (81.6%) of 38 cases, with a mean fecal hemoglobin concentration of 6.12 ng/mL (range, negative to 80.9 ng/mL) for this group of patients. Among patients with clinical active disease, FIT was negative in 16 (36.4%) out of 44 cases, with a mean fecal hemoglobin concentration > 167.4 ng/mL for this group of patients. FIT was positively correlated with endoscopic activity (r = 0.626, P < 0.01) and clinical activity (r = 0.496, P < 0.01). But, FIT did not correlate with the extent of disease (r = -0.047, P = 0.676)

CONCLUSION

Quantitative FIT can be a non-invasive and effective biomarker for evaluation of clinical and endoscopic activity in UC, but not predict the extent of disease.

Key words: Ulcerative colitis; Fecal immunochemical test; Mayo endoscopic subscore; Biomarker; Disease activity

Core tip: Until now, colonoscopy has been regarded as the gold standard to assess mucosal status and disease activity in patients with ulcerative colitis (UC). Recently, non-invasive markers of mucosal healing have been studied in UC. Fecal immunochemical test (FIT) is one of them suggested association with mucosa healing in some studies. Our results have identified that FIT correlated positively with endoscopic activity and clinical remission, but not with extent of disease. In particular a negative FIT could be regarded as an indication of endoscopic and clinical remission. FIT can be a non-invasive and economic biomarker in patients with UC.

Ryu DG, Kim HW, Park SB, Kang DH, Choi CW, Kim SJ, Nam HS. Assessment of disease activity by fecal immunochemical test in ulcerative colitis. *World J Gastroenterol* 2016; 22(48): 10617-10624 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10617.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10617

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disorder of the colorectum that is characterized by a clinical course of remission and relapse[1]. Assessment of the response to treatment and monitoring of disease activity are two important aspects of the clinical management inpatients with UC. Clinical indices do not always correlate with actual inflammation in UC patient and active mucosal inflammation is often present in asymptomatic patients. Therefore, recent opinions increasingly require to achieve both clinical response and endoscopic mucosal healing (MH) in the treatment of UC[2-4]. To date, the evaluation of mucosal inflammation with colonoscopy is the gold standard to assess disease activity in patients with UC. However, colonoscopic examination is difficult to frequently perform due to cost and inconvenience to patients. Therefore, identification of non-invasive biomarkers of disease activity in UC is a research priority.

Fecal calprotectin (FC) may provide a promising non-invasive marker of mucosal inflammation, with several studies having shown a correlation between FC and the severity of mucosal inflammation[5-9]. However, the measurement of FC requires the enzyme-linked immunosorbent assay technique which usually can only be performed in tertiary healthcare institutions, usually takes several hours to perform and is expensive[6]. In contrast, quantitative fecal immunochemical test (FIT) measures hemoglobin concentration in feces by using an antibody for human hemoglobin. FIT has been used to screen for colorectal cancer in the general population[10]. FIT quantifies blood in fecal samples simply and rapidly using automated equipment[11]. In patients with UC who are in clinical remission, occult blood can be present in stool samples due to residual mucosal inflammation. In fact, previous studies have shown that FIT correlates well with the mucosal status in patients with UC[12,13].

In this study, we measured FITs in patients with UC who had undergone colonoscopy with the aim of comparing endoscopic disease activities with FITs. Furthermore, unlike other studies thus far, we used FIT to compare clinical disease activity and extent of the disease.

MATERIALS AND METHODS

Patients and diagnosis of UC

Between February 2013 and November 2014 we retrospectively reviewed the medical records of patients with UCat the Pusan National University Yangsan Hospital, Korea. During the period, a total of 206 patients with suspected UC were evaluated. UC is diagnosed based on a comprehensive medical history and clinical features, in combination with typical endoscopic and biopsy findings according to current guidelines. UC is a chronic inflammatory condition causing continuous mucosal inflammation of the colon without granulomas on biopsy, affecting the rectum and a variable extent of the colon in continuity, which is characterized by relapsing and remitting course[14,15]. Patients are not clearly diagnosed with UC including inflammatory bowel disease unclassified (IBDU) and indeterminate colitis were excluded. IBDU is the cases where a definitive distinction between UCand Crohn's disease (CD), or other cause of colitis cannot be made after the history, endoscopic appearances, histopathology and appropriate radiology[15]. Indeterminate colitis is a term which has overlapping features of UC and CD[16]. Rule out other diseases in this process, a total of 187 patients were diagnosed

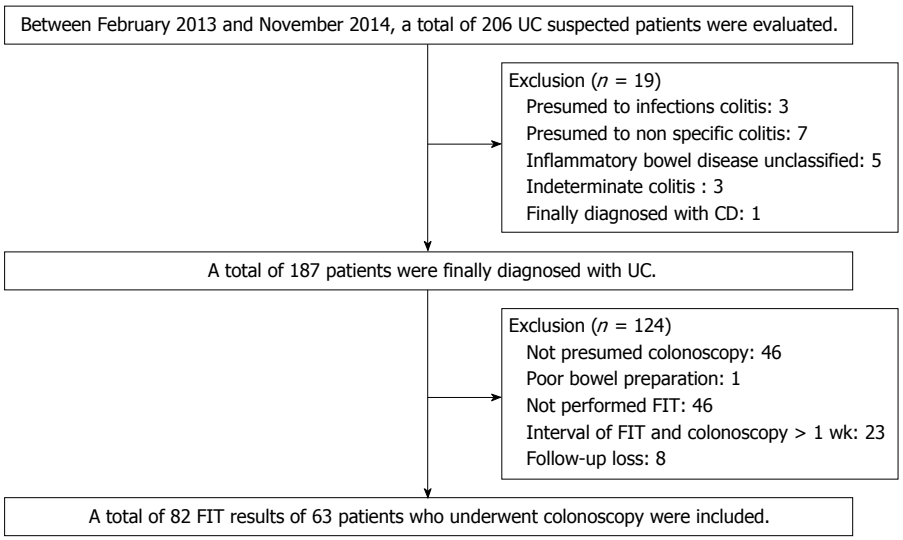

Figure 1 Flow chart of the enrolled patients. UC: Ulcerative colitis; FIT: Fecal immunochemical test.

with UC. Patients not performed colonoscopy or FIT were excluded and patients with the interval of FIT and colonoscopy was more than 1 wk were also excluded. Finally a total of 82 FIT results of 63 patients who underwent colonoscopy included in this study (Figure 1).

Fecal sampling and FIT analysis

Fecal samples were prepared on the morning of hospital visit or the previous day hospital visit. FIT results were obtained on the day of colonoscopy (56/82, 68.3%) or within 1 wk (before or after) of colonoscopy (26/82, 31.7%). About seasonally correlation FIT results and disease activity or mucosa status in UC patients is not yet known. But one study reported that FIT results and disease activity of patients with UC can deviate for 2-4 wk[17]. With reference to the study, patients with more than 1 wk intervals between FIT and colonoscopy were excluded. Submitted stool samples were immediately processed and examined using the HM-JACK system (Kyowa Medex, Japan), a fully automated quantitative FIT system. The HM-JACK system can accurately measure fecal hemoglobin concentration within a range of 7-300000 ng/mL. Stools with a hemoglobin concentration > 1000 ng/mL were classified as *bloody* stools. On the other hand, fecal specimens with a hemoglobin concentration < 7 ng/mL were classified as *negative* (0-7 ng/mL). The HM-JACK system cannot accurately differentiate hemoglobin concentrations < 7 ng/mL.

Assessment of clinical and endoscopic activity

Clinical and endoscopic disease activity were evaluated using the Mayo scoring system for UC[18]. Endoscopic remission and MH were defined bya Mayo endoscopic score (MES) of "0" or "1"[3]. Clinical remission was defined by a Mayo stool frequency subscore of "0" or "1" and a Mayo rectal bleeding subscore of "0"[3]. Patients with any other Mayo scores were considered to be active disease state. The extent of UC was based on the Montreal Classification, with patients classified as having either: proctitis (E1), left sided colitis (E2) or extensive colitis (E3)[15].

Statistical analysis

Statistical calculations were performed using SPSS version 21.0 for Windows (SPSS Inc., Chicago, IL, United States). Spearman's and Kendall's tau rank correlation were performed to determine the association among FIT, MES, the extent of disease and clinical remission. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), with associated 95%CI, for detecting mucosal status based on FIT results, were determined. Statistical significance was set at a *P* value of 0.05.

RESULTS

Patients characteristics

A total of 82 FITs were evaluated for 63 patients with UC, 60 males and 22 females, with a median age of 47.9 years. Baseline characteristics of our patient group are shown in Table 1. Mean disease duration was 33.5 mo (range, 0-217 mo). All patients were treated with suppository or oral 5-ASA, with 46 patients (56.1%) treated with additional therapy: 17 patients (20.7%) with systemic steroids; 23 patients (28%) with azathioprine; and 6 patients (7.3%) with anti TNF-alpha therapy. During the study period, 3 patients were hospitalized for severe UC. No patient underwent surgery for UC-related complications and no patient died.

Correlation between FIT and endoscopic finding

Colonoscopy was performed in all patients during the study period. Among the 82 colonoscopy, 38 (46.3%) were performed in patients in clinical remission and

Table 1 Characteristics of enrolled cases

Characteristics variables	Value
Age (yr), mean (range) ± SD	47.9 (22-75) ± 12.5
Male/Female, *n* (%)	60/22 (73.2/26.8)
Disease duration (mo), mean (range) ± SD	33.5 (0-217) ± 48.3
MES, *n* (%)	
0	21 (25.6)
1	39 (47.6)
2	15 (18.3)
3	7 (8.5)
Disease extent, *n* (%)	
Extensive colitis	32 (39.0)
Left sided colitis	30 (36.6)
Proctitis	20 (24.4)
Fecal hemoglobin concentrations (ng/mL), *n* (%)	
0-7, negative	47 (57.3)
7-100	21 (25.6)
100-1000	11 (13.4)
> 1000	3 (3.7)
Drug at study entry, *n* (%)	
Oral / Suppository 5-ASA	64 (100.0)/18 (22.0)
Systemic steroids	17 (20.7)
Azathioprine	23 (28.0)
Anti-TNFα	6 (7.3)

MES: Mayo endoscopic subscore; 5-ASA: 5-aminosalicylic acids; TNF: Tumor necrosis factor.

Table 2 Negative fecal immunochemical test probability (%) and mean fecal immunochemical test values according to mayo endoscopic subscore

MES	Negative FIT (%)	mean FIT (ng/mL)
0	21/21 (100)	0
1	44/60 (73.3)	33.94
2 or 3	4/22 (18.2)	> 134.89[1]

[1]Three cases in MES "2" or "3" were measured more than 1000 ng/mL fecal hemoglobin concentration. MES: Mayo endoscopic subscore; FIT: Fecal immunochemical test.

Table 3 Mayo endoscopic subscore according to fecal immunochemical test values

	n (%)
Negative FIT cases (*n* = 47)	
MES 0 or 1	43 (91.5)
MES 2 or 3	4 (8.5)
Positive FIT cases (*n* = 35)	
MES 0	0
MES 1	17 (48.6)
MES 2 or 3	18 (51.4)

MES: Mayo endoscopic subscore; FIT: Fecal immunochemical test.

the other 44 (53.7%) in patients with clinical active disease. The distribution of MES based on colonoscopic findings was as follows: MES of "0" in 21 patients (25.6%); MES of "1" in 39 (47.6%); MES of "2" in 15 (18.3%) patients; and MES of "3" in 7 (8.5%). All of patients with MES of "0" had negative FIT (< 7 ng/mL). Patients with MES of "2" or "3" had a mean fecal hemoglobin concentration of 134.89 ng/mL (range, negative to > 1000 ng/mL). Among patients with MES of "2" or "3", 18.2% (4 of 22 patients) had a negative FIT (Table 2).

The distribution of MES for the 47 negative FIT cases was as follows: MES of "0" in 21 patients (44.7%); MES of "1" in 22 (46.8%) patients; MES of "2" in 3 (6.4%) patients; and MES of "3" in 1 patient (2.1%). Therefore, a negative FIT identifies mucosa healing, assessed by endoscopy (MES of "0" or "1"), with a probability of 92%. For the 35 patients with a positive FIT, the distribution of MES was as follows: MES of "0" in 0 patient; MES of "1" in 17 patients (48.6%); MES of "2" in 12 patients (34.3%); and MES of "3" in 6 patients (17.1%). Among the three patients with FIT >1000 ng/mL, 2 patients had a MES of "2", with the other patient having a MES of "3" (Table 3).

When we consider only the 60 cases in whom endoscopy identified MH, FIT was negative in 44 of these 60 cases (73.33%). The sensitivity, specificity, PPV, NPV and accuracy of the fecal hemoglobin concentration in relation to MH are reported in Table 4 and summarized as follows: sensitivity, 73.33%; specificity, 81.82%; PPV, 91.49%; NPV, 51.43%; and accuracy, 73.17%. The change in these predictive values of FIT using a cutoff value of fecal hemoglobin concentration > 100 ng/mL, which is popularly used for colon cancer screening, is reported in Table 5. The predictive value of FIT specifically in patients with an active disease status, identified by endoscopy, is summarized as follows: sensitivity, 45.45%; specificity, 93.33%; PPV, 71.43%; NPV, 82.35%; and accuracy, 26.83%.

Table 4 Sensitivity, specificity and predictive value of fecal immunochemical test for mucosal healing (mayo endoscopic subscore 0 or 1)

	Negative FIT (< 7 ng/mL)
Sensitivity (95%CI)	0.73 (0.60-0.83)
Specificity (95%CI)	0.81 (0.59-0.94)
PPV (95%CI)	0.91 (0.80-0.97)
NPV (95%CI)	0.52 (0.35-0.70)
Accuracy (95%CI)	0.73 (0.62-0.82)

MES: Mayo endoscopic subscore; FIT: Fecal immunochemical test; PPV: Positive predictive value; NPV: Negative predictive value.

Table 5 Sensitivity, specificity and predictive value of fecal immunochemical test for endoscopic active disease (mayo endoscopic subscore 2 or 3)

	Positive FIT (> 100 ng/mL)
Sensitivity (95%CI)	0.45 (0.24-0.67)
Specificity (95%CI)	0.93 (0.83-0.98)
PPV (95%CI)	0.71 (0.41-0.91)
NPV (95%CI)	0.82 (0.71-0.90)
Accuracy (95%CI)	0.26 (0.17-0.37)

MES: Mayo endoscopic subscore; FIT: Fecal immunochemical test; PPV: Positive predictive value; NPV: Negative predictive value.

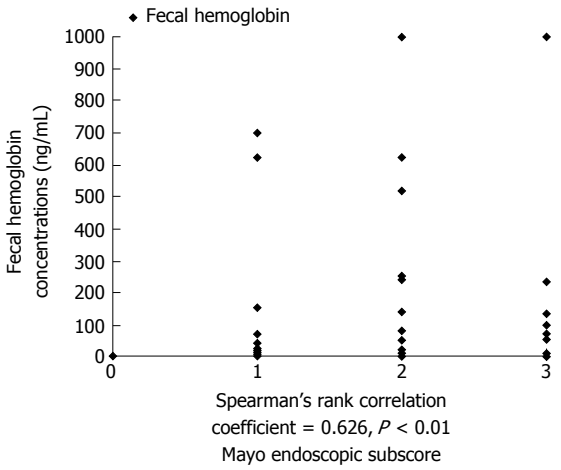

Figure 2 Correlation between fecal immunochemical test and mayo endoscopic subscore. FIT was positively correlated with endoscopic activity (Spearman's rank correlation coefficient = 0.626, $P < 0.01$). MES: Mayo endoscopic subscore; FIT: Fecal immunochemical test.

Table 6 Negative fecal immunochemical test probability (%) and mean fecal immunochemical test values according to clinical condition

Clinical condition	Negative FIT (%)	mean FIT (ng/mL)
Remission status (n = 38)	31/38 (81.6)	6.12
Active status (n = 44)	16/44 (36.4)	> 167.4[1]

[1]Three cases in clinical active were measured more than 1000 ng/mL fecal hemoglobin concentration. FIT: Fecal immunochemical test.

Table 7 Mayo endoscopic subscore according to clinical condition

Clinical condition	n (%)
Remission status (n = 38)	
MES 0 or 1	37 (97.4)
MES 2	1 (2.6)
MES 3	0
Active status (n = 44)	
MES 0 or 1	23 (52.3)
MES 2	14 (31.8)
MES 3	7 (15.9)

MES: Mayo endoscopic subscore.

As a result, FIT was positively correlated with endoscopic activity, with a Spearman's rho correlation coefficient of 0.626 and corresponding P value <0.01. The correlation between the FIT and findings of disease activity on endoscopy are shown in Figure 2.

Correlation between FIT and the extent of disease

Eighty two cases of colonoscopy were classified according to the extent of disease, where the extent of UC was defined using the Montreal Classification. The distribution of cases was as follows: 20 patients (24.4%) were classified in the E1 category; 30 patients (36.6%) in the E2 category; and 32 patients (39.0%) in the E3 category. Among the 47 patients with a negative FIT, the extent of disease was classified as E1 in 12 patients (25.6%), as E2 in 16 patients (34.0%), and as E3 in 19 patients (40.4%). Among the 35 patients with a positive FIT, the extent of disease was classified as E1 in 8 patients (22.9%), as E2 in 14 patients (40.0%), and as E3 in 13 patients (37.1%). As a result, FIT did not correlated with the extent of disease (r = -0.047, P = 0.676).

Correlation between FIT and clinical activity

Among the 82 colonoscopies performed, 38 (46.3%) were performed in patients with clinical remission, with the other 44 (53.7%) were performed in patients with clinical active disease. Among patients in clinical remission, FIT was negative in 31 (81.6%) of 38 cases, with a mean fecal hemoglobin concentration of 6.12 ng/mL (range, negative to 80.9 ng/mL) for this group of patients. Among patients in clinical active disease, FIT was negative in 16 (36.4%) out of 44 cases, with a mean fecal hemoglobin concentration > 167.4 ng/mL (range, negative to > 1000 ng/mL) for this group of patients. The probability of a negative FIT and mean FIT values according to clinical status of UC are reported in Table 6, with MES according to clinical status reported in Table 7. Overall, FIT positively correlated with clinical activity (r = 0.496, $P < 0.01$).

Correlation between FIT and conventional inflammatory markers

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were measured in all patients. In FIT negative group (n = 47), ESR was normal range (0-10 mm/h) in 15 cases (31.9%) and CRP was normal range (0-0.5 mg/dL) in 38 cases (80.9%). In FIT positive group (n = 35), ESR was higher than the normal ranges in 28 cases (80.0%) and CRP was higher than the normal ranges in 13 cases (37.1%). Mean ESR was 18.47 mm/hr (range, 2-83) in FIT negative group and 31.06 mm/hr (range, 2-118) in FIT positive group. Mean CRP was 0.338 mg/dL (range, 0.01-3.12) in FIT negative group and 1.286 mg/dL (range, 0.01-7.45) in FIT positive group. Statistically, FIT did not correlated with ESR (r = 0.183, P = 0.100) or CRP (r = 0.154, P = 0.167).

DISCUSSION

As the clinical course of UC is characterized by multiple phases of clinical remission and acute exacerbations, continued monitoring of disease status and treatment are required. In particular, identification of disease activity during asymptomatic periods is important inpredictingsubsequent acute exacerbation. With the accumulation of evidence on the value of the status of the mucosa in the disease process of UC, MH has been regarded as an important clinical goal to achieve in patients with UC[3,11]. Studies have in fact indicated

that MH can alter the course of UC, reducing the rate of hospitalization and surgery[19]. Therefore, the evaluation of MH is an important component of the treatment and follow-up of patients with UC. Until now, colonoscopy has been regarded as the gold standard to assess mucosal status in patients with UC. However, colonoscopy is burdensome for patients and carries the possibility of exacerbating the disease status. Furthermore in one study, colonoscopy itself may worsen the disease condition in IBD patients, even in remission state[20]. Therefore, frequent colonoscopy, as required for adequate monitoring of UC status, is difficult due to cost and the inconvenience to patients. To overcome this clinical problem, non-invasive markers of MH have been studied. Although ESR and CRP levels have been used as conventional markers of inflammation, the clinical application of these markers is limited as they reflect systemic inflammation and, therefore, are non-specific to inflammation of the mucosa. FC seems to be promising non-invasive marker of mucosal inflammation and several studies have shown its correlation to the severity of mucosal inflammation. The usefulness of FC has been proven in clinical practice, reliably identifying disease activity in patients with UC who had undergone endoscopicexamination and correlating well with the degree of mucosal inflammation[5-9]. Usually, however, FC can only be performed in tertiary institutions, takes several hours to provide results and is expensive. Moreover, it has been reported that FCresults can exhibit large variation, even between stool samples from the same patient collected on the same day[21].

FIT has been reported from several recent studies as another biomarker[12,13,22]. FC estimate the degree of inflammation in the bowel based on the amount of inflammatory cells, whereas FIT measures the amount of blood from the damaged bowel mucosa[22]. Our study demonstrates that fecal hemoglobin can be used as a marker of endoscopic and clinical disease activityin patients with UC, with a negative FIT accurately reflecting MH and disease remission. Therefore, FIT could provide an "easily available" evaluation of MH to assess the effectiveness of therapy aimed at inducing disease remission in patients with UC. FIT would also be appropriate for repeated evaluations of MH, which is required over the course of clinical remission in patients with UC. In this regard, FIT provides the fast results and at a low financial cost, which could allow physicians to monitor patients with UC more easily and frequently.

FC requires 5-10 g of fecal material for analysis while FIT only requires the probe to be placed in a small sample of stool[6,23]. FIT is available even in smaller institutions, including general primary healthcare. As well, FIT has a unique comparative cutoff value to differentiate disease activity and status, whereas a status cutoff value for FC is currently undetermined[23].

The definition of MH in UC has not been clearly established. Older studies had reported the MH to MES 0 or 1[3], whereas more recent studies have reported MH to MES 0 alone[12]. In recent one study, when the MH to MES 0, FIT appears to be more sensitive than FC for predicting MH (FIT, 95% sensitivity; FC, 82% sensitivity)[22].

The ideal biomarker will be able to detect early relapse in asymptomatic period. Our results also provide evidence of the future possibility of using FIT to identify aggravation of the disease status among patients with a positive FIT. As an example, among our patient group, a 57-year-old male patient, diagnosed with UC 5 years prior, was being treated using azathioprine and oral 5-ASA agents. The colonoscopy and FIT were performed while this patient was classified as being in clinical remission. Although endoscopy findings were negative, with MES of "1" and disease status of E1, a fecal hemoglobin concentration of 74.1 μg/dL was identified on the FIT. Three months later, the patient showed worsening of his clinical symptoms, including persistent bleeding. Sigmoidoscopy conducted at the time of disease exacerbation classified the mucosa as a MES of "3". The patient's symptoms improved after his doses of azathioprine and oral 5-ASA were adjusted. One previous study reported that FIT became higher prior to clinical relapse in some UC patients[17]. In order to accurately prove whether the FIT or FC can detect subclinical relapse in early, well designed studies are required.

The limitations of our study need to be acknowledged in the interpretation and application of our results. First, we conducted a retrospective, single center study that included a small absolute number of patients with UC. Second, although previous studies have used the OC-SENSOR neo system to measure hemoglobin concentrations < 50 ng/mL, in our study we used the HM-JACK system that can detect a negative FIT at < 7 ng/mL. With a lower discrimination threshold, a negative FIT can be a very sensitive test of disease status. Nevertheless, the correlation between the MES "0" and a negative FIT was 100% in our study. Third, all patients did not enforce the same day as the fecal sampling and colonoscopy. Although the fecal sampling 1 wk before or after the colonoscopy, FIT and mucosa state may not be exact match as temporally. Forth, only a single fecal sample was obtained from each patient thus individual variation and sampling variation can arise and lead to incorrect results.

In conclusion, our results show that FIT was a reliable tool to identify the inflammation status of colonic mucosa in patients with UC, especially for identifying clinical and endoscopic remission. As FIT was positively correlated with clinical status, a negative FIT could be regarded as an indication of endoscopic and clinical remission. With a positive FIT, careful observation and follow-up is recommended. Sequential testing using FIT could be helpful to monitor disease activity and to inform clinical decisions to modify treatment, including increasing dose of medication.

Therefore, FIT can bean effective test which can assess the disease activity in patients with UC.

COMMENTS

Background

To date, the evaluation of mucosal inflammation with colonoscopy is the gold standard to assess disease activity in patients with ulcerative colitis (UC). However, colonoscopic examination is difficult to frequently perform due to cost and inconvenience to patients. Therefore, identification of non-invasive biomarkers of disease activity in UC is a research priority.

Research frontiers

Fecal immunochemical test (FIT) is one of them suggested association with mucosa healing in some studies.

Innovations and breakthroughs

The presented results have identified that FIT correlated positively with endoscopic activity and clinical remission, but not with extent of disease. In particular a negative FIT could be regarded as an indication of endoscopic and clinical remission.

Applications

FIT can be a non-invasive and economic biomarker in patients with UC.

Peer-review

In this retrospective study, authors aimed to evaluate the efficacy of quantitative FIT as biomarker of disease activity in UC. This study shows that fit was a reliable tool to identify the inflammation status of colonic mucosa in patients with UC.

REFERENCES

1 **Langholz E**, Munkholm P, Davidsen M, Binder V. Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology* 1994; **107**: 3-11 [PMID: 8020674]

2 **Pineton de Chambrun G**, Peyrin-Biroulet L, Lémann M, Colombel JF. Clinical implications of mucosal healing for the management of IBD. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 15-29 [PMID: 19949430 DOI: 10.1038/nrgastro.2009.203]

3 **Colombel JF**, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, Marano CW, Strauss R, Oddens BJ, Feagan BG, Hanauer SB, Lichtenstein GR, Present D, Sands BE, Sandborn WJ. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* 2011; **141**: 1194-1201 [PMID: 21723220 DOI: 10.1053/j.gastro.2011.06.054]

4 **Ardizzone S**, Cassinotti A, Duca P, Mazzali C, Penati C, Manes G, Marmo R, Massari A, Molteni P, Maconi G, Porro GB. Mucosal healing predicts late outcomes after the first course of corticosteroids for newly diagnosed ulcerative colitis. *Clin Gastroenterol Hepatol* 2011; **9**: 483-489.e3 [PMID: 21195796 DOI: 10.1016/j.cgh.2010.12.028]

5 **Tibble JA**, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology* 2000; **119**: 15-22 [PMID: 10889150]

6 **Costa F**, Mumolo MG, Bellini M, Romano MR, Ceccarelli L, Arpe P, Sterpi C, Marchi S, Maltinti G. Role of faecal calprotectin as non-invasive marker of intestinal inflammation. *Dig Liver Dis* 2003; **35**: 642-647 [PMID: 14563186]

7 **Costa F**, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, Ricchiuti A, Marchi S, Bottai M. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut* 2005; **54**: 364-368 [PMID: 15710984 DOI: 10.1136/gut.2004.043406]

8 **Schoepfer AM**, Beglinger C, Straumann A, Trummler M, Renzulli P, Seibold F. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. *Inflamm Bowel Dis* 2009; **15**: 1851-1858 [PMID: 19462421 DOI: 10.1002/ibd.20986]

9 **Schoepfer AM**, Beglinger C, Straumann A, Safroneeva E, Romero Y, Armstrong D, Schmidt C, Trummler M, Pittet V, Vavricka SR. Fecal calprotectin more accurately reflects endoscopic activity of ulcerative colitis than the Lichtiger Index, C-reactive protein, platelets, hemoglobin, and blood leukocytes. *Inflamm Bowel Dis* 2013; **19**: 332-341 [PMID: 23328771 DOI: 10.1097/MIB.0b013e3182810066]

10 **Chen LS**, Yen AM, Chiu SY, Liao CS, Chen HH. Baseline faecal occult blood concentration as a predictor of incident colorectal neoplasia: longitudinal follow-up of a Taiwanese population-based colorectal cancer screening cohort. *Lancet Oncol* 2011; **12**: 551-558 [PMID: 21592859 DOI: 10.1016/S1470-2045(11)70101-2]

11 **Schnitzler F**, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, Hoffman I, Van Steen K, Vermeire S, Rutgeerts P. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 1295-1301 [PMID: 19340881 DOI: 10.1002/ibd.20927]

12 **Nakarai A**, Kato J, Hiraoka S, Kuriyama M, Akita M, Hirakawa T, Okada H, Yamamoto K. Evaluation of mucosal healing of ulcerative colitis by a quantitative fecal immunochemical test. *Am J Gastroenterol* 2013; **108**: 83-89 [PMID: 23007005 DOI: 10.1038/2012.315]

13 **Mooiweer E**, Fidder HH, Siersema PD, Laheij RJ, Oldenburg B. Fecal hemoglobin and calprotectin are equally effective in identifying patients with inflammatory bowel disease with active endoscopic inflammation. *Inflamm Bowel Dis* 2014; **20**: 307-314 [PMID: 24374878 DOI: 10.1097/01.MIB.0000438428.30800.a6]

14 **Dignass A**, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, Mantzaris G, Reinisch W, Colombel JF, Vermeire S, Travis S, Lindsay JO, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. *J Crohns Colitis* 2012; **6**: 965-990 [PMID: 23040452 DOI: 10.1016/j.crohns.2012.09.003]

15 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5A-36A [PMID: 16151544]

16 **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]

17 **Kuriyama M**, Kato J, Takemoto K, Hiraoka S, Okada H, Yamamoto K. Prediction of flare-ups of ulcerative colitis using quantitative immunochemical fecal occult blood test. *World J Gastroenterol* 2010; **16**: 1110-1114 [PMID: 20205282 DOI: 10.3748/wjg.v16.i9.1110]

18 **Schroeder KW**, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med* 1987; **317**: 1625-1629 [PMID: 3317057 DOI: 10.1056/NEJM198712243172603]

19 **Frøslie KF**, Jahnsen J, Moum BA, Vatn MH. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. *Gastroenterology* 2007; **133**: 412-422 [PMID: 17681162 DOI: 10.1053/j.gastro.2007.05.051]

20 **Menees S**, Higgins P, Korsnes S, Elta G. Does colonoscopy cause increased ulcerative colitis symptoms? *Inflamm Bowel Dis* 2007; **13**: 12-18 [PMID: 17206634 DOI: 10.1002/ibd.20049]

21 **Lasson A**, Stotzer PO, Öhman L, Isaksson S, Sapnara M, Strid H. The intra-individual variability of faecal calprotectin: a prospective study in patients with active ulcerative colitis. *J Crohns Colitis* 2015; **9**: 26-32 [PMID: 25008478 DOI: 10.1016/

j.crohns.2014.06.002]

22 **Kato J**, Hiraoka S, Nakarai A, Takashima S, Inokuchi T, Ichinose M. Fecal immunochemical test as a biomarker for inflammatory bowel diseases: can it rival fecal calprotectin? *Intest Res* 2016; **14**: 5-14 [PMID: 26884729 DOI: 10.5217/ir.2016.14.1.5]

23 **Vilkin A**, Rozen P, Levi Z, Waked A, Maoz E, Birkenfeld S, Niv Y. Performance characteristics and evaluation of an automated-developed and quantitative, immunochemical, fecal occult blood screening test. *Am J Gastroenterol* 2005; **100**: 2519-2525 [PMID: 16279909 DOI: 10.1111/j.1572-0241.2005.00231.x]

P- Reviewer: Yuksel I **S- Editor**: Yu J **L- Editor**: A
E- Editor: Zhang FF

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.v22.i48.10625

World J Gastroenterol 2016 December 28; 22(48): 10625-10630
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Capsule endoscopy and single-balloon enteroscopy in small bowel diseases: Competing or complementary?

Jing-Jing Ma, Ying Wang, Xiao-Min Xu, Jie-Wen Su, Wen-Yu Jiang, Jian-Xia Jiang, Lin Lin, Dao-Quan Zhang, Jing Ding, Li Chen, Ting Jiang, Ying-Hong Xu, Gui Tao, Hong-Jie Zhang

Jing-Jing Ma, Xiao-Min Xu, Jie-Wen Su, Wen-Yu Jiang, Jian-Xia Jiang, Lin Lin, Dao-Quan Zhang, Jing Ding, Li Chen, Ting Jiang, Ying-Hong Xu, Gui Tao, Hong-Jie Zhang, Department of Gastroenterology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Ying Wang, Department of Gastroenterology, Jiangsu Provincial Hospital, Nanjing 210024, Jiangsu Province, China

Author contributions: Ma JJ, Wang Y and Xu XM contributed equally to this study; Ma JJ and Wang Y performed the study; Ma JJ, Xu XM, Su JW and Jiang WY carried out data analysis and wrote the paper; Ma JJ, Wang Y, Jiang JX, Lin L, Zhang DQ, Ding J, Chen L, Jiang T, Xu YH, Tao G and Zhang HJ performed capsule endoscopy and single balloon enteroscopy; Ma JJ and Zhang HJ designed the study; Zhang HJ supervised the report; all authors read and approved the final version to be published.

Supported by the Key Medical Personnel of Jiangsu Province, No. RC2011063.

Institutional review board statement: This study was reviewed and approved by the institutional review board of the First Affiliated Hospital of Nanjing Medical University.

Informed consent statement: All patients, or their legal guardians, provided informed written consent prior to capsule endoscopy or single-balloon enteroscopy. Patients were not required to give informed consent for inclusion in the study, as the analysis used anonymous clinical data. Individuals cannot be identified according to the data presented.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Hong-Jie Zhang, PhD, Department of Gastroenterology, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, Jiangsu Province, China. hjzhang06@163.com
Telephone: +86-25-83718836-6920
Fax: +86-25-83674636

Received: August 21, 2016
Peer-review started: August 22, 2016
First decision: October 11, 2016
Revised: October 25, 2016
Accepted: November 14, 2016
Article in press: November 16, 2016
Published online: December 28, 2016

Abstract

AIM

To evaluate diagnostic yields of capsule endoscopy (CE) and/or single-balloon enteroscopy (SBE) in patients with suspected small bowel diseases.

METHODS

We retrospectively analyzed 700 patients with suspected small bowel diseases from September 2010 to March 2016. CE, SBE, or SBE with prior CE was performed in 401, 353, and 47 patients, respectively. Data from clinical and endoscopy records were collected for analysis. Indications, procedure times, diagnostic yields, and complications were summarized and evaluated.

RESULTS

The overall diagnostic yield for the CE group was 57.6%. The diagnostic yield of CE in patients with obscure gastrointestinal bleeding (OGIB) was significantly greater than that in patients with no bleeding (70.5% *vs* 43.8%, $P < 0.01$). The overall diagnostic yield of SBE was 69.7%. There was no difference in the diagnostic yield of SBE between patients with OGIB and those with no bleeding (72.5% *vs* 68.9%, $P = 0.534$). Forty-seven patients underwent CE prior to SBE. Among them, the diagnostic yield of SBE with positive findings on prior CE was 93.3%. In addition, SBE detected two cases with superficial ulcer and erosive lesions in the small bowel, which were missed by CE. However, one case with lymphoma and two with Crohn's disease were not confirmed by SBE. The rate of capsule retention was 2.0%. There were no significant complications during or after SBE examinations.

CONCLUSION

SBE is a safe and effective technique for diagnosing small bowel diseases. SBE with prior CE seemed to improve the diagnostic yield of small bowel diseases.

Key words: Capsule endoscopy; Small bowel diseases; Single-balloon enteroscopy; Diagnosis; Balloon-assisted enteroscopy

Core tip: The aims of this study were to evaluate diagnostic yields associated with capsule endoscopy (CE), single-balloon enteroscopy (SBE), or their combined use in patients with suspected small bowel diseases, as well as to demonstrate the appropriate diagnostic algorithms for diagnosing different small bowel diseases. This study revealed the diagnostic yield of SBE with positive findings on prior CE was high (93.3%). CE followed by SBE represents an especially effective strategy for determining the cause of small bowel disease when findings from an initial CE examination are indeterminate.

Ma JJ, Wang Y, Xu XM, Su JW, Jiang WY, Jiang JX, Lin L, Zhang DQ, Ding J, Chen L, Jiang T, Xu YH, Tao G, Zhang HJ. Capsule endoscopy and single-balloon enteroscopy in small bowel diseases: Competing or complementary? *World J Gastroenterol* 2016; 22(48): 10625-10630 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10625.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10625

INTRODUCTION

The small bowel has long been considered by gastroenterologists as a "black box" due to its length and complex anatomy. Before 2000, it was not possible to reach most of the small bowel using conventional endoscopic techniques, and thus the diagnosis of small bowel diseases has been a challenge for gastroenterologists. The development of capsule endoscopy (CE) and balloon-assisted enteroscopy (BAE) represents a decisive breakthrough in the field. CE is painless and can be used to explore the entire small bowel in a single examination, making it the best choice for an initial diagnostic examination when a patient is suspected of possessing small bowel disease[1-3]. However, CE has some technical limitations, including a lack of therapeutic capability and the risk of capsule retention.

BAE was introduced as a breakthrough technique for examining the deep small bowel, and comprises double-balloon endoscopy (DBE) and single-balloon enteroscopy (SBE). DBE was introduced in 2001 and is considered the standard technique of deep endoscopy for visualizing the small bowel and enabling endoscopists to perform therapeutic interventions; however, it must be noted that the preparation and handling of DBE is complex. SBE was introduced in 2008, which has a simpler and easier-to-handle small bowel endoscopy system. Although SBE may be less efficient in terms of depth of insertion and complete visualization of the small bowel when compared with DBE, some studies have shown that SBE is not inferior to DBE with respect to diagnostic yield[4]. Both CE and BAE are reported to have similarly high diagnostic yields for small bowel diseases[5,6]. DBE is considered an effective complementary technique which can be used after initial diagnostic CE examination[7]. However, there are comparatively limited data on the role of CE alone and in combination with SBE for assessment of small bowel diseases[8].

We performed a retrospective study with the aim to: (1) compare the diagnostic yields of CE, SBE, or their combined use; (2) determine their performance characteristics in patients with suspected small bowel diseases; and (3) demonstrate the appropriate diagnostic algorithms for different small bowel diseases.

MATERIALS AND METHODS

Study design and patient selection

We retrospectively analyzed the records of 700 patients suspected of small bowel diseases who underwent CE and/or SBE between September 2010 and March 2016 at the First Affiliated Hospital of Nanjing Medical University. All patients underwent routine clinical examinations and laboratory tests (including hemoglobin level and stool tests), abdominal ultrasound or computed tomography (CT), upper gastrointestinal endoscopy, and colonoscopy. CE and SBE were performed after obtaining informed consent from the patients. Indications for the study included obscure gastrointestinal bleeding (OGIB), abdominal pain, diarrhea, or other symptoms. The characteristics of all patients and procedures were extracted from

Table 1 Patient characteristics and indications for single-balloon enteroscopy and capsule endoscopy

	CE	SBE	Both (CE prior to SBE)	*P* value (CE *vs* SBE)
No. of patients	401	353	47	
Age (yr)	49.4 ± 16.0	42.1 ± 15.8	45.3 ± 15.1	< 0.01
Mean (range)	(13-85)	(11-84)	(15-77)	
Male/female	248/153	235/118	38/9	0.177
Main indications, *n* (%)				
OGIB	207 (51.6)	80 (22.7)	30 (63.8)	
Abdominal pain	133 (31.2)	184 (52.1)	11 (23.4)	
Diarrhea	30 (7.5)	52 (14.7)	1 (2.1)	
Other	31 (7.7)	37 (10.5)	5 (10.6)	

CE: Capsule endoscopy; SBE: Single-balloon enteroscopy; OGIB: Obscure gastrointestinal bleeding.

electronic medical records and the endoscopy reporting system. Final diagnosis was based on SBE findings, CE findings, surgical pathology, and/or clinical follow-up. Diagnostic yield was calculated by dividing the total number of patients who underwent the procedure by the number of cases with positive findings that could explain the patient's symptoms. This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University.

CE procedure

CE studies were performed using the OMOM CE system (Jinshan Science and Technology Company, Chongqing, China) or MiroCam™ system (IntroMedic, Seoul, South Korea). Each patient underwent bowel preparation with 3 L polyethylene glycol solution the day before the procedure, and then fasted overnight. Collected CE data included gastric transit time (GTT), small bowel transit time (SBTT), abnormal findings during the procedure, total recording time, quality of bowel preparation, and complete visualization rate of the small bowel. The complete video of each CE examination was viewed by two independent and experienced gastroenterologists.

SBE procedure

SBE procedures were performed using the SBE endoscope system (SIF-Q260; Olympus, Tokyo, Japan). For anterograde SBE, patients generally needed an overnight fast. For a retrograde approach, patients underwent bowel preparation with 3 L polyethylene glycol solution the day before the procedure, and then fasted overnight. The examination itself was carried out with conventional sedation with propofol and opioid. All procedures were performed by one of three experienced endoscopists, each of whom had previously conducted at least 50 SBE procedures. Procedures were carried out *via* the anterograde or retrograde approach, depending on whether the suspected pathology was in the proximal or distal small bowel.

Statistical analysis

Continuous data are expressed as mean ± SD and range, and categorical data are showed as percentages. Student's *t* test was used to compare age distributions between the CE and SBE groups. The χ^2 test was used to compare positive-detection rates and sex distribution between the CE and SBE groups. $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using SPSS version 20.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

Patient characteristics

Seven hundred patients who underwent CE and/or SBE were reviewed in the present study. Of these, 401 individuals (248 male, 153 female; mean age, 49.4 ± 16.0 years) underwent 404 CE procedures; 353 individuals (235 male, 118 female; mean age, 42.1 ± 15.8 years) underwent 419 SBE procedures; and 47 individuals (38 male, 9 female; mean age, 45.3 ± 15.1 years) underwent both CE and SBE (CE first) (Table 1). Main indications for CE and/or SBE were OGIB (37.1%, 243 with overt OGIB, and 17 with occult OGIB), chronic abdominal pain (42.6%), chronic diarrhea (11.3%), and other complaints (9.0%). The demographic data of these patients are shown in Table 1. The mean age of these patients was 46.1 ± 16.5 years (range, 11-85 years). There was no significant difference in sex distribution between the CE and SBE group ($P = 0.177$). The average age of the CE group was older compared with the SBE group ($P < 0.01$).

CE

A total of 401 patients underwent 404 CE procedures. Complete visualization of the small bowel was achieved in 73.5% (297/404). The mean recording time was 555 min ± 115 min (192-721 min). Mean GTT was 51 min ± 62 min (range 1-565 min) and mean SBTT was 352 min ± 157 min (range 33-715 min). The overall diagnostic yield for small bowel disease by CE was 57.6% (231/401). The main findings included: mucosal erosion and superficial ulcer in 98 patients (42.4%), angiodysplasia in 67 (29.0%), Crohn's disease (CD) in 26 (11.3%), and masses (tumors and polyps) in 24 (10.4%). Other findings were parasites in 5.6% (13/231), diverticulum in 2.6% (6/231), and ongoing bleeding in 2.2% (5/231) (Table 2, Supplementary

Table 2 Comparison of findings between single-balloon enteroscopy and capsule endoscopy *n* (%)

Findings	CE (*n* = 401)	SBE (*n* = 353)
Overall detection rate	231 (57.6)	246 (69.7)
Superficial ulcer and erosion	98 (42.4)	111 (45.1)
Angiodysplasia	67 (29.0)	21 (8.5)
Mass	24 (10.4)	26 (10.6)
Crohn's disease	26 (11.3)	86 (35.0)
Parasites	13 (5.6)	1 (0.4)
Diverticulum	6 (2.6)	3 (1.2)
Bleeding	5 (2.2)	0

CE: Capsule endoscopy; SBE: Single-balloon enteroscopy.

Figure 1). The diagnostic yield of CE in patients with OGIB was greater than that in those with no bleeding (70.5% *vs* 43.8%, $P < 0.01$) (Table 3). For eight patients, capsules were retained at the lesion sites, leading to a capsule-retention rate of 2.0%. Five of these patients were diagnosed with CD, two were diagnosed with lymphoma, and another patient had diverticulum with ulceration. Retained capsules were subsequently removed *via* surgery. We also examined whether there was any difference between the OMOM system and MiroCam system. Complete visualization of the small bowel was achieved in 72.4% (197/272) with OMOM and 78.8% (104/132) with MiroCam ($P = 0.169$). The overall diagnostic yield for small bowel diseases was 57.2% (155/271) by OMOM CE and 60.8% (79/130) by MiroCam ($P = 0.497$). The results showed no significant difference with regards to the rates of complete small-bowel examination or diagnostic yields between MiroCam and OMOM capsule endoscopy (Supplementary Table 1).

SBE

A total of 419 SBE procedures were performed in 353 patients: 98 anterograde and 321 retrograde procedures were conducted, as well as 24 combined anterograde and retrograde SBEs. No adverse events occurred during or after these procedures. The mean examination time was 65.5 min ± 26.6 min (15-120 min). The overall diagnostic yield for small bowel disease by SBE was 69.7% (246/353). The main findings were as follows: mucosal erosion and superficial ulceration in 111 patients (45.1%), CD in 86 (35.0%), angiodysplasia in 21 (8.5%), and masses (tumors and polyps) in 26 (10.6%). Other findings were diverticulum (1.2%, 3/246) and parasites (0.4%, 1/246) (Table 2, Supplementary Figure 2). The diagnostic yield for small bowel diseases by SBE was greater than that by CE (69.7% *vs* 57.6%). There was no significant difference in the diagnostic yield of SBE between patients with OGIB and those with no bleeding (72.5% *vs* 68.9%, $P = 0.534$). In a subgroup analysis, the diagnostic yield for OGIB by SBE was similar to by CE (72.5% *vs* 70.5%). In addition, the diagnostic yield for small bowel diseases in patients with no bleeding tended to be greater using SBE compared with CE (68.9% *vs* 43.8%, $P < 0.01$) (Table 3).

Table 3 Subgroup analysis of the diagnostic yield of capsule endoscopy or single-balloon enteroscopy in patients with obscure gastrointestinal bleeding or non-bleeding patients

	Diagnostic yield (%)		*P* value
	OGIB	Non-bleeding	
CE	70.5	43.8	< 0.01
SBE	72.5	68.9	0.534

OGIB: Obscure gastrointestinal bleeding; CE: Capsule endoscopy; SBE: Single-balloon enteroscopy.

CE combined with SBE

Forty-seven patients underwent CE (including 30 with OGIB, 11 with abdominal pain, 1 with diarrhea, and 5 with weight loss) and were subsequently subjected to SBE. The small intestinal findings on SBE in patients with negative evaluation or definite findings on CE are shown in Table 4. Of 47 patients, 45 had positive findings by CE examination followed by SBE and 42 had positive findings by SBE examination. The diagnostic yield of SBE with findings on prior CE was 93.3% (42/45), which was a high diagnostic yield. Two cases of superficial ulcer and mucosal erosion that were missed by CE were found by SBE. However, CE also detected one mass and two cases of CD that were not detected by SBE.

DISCUSSION

Current options for diagnosing small bowel diseases include push enteroscopy, CE, DBE, SBE, and intraoperative enteroscopy. Push enteroscopy has a limited depth of insertion. Intraoperative enteroscopy is the most invasive method and its use has diminished with the development of CE and BAE. CE is widely used to screen for various small bowel diseases, but is limited by a lack of therapeutic ability, as well as imprecise localization and the required collection of biopsy specimens. DBE is a deep enteroscopy technique that overcomes these shortcomings, but has the disadvantages of complex preparation and handling procedures. SBE was recently introduced as an alternative deep enteroscopy technique, with some studies demonstrating that it can provide a high diagnostic yield and enable therapeutic interventions[9-12]. In the present study, the diagnostic yield of SBE for small bowel diseases was 69.7%, which suggests that SBE has a high diagnostic yield. However, previous studies showed the complete visualization rate of the small bowel using SBE was lower than when using DBE; the rate of complete enteroscopy using DBE was 40%-80%, while using SBE was 0%-25%[13]. This indicated that CE, DBE, and SBE all have advantages and limitations. It is therefore important to select the

Table 4 Identification of positive findings on prior capsule endoscopy or single-balloon enteroscopy

Findings	CE: negative diagnosis SBE: definite diagnosis	CE: definite diagnosis SBE: definite diagnosis	CE: definite diagnosis SBE: negative diagnosis
Angiodysplasia		9	
Erosion and superficial ulcer	2	18	
Mass		3	1
Crohn's disease		11	2
Parasites		1	

CE: Capsule endoscopy; SBE: Single-balloon enteroscopy.

appropriate diagnostic algorithms when small bowel disease is suspected, and should be made on a case-by-case basis and dependent on clinical scenario, diagnostic yield, involved risks, availability, and patient preference.

In this study, OGIB was a common indication for small bowel endoscopy. Unless contraindicated, CE is recommended as the initial diagnostic test for patients with suspected OGIB[14], as it is minimally invasive, easily tolerated, and can theoretically visualize the entire small bowel. Here, the diagnostic yield of CE for small bowel abnormalities in patients with OGIB was 70.5%, with this result being supported by previous studies[15,16]. CE and BAE are also considered complementary procedures for the evaluation and treatment of OGIB[17-20]. Previous studies have supported using the non-invasive CE technique for patients with OGIB, with a subsequent DBE examination if necessary[21]. In the present study, CE found small bowel lesions in 30 patients with OGIB who were subsequently subjected to SBE. Twenty-eight patients had confirmed diagnosis by SBE examination. If false-negative rates were considered, our data suggested that both SBE and CE did miss some lesions. This study supports the belief that CE evaluation should remain the preferred initial strategy for patients with OGIB because of its relative non-invasiveness and acceptable diagnostic yield. However, SBE is useful in cases in which the CE result is ambiguous and further examination or a biopsy is required. For patients with no bleeding, previous studies have not detected a difference between DBE and CE in identifying small bowel abnormalities[22]. However, we found that for identifying small bowel abnormalities in patients with no bleeding, the diagnostic yield of SBE was higher than that of CE (68.9% *vs* 43.8%).

SBE has the potential to become a useful technique for deep enteroscopy, as it has a reasonable depth of insertion, can be administered using standard conscious sedation, and can be used with existing endoscopy systems. In addition, the SBE technique is easy to learn and can be rapidly incorporated into an endoscopy unit[23,24]. In our study, SBE generated a high diagnostic yield for small bowel diseases (overall diagnostic yield, 69.7%), as well as for patients with OGIB and those without bleeding (72.5% *vs* 68.5%). A previous study recommended an initial CE examination that should be followed by DBE if necessary[7]. Here, we combined CE and SBE techniques to detect small bowel diseases and found that 45 patients had positive findings by CE examination followed by SBE, and 42 patients had positive findings by SBE examination. The diagnostic yield of SBE with prior CE was 93.3% (42/45), which was a high diagnostic yield.

In summary, SBE appears to be a safe and effective method for diagnosing small bowel disease, especially for patients with OGIB. CE followed by SBE represents an especially effective strategy for determining the cause of small bowel disease when findings from initial CE examinations are indeterminate.

COMMENTS

Background

The diagnosis of small bowel diseases was difficult until the advent of capsule endoscopy (CE) and balloon-assisted enteroscopy (BAE). Both CE and BAE were reported to have similarly high diagnostic yields of small bowel disorders. Single balloon enteroscopy (SBE), which is an alternative technique of double balloon enteroscopy (DBE) for examining the deep small bowel, is simpler and easier to handle. There is limited data on the role of CE, both in comparison and combination with SBE, in the assessment of small bowel diseases.

Research frontiers

In this study, the authors aimed to evaluate the diagnostic yields associated with CE, SBE, or their combined use in patients with suspected small bowel diseases, as well as demonstrate the appropriate selection for different small bowel diseases.

Innovations and breakthroughs

This study was a single-center experience in China involving 700 patients who underwent CE and/or SBE. The diagnostic yield difference in detecting small bowel diseases between CE and SBE was evaluated. The diagnostic yields of different indications and findings of CE and/or SBE were analyzed in detail. At the same time, the advantage of SBE combined with prior CE was also evaluated.

Applications

Both CE and SBE have high diagnostic yields of small bowel disorders. SBE has a similar diagnostic yield for patients with obscure gastrointestinal bleeding and a higher diagnostic yield with non-bleeding compared with CE. CE followed by SBE represents an especially effective strategy for determining small bowel disease.

Terminology

CE refers to a miniature capsule-shaped camera that takes multiple pictures as its passes through the small intestine. SBE is a method of enteroscopy that can lead to the observation of the small intestine via the mouth or anus with the help of a balloon attached to the distal end of a soft overtube.

Peer-review

This is an interesting study that shows the role of small bowel evaluation in CE and SBE. It deserves to be published, as it will add to the literature on the subject. It shows very good language and presentation of data.

REFERENCES

1 **Rey JF**, Ladas S, Alhassani A, Kuznetsov K. European Society of Gastrointestinal Endoscopy (ESGE). Video capsule endoscopy: update to guidelines (May 2006). *Endoscopy* 2006; **38**: 1047-1053 [PMID: 17058174 DOI: 10.1055/s-2006-944874]

2 **Ginsberg GG**, Barkun AN, Bosco JJ, Isenberg GA, Nguyen CC, Petersen BT, Silverman WB, Slivka A, Taitelbaum G. Wireless capsule endoscopy: August 2002. *Gastrointest Endosc* 2002; **56**: 621-624 [PMID: 12397265 DOI: 10.1016/S0016-5107(02)70106-5]

3 **Leighton JA**, Goldstein J, Hirota W, Jacobson BC, Johanson JF, Mallery JS, Peterson K, Waring JP, Fanelli RD, Wheeler-Harbaugh J, Baron TH, Faigel DO. Obscure gastrointestinal bleeding. *Gastrointest Endosc* 2003; **58**: 650-655 [PMID: 14595294 DOI: 10.1016/S0016-5107(03)01995-3]

4 **Domagk D**, Mensink P, Aktas H, Lenz P, Meister T, Luegering A, Ullerich H, Aabakken L, Heinecke A, Domschke W, Kuipers E, Bretthauer M. Single- vs. double-balloon enteroscopy in small-bowel diagnostics: a randomized multicenter trial. *Endoscopy* 2011; **43**: 472-476 [PMID: 21384320 DOI: 10.1055/s-0030-1256247]

5 **Kalra AS**, Walker AJ, Benson ME, Soni A, Guda NM, Misha M, Gopal DV. Comparison of Capsule Endoscopy Findings to Subsequent Double Balloon Enteroscopy: A Dual Center Experience. *Diagn Ther Endosc* 2015; **2015**: 438757 [PMID: 26420979]

6 **Hong SN**, Kim ER, Ye BD, Jang HJ, Jeon SR, Park SJ, Im JP, Kim JH, Choi CH, Choi H, Chang DK. Indications, diagnostic yield, and complication rate of balloon-assisted enteroscopy (BAE) during the first decade of its use in Korea. *Dig Endosc* 2015; Epub ahead of print [PMID: 26688319]

7 **Sidhu R**, McAlindon ME, Drew K, Hardcastle S, Cameron IC, Sanders DS. Evaluating the role of small-bowel endoscopy in clinical practice: the largest single-centre experience. *Eur J Gastroenterol Hepatol* 2012; **24**: 513-519 [PMID: 22330235 DOI: 10.1097/MEG.0b013e328350fb05]

8 **Sethi S**, Cohen J, Thaker AM, Garud S, Sawhney MS, Chuttani R, Pleskow DK, Falchuk K, Berzin TM. Prior capsule endoscopy improves the diagnostic and therapeutic yield of single-balloon enteroscopy. *Dig Dis Sci* 2014; **59**: 2497-2502 [PMID: 24798998 DOI: 10.1007/s10620-014-3178-3]

9 **Frantz DJ**, Dellon ES, Grimm IS, Morgan DR. Single-balloon enteroscopy: results from an initial experience at a U.S. tertiary-care center. *Gastrointest Endosc* 2010; **72**: 422-426 [PMID: 20541189 DOI: 10.1016/j.gie.2010.03.1117]

10 **Ramchandani M,** Reddy DN, Gupta R, Lakhtakia S, Tandan M, Rao GV, Darisetty S. Diagnostic yield and therapeutic impact of single-balloon enteroscopy: series of 106 cases. *J Gastroenterol Hepatol* 2009; **24**: 1631-1638 [PMID: 19686408 DOI: 10.1111/j.1440-1746.2009.05936.x]

11 **Upchurch BR**, Sanaka MR, Lopez AR, Vargo JJ. The clinical utility of single-balloon enteroscopy: a single-center experience of 172 procedures. *Gastrointest Endosc* 2010; **71**: 1218-1223 [PMID: 20409544 DOI: 10.1016/j.gie.2010.01.012]

12 **Aktas H**, de Ridder L, Haringsma J, Kuipers EJ, Mensink PB. Complications of single-balloon enteroscopy: a prospective evaluation of 166 procedures. *Endoscopy* 2010; **42**: 365-368 [PMID: 20178072 DOI: 10.1055/s-0029-1243931]

13 **Teshima CW**. Small bowel endoscopy for obscure GI bleeding. *Best Pract Res Clin Gastroenterol* 2012; **26**: 247-261 [PMID: 22704568 DOI: 10.1016/j.bpg.2012.01.020]

14 **Sidhu R**, Sanders DS, Morris AJ, McAlindon ME. Guidelines on small bowel enteroscopy and capsule endoscopy in adults. *Gut* 2008; **57**: 125-136 [PMID: 18094205 DOI: 10.1136/gut.2007.129999]

15 **Nakamura M**, Niwa Y, Ohmiya N, Miyahara R, Ohashi A, Itoh A, Hirooka Y, Goto H. Preliminary comparison of capsule endoscopy and double-balloon enteroscopy in patients with suspected small-bowel bleeding. *Endoscopy* 2006; **38**: 59-66 [PMID: 16429356 DOI: 10.1055/s-2005-870446]

16 **Hadithi M**, Heine GD, Jacobs MA, van Bodegraven AA, Mulder CJ. A prospective study comparing video capsule endoscopy with double-balloon enteroscopy in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2006; **101**: 52-57 [PMID: 16405533 DOI: 10.1111/j.1572-0241.2005.00346.x]

17 **Arakawa D**, Ohmiya N, Nakamura M, Honda W, Shirai O, Itoh A, Hirooka Y, Niwa Y, Maeda O, Ando T, Goto H. Outcome after enteroscopy for patients with obscure GI bleeding: diagnostic comparison between double-balloon endoscopy and videocapsule endoscopy. *Gastrointest Endosc* 2009; **69**: 866-874 [PMID: 19136098 DOI: 10.1016/j.gie.2008.06.008]

18 **Li X**, Dai J, Lu H, Gao Y, Chen H, Ge Z. A prospective study on evaluating the diagnostic yield of video capsule endoscopy followed by directed double-balloon enteroscopy in patients with obscure gastrointestinal bleeding. *Dig Dis Sci* 2010; **55**: 1704-1710 [PMID: 19672712 DOI: 10.1007/s10620-009-0911-4]

19 **Kameda N**, Higuchi K, Shiba M, Machida H, Okazaki H, Yamagami H, Tanigawa T, Watanabe K, Watanabe T, Tominaga K, Fujiwara Y, Oshitani N, Arakawa T. A prospective, single-blind trial comparing wireless capsule endoscopy and double-balloon enteroscopy in patients with obscure gastrointestinal bleeding. *J Gastroenterol* 2008; **43**: 434-440 [PMID: 18600387 DOI: 10.1007/s00535-008-2182-9]

20 **Bar-Meir S**. Video capsule endoscopy or double-balloon enteroscopy: are they equivalent? *Gastrointest Endosc* 2009; **69**: 875-876 [PMID: 19327474 DOI: 10.1016/j.gie.2008.07.051]

21 **Shishido T**, Oka S, Tanaka S, Aoyama T, Watari I, Imagawa H, Yoshida S, Chayama K. Diagnostic yield of capsule endoscopy vs. double-balloon endoscopy for patients who have undergone total enteroscopy with obscure gastrointestinal bleeding. *Hepatogastroenterology* 2012; **59**: 955-959 [PMID: 22580642]

22 **Li XB**, Ge ZZ, Dai J, Gao YJ, Liu WZ, Hu YB, Xiao SD. The role of capsule endoscopy combined with double-balloon enteroscopy in diagnosis of small bowel diseases. *Chin Med J* (Engl) 2007; **120**: 30-35 [PMID: 17254484]

23 **Tsujikawa T**, Saitoh Y, Andoh A, Imaeda H, Hata K, Minematsu H, Senoh K, Hayafuji K, Ogawa A, Nakahara T, Sasaki M, Fujiyama Y. Novel single-balloon enteroscopy for diagnosis and treatment of the small intestine: preliminary experiences. *Endoscopy* 2008; **40**: 11-15 [PMID: 18058613 DOI: 10.1055/s-2007-966976]

24 **Kawamura T**, Yasuda K, Tanaka K, Uno K, Ueda M, Sanada K, Nakajima M. Clinical evaluation of a newly developed single-balloon enteroscope. *Gastrointest Endosc* 2008; **68**: 1112-1116 [PMID: 18599052 DOI: 10.1016/j.gie.2008.03.1063]

P- Reviewer: Christodoulou DK, de'Angelis GL, Kawano S
S- Editor: Yu J **L- Editor**: Rutherford A **E- Editor**: Liu WX

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.v22.i48.10631

World J Gastroenterol 2016 December 28; 22(48): 10631-10642
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Clinical Trials Study

Irritable bowel syndrome symptom severity improves equally with probiotic and placebo

Anna Lyra, Markku Hillilä, Teppo Huttunen, Sofia Männikkö, Mikko Taalikka, Julia Tennilä, Anneli Tarpila, Sampo Lahtinen, Arthur C Ouwehand, Lea Veijola

Anna Lyra, Julia Tennilä, Anneli Tarpila, Sampo Lahtinen, Arthur C Ouwehand, DuPont Nutrition and Health, Global Health and Nutrition Science, Danisco Sweeteners Oy, 02460 Kantvik, Finland

Markku Hillilä, Clinic of Gastroenterology, University of Helsinki and University Hospital, 00290 Helsinki, Finland

Teppo Huttunen, Sofia Männikkö, Mikko Taalikka, 4Pharma Ltd, Lemminkäisenkatu 1, 20520 Turku, Finland

Lea Veijola, Mehiläinen Oy, Pohjoinen Hesperiankatu 17, 00260 Helsinki, Finland

Author contributions: Lyra A, Hillilä M, Huttunen T, Männikkö S, Taalikka M, Tennilä J, Tarpila A, Lahtinen S, Ouwehand AC and Veijola L designed the study; Veijola L was the principal investigator and Hillilä M was the co-investigator; Lyra A and Ouwehand AC managed the trial; Huttunen T and Männikkö S were responsible for the statistics; Taalikka M performed the data management; Tennilä J and Tarpila A monitored the trial; Lyra A, Hillilä M, Huttunen T, Männikkö S, Taalikka M, Tennilä J, Ouwehand AC, and Veijola L wrote the manuscript; all authors have read and approved the manuscript.

Institutional review board statement: This study was reviewed and approved by the Hospital District of Helsinki and Uusimaa (HUS) Ethics Committee of Medicine (initial ethical approval 251/13/03/01/2012 dated 15.8.2012, received 17.8.2012).

Clinical trial registration statement: This study has been registered at Clinicaltrials.gov. The registration identification number is NCT01728610. The study was registered before study recruitment commenced.

Informed consent statement: All volunteers provided written informed consent before any study-specific procedures or assessments were performed.

Conflict-of-interest statement: Anna Lyra, Julia Tennilä, Anneli Tarpila, Sampo Lahtinen, and Arthur C Ouwehand are present or former employees of the sponsor, DuPont Nutrition and Health.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Anna Lyra, PhD, Senior Scientist, DuPont Nutrition and Health, Global Health and Nutrition Science, Danisco Sweeteners Oy, Sokeritehtaantie 20, 02460 Kantvik, Finland. anna.lyra@dupont.com
Telephone: +358-40-8241732
Fax: +358-10-4315555

Received: April 13, 2016
Peer-review started: April 14, 2016
First decision: June 20, 2016
Revised: July 13, 2016
Accepted: July 31, 2016
Article in press: August 1, 2016
Published online: December 28, 2016

Abstract

AIM

To determine the effects of *Lactobacillus acidophilus* NCFM on irritable bowel syndrome (IBS) symptoms and quality of life (QoL).

METHODS

In this randomized triple-blind trial, adult IBS volunteers

who were recruited according to Rome Ⅲ criteria received 10^9 or 10^{10} colony-forming units of NCFM or placebo daily for 12 wk. IBS Symptom Severity Score (IBS-SSS), which constituted the primary outcome, and secondary outcomes, including individual IBS symptoms, IBS-related QoL questionnaire, anxiety and depression, defecation frequency, and stool consistency, were assessed at baseline at the end of the 8-wk run-in period, after 4 and 12 wk of intervention, and after a 4-wk washout.

RESULTS

A total of 340 of 391 randomized volunteers completed the trial. IBS-SSS improved over 12 wk of treatment in all treatment groups, decreasing by a mean ± SD of 44.0 ± 80.2, 50.8 ± 82.4, and 48.3 ± 72.2 in the placebo, active low-dose, and active high-dose groups, respectively. Similarly, secondary outcomes did not differ between treatment groups. However, in a post hoc analysis of volunteers with moderate to severe abdominal pain at baseline (VAS > 35/100), the treatment significantly reduced the sensation of abdominal pain. Pain scores fell by 20.8 ± 22.8, 29.4 ± 17.9, and 31.2 ± 21.9 in the placebo, active low-dose, and active high-dose groups, respectively (*P* value for placebo *vs* combined active doses = 0.0460).

CONCLUSION

NCFM alleviates moderate to severe abdominal pain, consistent with earlier observations of this strain mitigating visceral pain through increased analgesic receptor expression.

Key words: Irritable bowel syndrome; Functional bowel disorder; Symptom questionnaire; Quality of life; Visceral pain; Abdominal pain; *Lactobacillus acidophilus*; Probiotic; Intervention

Core tip: Symptoms of irritable bowel syndrome (IBS) might benefit from probiotic interventions, although mechanistic insights into probiotic function are seldom available. *Lactobacillus acidophilus* NCFM induces human colonic mucosal opioid receptor expression - the putative mechanism by which visceral pain is alleviated. In this study, 391 volunteers with IBS were treated with 10^9 or 10^{10} colony-forming units of NCFM or placebo and evaluated using symptom questionnaires. NCFM was not superior to placebo in improving the composite IBS symptom score, whereas abdominal pain - as an individual symptom - declined in IBS volunteers with moderate to severe pain at baseline.

Lyra A, Hillilä M, Huttunen T, Männikkö S, Taalikka M, Tennilä J, Tarpila A, Lahtinen S, Ouwehand AC, Veijola L. Irritable bowel syndrome symptom severity improves equally with probiotic and placebo. *World J Gastroenterol* 2016; 22(48): 10631-10642 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10631.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10631

INTRODUCTION

With a prevalence of 5% to 16% in northern Europe, irritable bowel syndrome (IBS) imposes considerable health care costs on society[1,2]. IBS affects adult age groups evenly but is more common in women[2]. According to the European Food Safety Authority, IBS volunteers can be recruited as an appropriate group for studying bowel discomfort symptoms that also affect the general population[3], allowing the results to be extrapolated to a wider potential market.

A host's gastrointestinal (GI) microbiota can contribute to IBS etiology and symptomology through changes in bacterial abundance and fermentation products, lower diversity, and instability over time, which are associated with increased epithelial permeability, aberrations in immunity and brain-gut interactions, and altered GI neuromuscular function[4-7]. Thus, manipulation of the GI microbiota with probiotics is a putative therapeutic option for IBS[4,8].

Probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"[9], have shown efficacy in selected randomized clinical trials in relieving IBS symptoms and are generally well tolerated[10-12]. However, the quality of several probiotic intervention studies has been limited due to inadequate sample sizes and intervention periods, poor trial design, and undefined or unstable probiotic products[13]. Moreover, each strain or combination of strains and each dose requires a separate clinical trial to show efficacy[9,10].

Our aim was to perform a high-quality, randomized, triple-blind, and placebo-controlled dose-response clinical trial of 3 statistically adequately sized parallel groups. The supplement that we examined is a well-characterized and stable probiotic strain, mechanistic studies of which have reported putative efficacy in alleviating visceral pain. *Lactobacillus acidophilus* NCFM increases the visceral pain threshold in a rat model by 44% through the opioid pathway[14] and upregulates μ-opioid receptor (MOR) in humans[15].

Participant-reported severity of functional bowel symptoms, however, has merely been evaluated for a high-concentration combination of *L. acidophilus* NCFM and *Bifidobacterium animalis* subsp. *lactis* Bi-07[16], which is significantly less effective in increasing MOR expression than *L. acidophilus* NCFM alone[15]. Therefore, assessment of the clinical effects of *L. acidophilus* NCFM as a single-strain supplement for functional bowel symptoms, including visceral pain, was essential. Two doses, 10^9 and 10^{10} colony-forming units (CFU), were selected as clinically adequate and

applicable for use in consumer products.

MATERIALS AND METHODS

Study volunteers

This trial was performed at 2 private clinics in Helsinki and Turku, Finland, from October 2012 to November 2014. Newspaper and radio advertisements, followed by prescreening phone calls, were used to invite potential eligible volunteers to screening visits that were held by gastroenterologists who were experienced in functional bowel disorders, including IBS. The recruited volunteers were adults (18-65 years) who were diagnosed with IBS according to Rome Ⅲ criteria[17]. Volunteers who suffered from severe IBS symptoms were excluded due to the use of medications (strong pain medication, diarrhea medication, and laxatives) that might have confounded the primary outcome measure. Other inclusion criteria were sufficient general health and orientation for participation in the study, adequate Finnish language skills for being interviewed and completing questionnaires, high likelihood of compliance with and completion of the study, and a body mass index (BMI) between 19 and 35.

Volunteers were excluded if they had participated in a clinical trial with an investigational product (IP) or drug within 3 mo prior to the screening, were likely to be noncompliant with the protocol or judged to be unsuitable for study participation by the investigator for any reason, were planning major changes in lifestyle (*e.g.*, diet, dieting, exercise level, travel), had a history of drug or alcohol abuse, were pregnant or breastfeeding, were diagnosed with or suspected of having organic GI disease (*e.g.*, colitis, Crohn's disease, celiac disease, bowel surgery, recurrent diverticulitis), or had severely impaired general health, including cancer and cancer therapy. Lactose-intolerant volunteers were allowed to enter the trial if they followed a non lactose diet. Any previous allergic reaction to any substance in the study product was also considered an exclusion criterion.

Medications that could affect the outcomes, including anticholinergic medications, antibiotics (including use during the 3 mo prior to the start of the study), pain medications that contained opiates or morphine, weight loss medication, misoprostol, 5-HT3 receptor antagonists, antacids with magnesium or aluminum, diarrhea medication, medication that accelerates the emptying of the stomach, sulfasalazine, laxatives, cholestyramine, cytostatics, biological medications, oral steroids (3 mo prior to and during the study), and probiotic products, excluded subjects from participation in the trial. Iron supplements, antidepressants, fiber supplements, statins, thyroxine, coxibs, acid medication, inhaled steroids, and other non excluding medications that did not affect outcome measures in the clinician's opinion were allowed during the trial if they had been consumed for at least 30 d at the same dose.

During the screening visit, thorough demographic data were collected, including lifestyle habits, diet, medical history, and family history of GI disturbances. Screening safety blood tests included a basic blood count, C-reactive protein (CRP), celiac test, lactase gene test, and thyroid-stimulating hormone for constipated IBS volunteers. The test results complied with the inclusion and exclusion criteria and were clinically within normal-range values according to the recruiting gastroenterologists.

Study design

The trial was a randomized, triple-blind (volunteers, investigators, and statisticians blinded), placebo-controlled, dose-response intervention to determine the efficacy of a probiotic supplement in reducing IBS Symptom Severity Score (IBS-SSS)[18]. The trial comprised an 8-wk run-in period, a 12-wk intervention phase, and a 4-wk washout period (Figure 1). Outcome measures were assessed using questionnaires, and adverse events (AEs) were recorded through phone calls. Volunteers who withdrew were not replaced.

The primary outcome was the change in IBS-SSS from baseline at the end of the run-in period to after 12 wk of treatment[18]. IBS-SSS is a composite score of abdominal pain, number of days with abdominal pain, bloating/distension, satisfaction with bowel habits, and IBS-related quality of life (QoL). Each measure is rated from 0 to 100, with total scores ranging from 0 to 500. Based on previous trials[19,20], a 15% change in the IBS-SSS was determined to be clinically significant in measuring efficacy. QoL was evaluated with a thorough 34-item IBS-related QoL questionnaire (IBS-QoL), analyzed as a total score and as subscales on dysphoria, interference with activity, body image, health-related worries, food avoidance, social reactions, sexuality, and relationships[21]. Psychological comorbidities were evaluated with the Hospital Anxiety and Depression Scale (HADS) questionnaire[22].

IBS-SSS, IBS-QoL, and HADS data were collected at the end of the run-in, after 4 and 12 wk of treatment, and after the 4-wk washout. Weekly bowel movement frequency and consistency were recorded prior to each outcome assessment time point with an in-house questionnaire, based on the Bristol Stool Form Scale[23]. For analysis, stool consistencies were grouped into constipation (Bristol Scale 1 or 2), diarrhea (Bristol Scale 6 or 7), and normal (Bristol Scale 3, 4, or 5). Overall satisfaction with the treatment with regard to IBS symptoms was measured with a dichotomous adequate relief (AR) question[24]. The volunteers were instructed to compare AR from IBS symptoms during the past week to their symptom severity prior to consuming IPs. Volunteers who reported AR for at least half of the intervention weeks were considered to be responders. AR data were collected throughout the 12-wk intervention.

Prior to each visit (end of run-in, week 4, week 12,

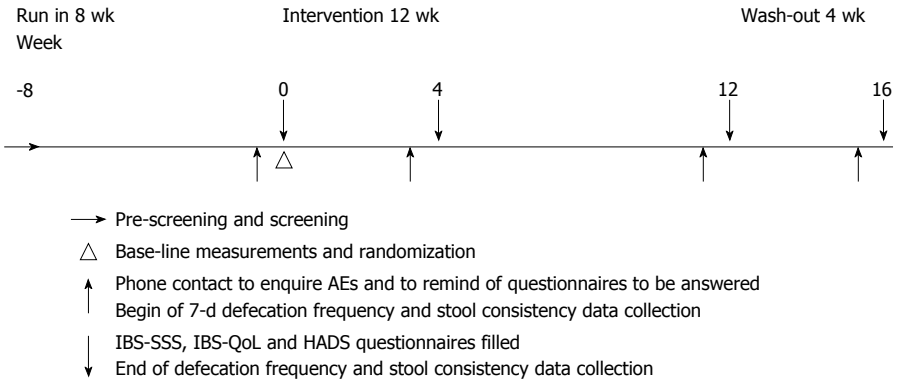

Figure 1 Study outline. Volunteers were selected for screening visits by a prescreen for compliance over the phone. Eligible volunteers entered an 8-wk run-in period without any investigational product (IP) consumption and were thereafter randomized to receive low- or high-dose active IP or a placebo IP for 12 wk. The intervention period was followed by a 4-wk washout period without any IP consumption. Preceding each time point (weeks 0, 4, 12, and 16), volunteers recorded their stool consistency during each defecation event for 7 d. At each time point, questionnaires [Irritable Bowel Syndrome Symptom Severity Score (IBS-SSS); IBS-related Quality of Life (IBS-QoL); Hospital Anxiety and Depression Score (HADS)] were filled out. All volunteers were contacted by phone prior to each sampling time point to inquire about adverse events (AEs) and to remind of the sampling procedures.

and end of washout), research nurses contacted the volunteers by phone to inquire about AEs and remind them of the sampling time point procedures.

Study treatment

The IPs were administered orally in 1 daily capsule that contained 10^9 (low dose) or 10^{10} (high dose) CFU of *L. acidophilus* NCFM (ATCC 700396), with microcrystalline cellulose as the excipient or microcrystalline cellulose as placebo. All treatments were supplied by Danisco USA (Madison, Wisconsin, United States). The formulations of all 3 treatments were similar in smell, taste, and appearance. The IPs were stored at -20 ℃ until they were distributed to the clinics, where they were refrigerated below 6 ℃. Volunteers were allowed to store the IPs refrigerated or at room temperature. The CFU counts were checked in the active and placebo products during and after the trial.

Laboratory measures

Screening safety tests were performed by certified clinical laboratories of the private clinics Mehiläinen Töölö (Helsinki, Finland) and Mehiläinen Turku (Turku, Finland).

Compliance testing

Volunteers received 84 capsules and were instructed to return the container after the 84-d treatment period. Leftover capsules were counted to estimate compliance.

Ethical considerations

This study was conducted according to the 2008 Sixth Revision of the Declaration of Helsinki, the EMA Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95 - in operation 17.01.97), and laws and regulations for clinical research in Finland. Written informed consent was obtained from each volunteer before any study-specific procedures. The trial was registered at Clinicaltrials.gov under the identifier NCT01728610.

Data quality assurance

Case report forms were 100% monitored and double-entered independently into a database that was created with SAS®. Queries were raised in cases of illegible, missing, or inconsistent data. All discrepancies were resolved before the database was locked. Data management and statistics were performed by 4Pharma Ltd.

Statistical analyses

Determination of sample size: The primary analysis variable was the change in IBS-SSS from baseline to week 12. Baseline levels were estimated to be approximately 250 (halfway on the 0-500 scale). A difference of 15% - *i.e.*, 37.5 points - was used in the sample size calculation[20]. Using 80% power and a 2-sided alpha level of 0.05, the sample size was calculated to be 104 for each group. Taking into account a discontinuation rate of 20%, approximately 390 patients were needed for randomization. The sample sizes were calculated using NQuery Advisor, version 7.0.

Analysis sets: The intent-to-treat dataset (ITT) included all randomized volunteers who received at least 1 dose of the IP and had at least 1 post baseline efficacy measurement available. The per protocol dataset (PP) was a subset of the ITT dataset, excluding volunteers and measurements for a given volunteer with major protocol violation(s) or concomitant medication use that was expected to alter the primary outcome. Volunteer classification into the ITT and PP populations was detailed after locking the database and completed before the study was unmasked. In addition, post hoc analyses of volunteers with IBS-SSS pain score VAS > 35/100 were performed.

Randomization: Volunteers were randomized at the end of the 8-wk run-in phase, because the long run-in period created risk of a high dropout rate. Randomization was performed with Research Randomizer[25], applying non repeating numbers in blocks of 6. Volunteers, investigators, statisticians, and monitors were blinded until the database was locked and the Statistical Analyses Plan was completed. The clinics were supplied with sealed, volunteer-specific envelopes for revealing the randomization code, if required by the investigators. None of the envelopes was opened during the trial.

Analysis of efficacy: Descriptive statistics for all variables were computed, and the disposition of volunteers was summarized by treatment. The primary efficacy variable (change in IBS-SSS from baseline) was analyzed using a repeated measures analysis of covariance (RM ANCOVA) model. The differences (low dose *vs* placebo and high dose *vs* placebo) in changes from baseline in IBS symptom score at 12 wk and their 95%CI were estimated with the RM ANCOVA model. Explorative comparisons were performed for high dose *vs* low dose and combined active group *vs* placebo and for within-group changes from baseline.

The individual domains of the IBS-SSS were analyzed with a similar RM ANCOVA model that was applied for IBS-SSSs. A logistic regression model was used to compare treatment groups against placebo for AR responder status. Changes in HADS and IBS-QOL scores from baseline were analyzed using an RM ANCOVA model. The stool consistencies (Bristol Stool Form) and defecation frequencies were summarized descriptively. Simple *t*-test was used in the post hoc analyses of subgroups (*e.g.*, pain score VAS > 35/100).

All statistical analyses and volunteer data listings were performed at 4Pharma Ltd using SAS, version 9.3 (SAS Institute Inc., Cary, NC, United States).

Safety analyses: All randomized volunteers who received the study treatment were included in the safety analysis. AEs were counted by volunteer, event, type, treatment, severity, and causality. Each symptom of an AE or serious adverse event (SAE) case was recorded separately.

The statistical methods were reviewed by statistician Teppo Huttunen, 4Pharma Ltd.

RESULTS

Altogether, 618 volunteers were prescreened by telephone interview for compliance with the inclusion and exclusion criteria, and thereafter, the eligibility of 529 potential volunteers was confirmed by gastroenterologists. A total of 471 volunteers entered the trial, of whom 391 were randomized after the 8-wk run-in phase, with 340 (87%) completing the trial (Figure 2). The results are presented for the ITT dataset; those for the PP dataset were comparable.

Demographics and baseline characteristics

The volunteers from Helsinki (n = 276; 34 dropouts) and Turku (n = 115; 17 dropouts) were randomized evenly to the 3 treatments. The treatment groups were comparable with regards to age, sex, BMI, and lifestyle habits, including type of diet, exercise level, alcohol consumption, smoking (Table 1), and IBS symptom characteristics (Table 2). All groups were predominantly female (71.8% to 79.4% female), and men had a higher BMI (60.6% and 36.6% of male and female volunteers, respectively, had a BMI > 25). All findings on vital signs and the safety blood tests taken at screening were evaluated for their clinical significance in relation to the inclusion and exclusion criteria.

Prior and concomitant medications

Prior and concomitant medications for the alimentary tract, pain, and anxiety/depression were recorded, with gastroenterologists evaluating any putative bias of them on efficacy measures. The most common medications used before and during the study were drugs for gastric acid disorders, nonsteroidal anti-inflammatory drugs, and antidepressants/anxiolytics. Also, 36 volunteers were on thyroxine medication due to hypothyreosis.

IP quality check and compliance

The *L. acidophilus* NCFM CFU count was confirmed to be adequate for both active treatment doses (> 1.04×10^{10} and > 9.8×10^{9} CFU/capsule for the high and low doses, respectively). For the placebo, the *L. acidophilus* NCFM count was below < 3.2×10^{2} CFU/capsule. According to the number of returned capsules 95%, 95%, and 94% of IP capsules were consumed in the placebo and low-dose and high-dose treatment groups, respectively.

Efficacy

The IBS-SSS improved significantly from baseline to the end of the intervention by a mean ± SD of 44.0 ± 80.2, 50.8 ± 82.4, and 48.3 ± 72.2 in the placebo, low-dose, and high-dose groups, respectively (P < 0.001 for all groups), with no significance between the placebo and active groups (Figure 3). Results for individual IBS-SSS item scores were comparable between groups (Table 3). However, in a post hoc analysis of a subgroup of volunteers who suffered from moderate to severe pain (pain score VAS > 35/100 at baseline), *L. acidophilus* NCFM significantly reduced abdominal pain in the combined active groups compared with placebo (Table 4).

During the intervention period, 28.4%, 25.0%, and 26.5% of volunteers considered their IBS symptoms to have been adequately relieved with the placebo,

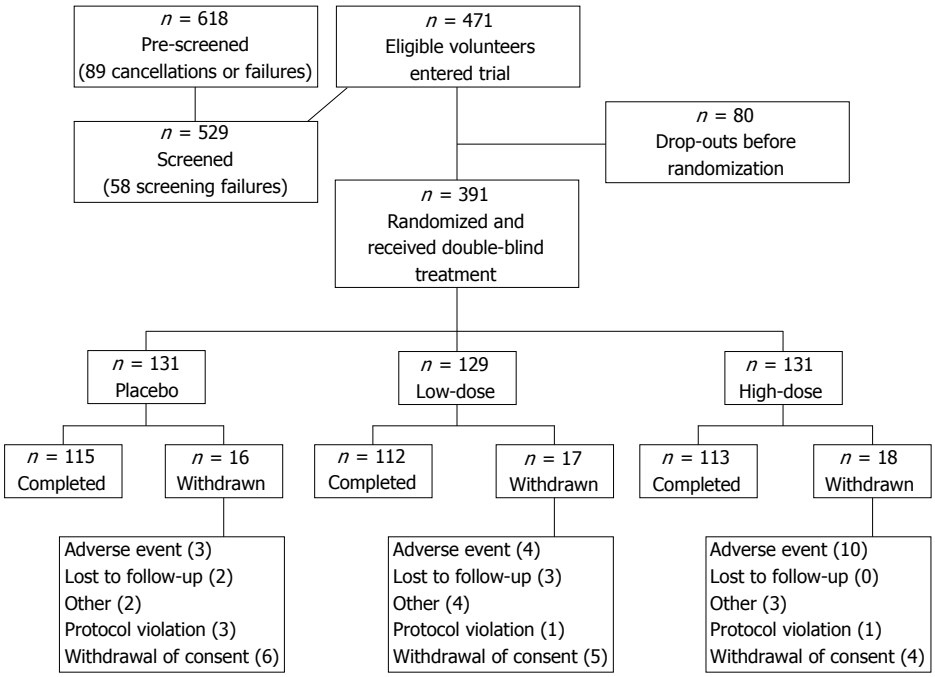

Figure 2 Disposition of volunteers. During the 8-wk run-in period prior to randomization, 17% of eligible volunteers dropped out. After randomization, most volunteers (87%) completed the trial. Withdrawals were evenly distributed between treatment groups. Low- and high-dose treatment groups received 10^9 or 10^{10} CFU *Lactobacillus acidophilus* NCFM daily.

Table 1 Baseline demographics mean ± SD or *n* (%)

Characteristics	Placebo (*n* = 131)	Low-dose (*n* = 129)	High-dose (*n* = 131)
Age (yr)	49.4 ± 12.9	47.1 ± 13.3	47.2 ± 12.5
Gender			
Male	37 (28.2)	35 (27.1)	27 (20.6)
Female	94 (71.8)	94 (72.9)	104 (79.4)
BMI (kg/m^2)	24.9 ± 3.7	24.7 ± 3.7	24.5 ± 3.9
Diet			
Low-carbohydrate	2 (1.5)	0 (0.0)	0 (0.0)
Vegetarian	1 (0.8)	2 (1.6)	0 (0.0)
Non-lactose	26 (19.8)	32 (24.8)	26 (19.8)
Regular	77 (58.8)	63 (48.8)	67 (51.1)
Probiotics			
Daily user	24 (18.3)	25 (19.4)	18 (13.7)
Irregular user	18 (13.7)	17 (13.2)	29 (22.1)
History of using	65 (49.6)	62 (48.1)	66 (50.4)
No use	23 (17.6)	25 (19.4)	18 (13.7)
N/A	1 (0.8)	0 (0.0)	0 (0.0)
Exercise level			
> 30 min 3 times a week	62 (47.3)	63 (48.8)	59 (45.0)
⩽ 30 min 3 times a week	57 (43.5)	56 (43.4)	63 (48.1)
No exercise	12 (9.2)	10 (7.8)	9 (6.9)
Alcohol consumption			
> 14 units/wk	1 (0.8)	0 (0.0)	0 (0.0)
⩽ 14 units/wk	96 (73.3)	90 (69.8)	90 (68.7)
Non-drinker	34 (26.0)	39 (30.2)	41 (31.3)
Tobacco smoking			
Current use	11 (8.4)	11 (8.5)	14 (10.7)
Never used	80 (61.1)	86 (66.7)	88 (67.2)
History of use	40 (30.5)	32 (24.8)	29 (22.1)
Drug abuse			
Never used	131 (100)	129 (100)	131 (100)

Table 2 Irritable bowel syndrome symptom characteristics *n* (%)

	Placebo (*n* = 131)	Low-dose (*n* = 129)	High-dose (*n* = 131)
IBS subtype			
IBS-C	25 (19.1)	20 (15.5)	20 (15.3)
IBS-D	49 (37.4)	51 (39.5)	52 (39.7)
IBS-M	56 (42.7)	58 (45.0)	58 (44.3)
IBS-U	1 (0.8)	0 (0.0)	1 (0.8)
Postinfectious IBS	9 (6.9)	4 (3.1)	7 (5.3)
Psychological comorbidities	9 (6.9)	6 (4.7)	13 (9.9)
Symptoms provoked by specific food	103 (79.8)[1]	116 (89.9)	120 (91.6)
Family history of intestinal disorders or diseases	95 (72.5)	97 (75.2)	92 (70.8)[2]

[1]Two volunteers not analysed; [2]One volunteer not analyzed. IBS: Irritable bowel syndrome.

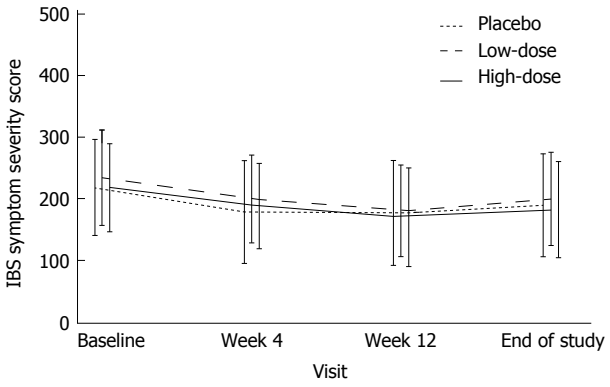

Figure 3 Irritable bowel syndrome symptom severity score over time. IBS-SSS is a 5-item composite score inquiring about the severity of abdominal pain, bloating/distension, satisfaction with bowel habits, and IBS-related quality of life on a 10-cm VAS scale and the number of days with abdominal pain over the past 10 d[18]. All items are scored from 0 to 100, allowing for IBS-SSSs to range from 0 to 500. Francis and colleagues have validated non-IBS controls and volunteers suffering from mild, moderate, and severe IBS symptoms to range between 0-75, 75-175, 175-300, and 300-500, respectively[18]. The composite symptom score declined similarly in all treatment groups during the intervention, showing no treatment effect for the active groups (low-dose and high-dose) receiving *Lactobacillus acidophilus* NCFM. The severity scores are given as mean ± SD. Differences from baseline to week 12 were not significant between treatment groups. All within-group comparisons to baseline (week 4, week 12, and washout) were significant ($P < 0.001$). IBS-SSS: Irritable bowel syndrome symptom severity score; IBS: Irritable bowel syndrome.

low-dose, and high-dose treatments, respectively ($P = 0.8371$ between groups by logistic regression). The IBS-QoL reflected a higher QoL at the end of the intervention in all treatment groups (Table 5), although neither active treatment dose was superior to placebo. The HADS total score declined significantly from baseline to the end of the intervention in both active treatment groups, HADS anxiety improved significantly in all treatment groups, but HADS depression improved significantly only in the high-dose group. However, none of the between-group comparisons reached statistical significance, although total HADS and HADS anxiety were slightly lower in the high-dose group compared with placebo (Table 5).

None of the treatments had undesired effects on stool consistency (Table 6).

Safety results

AEs were evenly distributed in all groups, with 81, 57, and 61 AEs recorded during the treatment period in the placebo, low-dose, and high-dose treatment groups, respectively. The most common treatment-emergent AEs were GI disorders (abdominal discomfort, abdominal distension, abdominal pain, constipation, diarrhea, flatulence), gastroenteritis, and influenza. Potentially IP-related AEs - mild GI symptoms, which might also have been due to all volunteers having IBS that presented with similar symptoms - were recorded for 7, 7, and 9 volunteers in the placebo, low-dose, and high-dose treatment groups, respectively.

AEs led to discontinuation 10, 4, and 3 times in the high-dose, low-dose, and placebo groups, respectively, but these cases were not considered IP-related safety issues, based on the possibility of the IP-related cases being general IBS symptoms. Two SAE cases that presented with 3 symptoms were encountered: pneumonia with fever and cough and a case of syncope that resulted in a hospital visit; neither was associated with the IP or any trial procedure. The case of pneumonia, which involved hospitalization and antibiotic treatment, led to discontinuation.

DISCUSSION

This study is the first adequately powered clinical trial to determine the effects of *L. acidophilus* NCFM on IBS symptoms using patient-reported outcomes. The *L. acidophilus* NCFM strain is well characterized[26] and has been commercially available as a probiotic for several decades. It has been associated with the alleviation of the perception of visceral pain as a 2-strain blend with *B. lactis* Bi-07 in volunteers with bloating and among colonoscopy patients[14,15,27]. In a mechanistic study, however, *L. acidophilus* NCFM, as a single-strain supplement, was more effective than the combination in enhancing human colonic MOR expression and activity, although both treatments relieved bowel symptoms, albeit insignificantly[15,16].

L. acidophilus NCFM has been shown to elevate the visceral pain threshold in a rat model by 44%

Table 3 Irritable bowel syndrome symptom severity score item scores at baseline and end of intervention

Treatment	Baseline		Week 12		Change from baseline	Within-group comparison	Comparison with placebo	Comparison with low- and high-dose
	n	mean ± SD	*n*	mean ± SD	mean ± SD	*P* value	*P* value	*P* value
Severity of pain								
Placebo	121	20.2 ± 20.6	118	18.5 ± 20.7	-2.2 ± 21.8	0.024	NA	0.303
Low-dose	124	24.1 ± 22.3	110	18.3 ± 18.6	-5.2 ± 24.4	0.005	0.640	NA
High-dose	122	24.3 ± 21.5	113	16.4 ± 17.8	-7.9 ± 21.8	< 0.001	0.189	NA
Number of days with pain over 10 d								
Placebo	114	3.8 ± 2.8	111	2.8 ± 2.8	-1.0 ± 2.7	< 0.001	NA	0.234
Low-dose	115	4.4 ± 2.8	105	3.3 ± 2.6	-1.1 ± 2.9	< 0.001	0.470	NA
High-dose	121	4.1 ± 2.8	106	2.9 ± 2.4	-1.2 ± 2.5	< 0.001	0.634	NA
Bloating/Distension								
Placebo	121	39.0 ± 28.0	118	30.7 ± 25.6	-8.3 ± 23.6	< 0.001	NA	0.669
Low-dose	122	40.5 ± 29.9	110	31.0 ± 25.7	-9.4 ± 29.6	< 0.001	0.905	NA
High-dose	122	37.1 ± 26.6	113	31.0 ± 27.3	-6.1 ± 25.8	0.002	0.535	NA
Satisfaction with bowel habits								
Placebo	121	57.4 ± 22.6	118	47.3 ± 24.2	-10.3 ± 21.4	< 0.001	NA	0.964
Low-dose	124	59.4 ± 22.7	110	46.3 ± 19.8	-11.8 ± 22.4	< 0.001	0.701	NA
High-dose	122	55.0 ± 19.7	113	46.4 ± 22.6	-8.3 ± 23.3	< 0.001	0.757	NA
Interference of IBS with quality of life								
Placebo	121	62.4 ± 17.0	118	48.6 ± 22.1	-13.9 ± 20.3	< 0.001	NA	0.210
Low-dose	124	60.8 ± 16.9	110	50.1 ± 19.4	-9.8 ± 16.5	< 0.001	0.133	NA
High-dose	122	59.8 ± 13.5	113	47.9 ± 16.5	-11.7 ± 13.6	< 0.001	0.509	NA

IBS: Irritable bowel syndrome.

Table 4 Change in pain score for volunteers with moderate or severe abdominal pain at baseline

Treatment	*n*	Baseline	Week 12	Change from baseline	Mean difference for combined active doses	*P* value
		mean ± SD	mean ± SD	mean ± SD	95%CI	
Placebo	29	51.1 (9.3)	30.3 (22.9)	-20.8 (22.8)		
Low-dose	36	53.6 (10.9)	24.4 (19.4)	-29.4 (17.9)	-9.5 (-18.8; -0.17)	0.046
High-dose	34	52.1 (10.7)	21.9 (20.6)	-31.2 (21.9)		

through the MOR-mediated pathway with an efficacy that is comparable with that of 1 mg/kg subcutaneous morphine[14]. Thus, there was tremendous interest in determining the efficacy of *L. acidophilus* NCFM as a single-strain supplement in mitigating functional bowel symptoms.

The volunteer recruitment was successful, and the trial completion rate and IP compliance were high. All 391 randomized volunteers fulfilled the Rome Ⅲ criteria for IBS, with an even distribution of IBS subtypes and demographics between groups. Blood tests were performed at the screening visit (basic blood count and CRP) to rule out inflammatory causes of the bowel symptoms. Most volunteers (57%) had undergone a colonoscopy in the past due to chronic GI symptoms to rule out organic or inflammatory causes, but endoscopy was not performed during the screening.

Based on a crossover trial that raised concerns over the inadequate length of a 1-mo run-in period[28], all volunteers refrained from using any commercial or trial-related probiotics over an 8-wk run-in period to reduce any putative carryover effects. A total of 83% of randomized volunteers had a history of consuming commercial probiotics. The intervention period was designed to last for 12 wk to follow up on the efficacy and AEs for a sufficient period of time[10] and to limit the placebo effect toward the end of the trial. Altogether, we recruited an adequate number of reliably diagnosed, extensively examined volunteers who were not using any concomitant medications or supplements that could have affected outcomes. The treatment was a well-characterized probiotic strain that was supplemented in 2 common doses for an adequately long intervention period.

However, in this trial, based on a composite score of IBS symptom severity (IBS-SSS), *L. acidophilus* NCFM was not superior to placebo. No significant differences in the secondary outcomes were observed between groups, and none of the outcomes showed a dose-response effect. Nevertheless, in post hoc analyses of a subgroup that presented with moderate to severe abdominal pain at baseline (IBS-SSS pain score on VAS > 35/100 at baseline; active groups combined *vs* placebo), the level of abdominal pain declined in the active groups at week 12. These data are consistent with previous mechanistic findings of greater MOR expression and activity during *L. acidophilus* NCFM treatment in humans and rats[14,15] and earlier recovery from colonoscopy-associated pain[27].

Table 5 Change in irritable bowel syndrome-related quality of life and level of anxiety and depression (Hospital Anxiety and Depression Score)

Treatment	Baseline		Week 12		Change from baseline	Within-group comparison	Comparison with placebo	Comparison with low- and high-dose
	n	mean ± SD	*n*	mean ± SD	mean ± SD	*P* value	*P* value	*P* value
IBS-QoL								
Placebo	121	66.4 ± 17.5	118	73.2 ± 19.0	7.0 ± 12.3	< 0.001	NA	0.412
Low-dose	124	63.9 ± 19.0	110	71.6 ± 19.3	7.4 ± 12.3	< 0.001	0.812	NA
High-dose	122	68.2 ± 16.5	113	76.5 ± 15.8	8.5 ± 8.8	< 0.001	0.238	NA
HADS total score								
Placebo	119	9.2 ± 5.6	110	8.6 ± 6.6	-0.4 ± 4.3	0.302	NA	0.134
Low-dose	122	10.1 ± 5.7	109	9.2 ± 6.0	-1.0 ± 4.4	0.034	0.435	NA
High-dose	118	9.7 ± 5.5	109	8.2 ± 5.8	-1.5 ± 3.9	< 0.001	0.071	NA
HADS-Anxiety								
Placebo	121	5.9 ± 3.5	114	5.3 ± 3.6	-0.4 ± 2.6	0.036	NA	0.246
Low-dose	122	6.1 ± 3.3	109	5.5 ± 3.1	-0.6 ± 2.7	0.011	0.726	NA
High-dose	119	6.2 ± 3.3	109	5.0 ± 3.1	-1.0 ± 2.2	< 0.001	0.099	NA
HADS-Depression								
Placebo	119	3.4 ± 2.9	111	3.4 ± 3.6	0.0 ± 2.3	0.906	NA	0.162
Low-dose	124	4.0 ± 3.0	109	3.7 ± 3.4	-0.3 ± 2.2	0.258	0.376	NA
High-dose	119	3.5 ± 2.9	110	3.2 ± 3.1	-0.4 ± 2.4	0.041	0.125	NA

HADS: Hospital anxiety and depression score; IBS-QoL: Irritable bowel syndrome quality of life.

Table 6 Change in stool consistency from baseline according to Bristol stool scale[1] *n* (%)

	n	Change to optimal from constipation or diarrhea	Change to constipation or diarrhea from optimal
Placebo	131		
Week 4		18 (13.7)	15 (11.5)
Week 12		21 (16.0)	18 (13.7)
Washout		20 (15.3)	17 (13.0)
Low-dose	129		
Week 4		26 (20.2)	13 (10.1)
Week 12		27 (20.9)	14 (10.9)
Washout		14 (10.9)	12 (9.3)
High-dose	131		
Week 4		14 (10.7)	16 (12.2)
Week 12		18 (13.7)	20 (15.3)
Washout		14 (10.7)	18 (13.7)
Total	391	172 (44.0)	143 (36.6)

[1]Bristol stool scale types 1 and 2 were classified as constipation; 3, 4, and 5 were classified as optimal stool consistency; and 6 and 7 were classified as diarrhea.

IBS is associated with significant placebo effects, in part due to the subjective nature of the outcome measures (Shah and Pimentel, 2014). Our trial had an 8-wk run-in period during which volunteers did not consume any product (including placebo); thus, there was no pre-randomization selection for high placebo responders. The study products were administered daily, which should be beneficial for minimizing a placebo effect. However, the high frequency of contact by research nurses might have heightened the placebo response, although their attention and care were targeted originally toward evaluating safety adequately and ensuring high compliance with the protocol.

Our principal challenge was the significant placebo effect, which was comparable with the efficacy of the active treatments. A decrease of > 50 in IBS-SSS indicates clinical improvement of symptoms[18]. In the present trial, the IBS-SSS decreased from 44.0 to 50.8 in the 3 different treatment groups suggesting a borderline clinically significant effect. Similarly, IBS-QoL scores corresponded to moderate symptom severity at baseline *vs* mild symptom severity by the end of treatment in all groups[21]. Also, with the AR questionnaire, the placebo was as effective as the active treatment. Volunteers had had difficulty comprehending whether the weekly AR question should be referenced to the base-line period, as intended, or to the previous calendar week. Thus some participants compared consecutive intervention weeks rather than treatment to baseline in the AR questionnaire.

HADS was applied to subgroup participants by psychological comorbidity which has previously been associated with characteristics of intestinal microbiota[29]. Statistically significant reductions in HADS were observed in the active treatment groups. However, these changes were small and likely of no clinical relevance; further, the prevalence of anxiety and depression was low, and for all treatment groups HADS averages throughout the study were at normal levels[22]. No subgroup analyses were implemented. Nevertheless, the changes in HADS indicate a target for future study in a more appropriate population.

Changes in stool consistency are not unexpected for an IBS population[30], and none of the treatments appeared to cause undesirable alterations in stool form. Defecation frequencies were close to normal limits, suggesting that diarrhea and constipation in IBS volunteers are subjective phenomena that are related to defecation events, rather than a result of hard stool and slow transit or loose stool and accelerated transit. Thus recruitment of all IBS symptom subtypes is justifiable.

Selecting volunteers from only 1 IBS symptom subgroup or setting a threshold for baseline symptom severity as applied by Sisson and colleagues[31] can reduce variation in the response to treatment. However, our objective was to recruit IBS volunteers as a representation of the general population[3], which would have been distorted by selecting subgroups or IBS patients with more severe symptoms. In addition, because the etiology of IBS is multifactorial and, to a large extent, unknown and because symptom subgroups tend to vary over time in each volunteer[30], selecting between symptom subgroups is challenging. Moreover, excluding volunteers whom consume diarrhea medication, laxatives, and opiate- or morphine-containing pain medications was necessary, because these agents influence the outcome, but it also effectively excluded volunteers with severe IBS symptoms.

Another shortcoming in assessing the efficacy of bowel symptoms was the infrequent and retrospective evaluation of IBS symptoms[32]. The symptom questionnaires were administered 4 times in total and only twice during the 12-wk intervention period. More frequent assessments were initially considered to be a risk for noncompliance due to the laboriousness of answering so many questionnaires. Moreover, although the IBS-SSS questionnaire inquires about symptom severity over the past 10 d, the volunteers' symptoms, everyday events, and mood when they complete the questionnaires are likely to bias the answers, resulting in potentially irrelevant variations in scores. Because volunteers were not severely symptomatic (mean IBS-SSS at baseline corresponded to moderate for all treatment groups), inquiring about symptom frequency instead of severity might have been more sensitive in measuring efficacy[33].

AEs were evenly distributed between groups. The slightly higher number of volunteer discontinuations due to AEs in the active high-dose group was not treatment-related. Digestive symptoms were the chief manifestation of the inclusion criteria of the study population; thus, GI discomfort, recorded as a possibly treatment-related AE, might have also been part of their normal symptomology.

L. acidophilus NCFM can be consumed safely by adult IBS volunteers over a 3-mo period but is ineffective against IBS symptoms in general compared to placebo. However, *L. acidophilus* NCFM treatment alleviated abdominal pain in IBS volunteers with at least moderately severe visceral pain. More frequent - preferably daily - assessment of bowel symptoms with a user-friendly application is recommended for future trials in this area. Moreover, enquiring individual symptoms rather than a composite score sum may be more applicable. Among the recruited IBS participants with moderate symptom severity at baseline bowel movement frequency appeared normal regardless of stool consistency and anxiety and depression levels were not clinically notable.

ACKNOWLEDGMENTS

The IBS-SSS questionnaire (Francis *et al*[18], 1997) was back-translated into Finnish by the medical translation company Anita Paajanen-Mannila Oy and kindly provided for use in this study by Oy Verman Ab and Kajsa Kajander. We thank research nurses Päivi Kuusniemi for coordinating the clinics and Laura Lehtonen for her substantial efforts in archiving the trial according to GCP. We gratefully acknowledge Tad Stewart and Angela Paulsen for manufacturing the IP, Kaisa Olli for monitoring and Nicolas Yeung for providing the audio Core Tip. The authors would also like to thank gastroenterologists Tiina Kojo, Helena Kemppainen, and Janne Rajala for their efforts in screening participants and the volunteers for their commitment and participation.

COMMENTS

Background

Functional bowel symptoms are a common disturbance encountered transiently by most humans and in a chronic manner by many. Irritable bowel syndrome (IBS) is a functional bowel disorder with abdominal pain as its core symptom. In the present trial, *Lactobacillus acidophilus* NCFM, a probiotic bacterium that is known to enhance analgesic receptor expression in the gastrointestinal (GI) tract of rodents and humans, was evaluated for its ability to alleviate IBS symptoms.

Research frontiers

In probiotic research there is a great demand for high quality clinical trials to show potential health efficacy for well-defined probiotic strains. This applies to an array of health areas, including GI wellbeing. On the other hand, also mechanistic insight into the modes of action of probiotics is required to enlighten their efficacy.

Innovations and breakthrough

The present clinical trial was conducted with high quality applying an adequate population for statistical power and a well characterized probiotic strain with prior mechanistic efficacy data. A thorough set of participant-reported outcomes were evaluated on bowel symptoms, quality of life, psychological wellbeing and defecation habit and stool consistency. The participants complied well with the trial protocol allowing analysis of a comparatively large and complete dataset.

Applications

In the present trial the tested strain was not superior to placebo on IBS symptom alleviation in general. However, abdominal pain was relieved among participants with moderate to severe abdominal pain at baseline. The trial also gives insight into design and conduct of probiotic clinical trials on functional bowel disorders.

Peer-review

This article describes a very well designed clinical trial on the effect of the probiotic strain *L. acidophilus* NCFM in two doses on symptoms in IBS patients. The trial design is of high-quality including the use of a well-defined strain, as well as an adequate number of participants and a long enough intervention period.

REFERENCES

1 **Hillilä MT**, Färkkilä MA. Prevalence of irritable bowel syndrome

according to different diagnostic criteria in a non-selected adult population. *Aliment Pharmacol Ther* 2004; **20**: 339-345 [PMID: 15274671 DOI: 10.1111/j.1365-2036.2004.02034.x]
2 **Krogsgaard LR**, Engsbro AL, Bytzer P. The epidemiology of irritable bowel syndrome in Denmark. A population-based survey in adults ≤50 years of age. *Scand J Gastroenterol* 2013; **48**: 523-529 [PMID: 23506174 DOI: 10.3109/00365521.2013.775328]
3 **EFSA**. Guidance on the scientific requirements for health claims related to gut and immune function. *EFSA* 2011; **9**: 1984 [DOI: 10.2903/j.efsa.2011.1984]
4 **Hyland NP**, Quigley EM, Brint E. Microbiota-host interactions in irritable bowel syndrome: epithelial barrier, immune regulation and brain-gut interactions. *World J Gastroenterol* 2014; **20**: 8859-8866 [PMID: 25083059 DOI: 10.3748/wjg.v20.i27.8859]
5 **Kennedy PJ**, Cryan JF, Dinan TG, Clarke G. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? *World J Gastroenterol* 2014; **20**: 14105-14125 [PMID: 25339800 DOI: 10.3748/wjg.v20.i39.14105]
6 **Rajilić-Stojanović M**, Jonkers DM, Salonen A, Hanevik K, Raes J, Jalanka J, de Vos WM, Manichanh C, Golic N, Enck P, Philippou E, Iraqi FA, Clarke G, Spiller RC, Penders J. Intestinal microbiota and diet in IBS: causes, consequences, or epiphenomena? *Am J Gastroenterol* 2015; **110**: 278-287 [PMID: 25623659 DOI: 10.1038/ajg.2014.427]
7 **Moloney RD**, Johnson AC, O'Mahony SM, Dinan TG, Greenwood-Van Meerveld B, Cryan JF. Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. *CNS Neurosci Ther* 2016; **22**: 102-117 [PMID: 26662472 DOI: 10.1111/cns.12490]
8 **Bonfrate L**, Tack J, Grattagliano I, Cuomo R, Portincasa P. Microbiota in health and irritable bowel syndrome: current knowledge, perspectives and therapeutic options. *Scand J Gastroenterol* 2013; **48**: 995-1009 [PMID: 23964766 DOI: 10.3109/00365521.2013.799220]
9 **Hill C**, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 506-514 [PMID: 24912386 DOI: 10.1038/nrgastro.2014.66]
10 **Ford AC**, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BM, Moayyedi P. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Am J Gastroenterol* 2014; **109**: 1547-1561; quiz 1546, 1562 [PMID: 25070051 DOI: 10.1038/ajg.2014.202]
11 **Hungin AP**, Mulligan C, Pot B, Whorwell P, Agréus L, Fracasso P, Lionis C, Mendive J, Philippart de Foy JM, Rubin G, Winchester C, de Wit N. Systematic review: probiotics in the management of lower gastrointestinal symptoms in clinical practice -- an evidence-based international guide. *Aliment Pharmacol Ther* 2013; **38**: 864-886 [PMID: 23981066 DOI: 10.1111/apt.12460]
12 **Wall GC**, Bryant GA, Bottenberg MM, Maki ED, Miesner AR. Irritable bowel syndrome: a concise review of current treatment concepts. *World J Gastroenterol* 2014; **20**: 8796-8806 [PMID: 25083054 DOI: 10.3748/wjg.v20.i27.8796]
13 **Sanders ME**, Guarner F, Guerrant R, Holt PR, Quigley EM, Sartor RB, Sherman PM, Mayer EA. An update on the use and investigation of probiotics in health and disease. *Gut* 2013; **62**: 787-796 [PMID: 23474420 DOI: 10.1136/gutjnl-2012-302504]
14 **Rousseaux C**, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E, Geboes K, Chamaillard M, Ouwehand A, Leyer G, Carcano D, Colombel JF, Ardid D, Desreumaux P. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* 2007; **13**: 35-37 [PMID: 17159985 DOI: 10.1038/nm1521]
15 **Ringel-Kulka T**, Goldsmith JR, Carroll IM, Barros SP, Palsson O, Jobin C, Ringel Y. Lactobacillus acidophilus NCFM affects colonic mucosal opioid receptor expression in patients with functional abdominal pain - a randomised clinical study. *Aliment Pharmacol Ther* 2014; **40**: 200-207 [PMID: 24853043 DOI: 10.1111/apt.12800]
16 **Ringel-Kulka T**, Palsson OS, Maier D, Carroll I, Galanko JA, Leyer G, Ringel Y. Probiotic bacteria Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. *J Clin Gastroenterol* 2011; **45**: 518-525 [PMID: 21436726 DOI: 10.1097/MCG.0b013e31820ca4d6]
17 **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]
18 **Francis CY**, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther* 1997; **11**: 395-402 [PMID: 9146781 DOI: 10.1046/j.1365-2036.1997.142318000.x]
19 **Simrén M**, Ohman L, Olsson J, Svensson U, Ohlson K, Posserud I, Strid H. Clinical trial: the effects of a fermented milk containing three probiotic bacteria in patients with irritable bowel syndrome - a randomized, double-blind, controlled study. *Aliment Pharmacol Ther* 2010; **31**: 218-227 [PMID: 19863495 DOI: 10.1111/j.1365-2036.2009.04183.x.]
20 **Williams EA**, Stimpson J, Wang D, Plummer S, Garaiova I, Barker ME, Corfe BM. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Aliment Pharmacol Ther* 2009; **29**: 97-103 [PMID: 18785988 DOI: 10.1111/j.1365-2036.2008.03848.x.]
21 **Patrick DL**, Drossman DA, Frederick IO, DiCesare J, Puder KL. Quality of life in persons with irritable bowel syndrome: development and validation of a new measure. *Dig Dis Sci* 1998; **43**: 400-411 [PMID: 9512138]
22 **Zigmond AS**, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983; **67**: 361-370 [PMID: 6880820 DOI: 10.1111/j.1600-0447.1983.tb09716.x]
23 **Lewis SJ**, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 1997; **32**: 920-924 [PMID: 9299672 DOI: 10.3109/00365529709011203]
24 **Passos MC**, Lembo AJ, Conboy LA, Kaptchuk TJ, Kelly JM, Quilty MT, Kerr CE, Jacobson EE, Hu R, Friedlander E, Drossman DA. Adequate relief in a treatment trial with IBS patients: a prospective assessment. *Am J Gastroenterol* 2009; **104**: 912-919 [PMID: 19293784 DOI: 10.1038/ajg.2009.13]
25 **Urbaniak G**, Plous S. Research Randomizer. 4.0 ed, 2012: Computer software. Available at: URL: https://www.randomizer.org/
26 **Altermann E**, Russell WM, Azcarate-Peril MA, Barrangou R, Buck BL, McAuliffe O, Souther N, Dobson A, Duong T, Callanan M, Lick S, Hamrick A, Cano R, Klaenhammer TR. Complete genome sequence of the probiotic lactic acid bacterium Lactobacillus acidophilus NCFM. *Proc Natl Acad Sci USA* 2005; **102**: 3906-3912 [PMID: 15671160 DOI: 10.1073/pnas.0409188102]
27 **D'Souza B**, Slack T, Wong SW, Lam F, Muhlmann M, Koestenbauer J, Dark J, Newstead G. Randomized controlled trial of probiotics after colonoscopy. *ANZ J Surg* 2015; Epub ahead of print [PMID: 26183594 DOI: 10.1111/ans.13225]
28 **Childs CE**, Röytiö H, Alhoniemi E, Fekete AA, Forssten SD, Hudjec N, Lim YN, Steger CJ, Yaqoob P, Tuohy KM, Rastall RA, Ouwehand AC, Gibson GR. Xylo-oligosaccharides alone or in synbiotic combination with Bifidobacterium animalis subsp. lactis induce bifidogenesis and modulate markers of immune function in healthy adults: a double-blind, placebo-controlled, randomised, factorial cross-over study. *Br J Nutr* 2014; **111**: 1945-1956 [PMID: 24661576 DOI: 10.1017/S0007114513004261]
29 **Jeffery IB**, O'Toole PW, Öhman L, Claesson MJ, Deane J, Quigley EM, Simrén M. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut* 2012; **61**:

997-1006 [PMID: 22180058 DOI: 10.1136/gutjnl-2011-301501]
30 **Engsbro AL**, Simren M, Bytzer P. Short-term stability of subtypes in the irritable bowel syndrome: prospective evaluation using the Rome III classification. *Aliment Pharmacol Ther* 2012; **35**: 350-359 [PMID: 22176384 DOI: 10.1111/j.1365-2036.2011.04948.x]
31 **Sisson G**, Ayis S, Sherwood RA, Bjarnason I. Randomised clinical trial: A liquid multi-strain probiotic vs. placebo in the irritable bowel syndrome--a 12 week double-blind study. *Aliment Pharmacol Ther* 2014; **40**: 51-62 [PMID: 24815298 DOI: 10.1111/apt.12787]
32 **Lackner JM**, Jaccard J, Keefer L, Firth R, Carosella AM, Sitrin M, Brenner D. The accuracy of patient-reported measures for GI symptoms: a comparison of real time and retrospective reports. *Neurogastroenterol Motil* 2014; **26**: 1802-1811 [PMID: 25424582 DOI: 10.1111/nmo.12466]
33 **Azpiroz F**, Guyonnet D, Donazzolo Y, Gendre D, Tanguy J, Guarner F. Digestive Symptoms in Healthy People and Subjects With Irritable Bowel Syndrome: Validation of Symptom Frequency Questionnaire. *J Clin Gastroenterol* 2015; **49**: e64-e70 [PMID: 25014236 DOI: 10.1097/MCG.0000000000000178]

P- Reviewer: Ducrotte P, Konig J, Seow-Choen F, Lakatos PL
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Zhang FF

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.v22.i48.10643

World J Gastroenterol 2016 December 28; 22(48): 10643-10652
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Prevalence of upper gastrointestinal bleeding risk factors among the general population and osteoarthritis patients

Sang Hyuck Kim, Jae Moon Yun, Chong Bum Chang, Heng Piao, Su Jong Yu, Dong Wook Shin

Sang Hyuck Kim, Jae Moon Yun, Dong Wook Shin, Department of Family Medicine, Seoul National University Hospital, Seoul 03080, South Korea

Chong Bum Chang, Department of Orthopedic Surgery, Seoul Municipal Government-Seoul National University Boramae Medical Center, Seoul 03080, South Korea

Heng Piao, Department of Family Medicine, Graduate School, Seoul National University, Seoul 03080, South Korea

Su Jong Yu, Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul 03080, South Korea

Author contributions: All authors contributed to this work; Kim SH had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis; Kim SH, Yun JM, Shin DW concepted and designed this study; Kim SH, Yun JM, Shin DW, Piao PH acquisited data; all authors analysis data, or interpretation; all authors preparated and approved this manuscript.

Supported by Pfizer Pharmaceutical Korea Ltd, No. A3191378.

Institutional review board statement: This study was approved by the institutional review board of Seoul National University Hospital, No. E-1508-002-689.

Informed consent statement: As this study was an observational study and we used data from the public repository (National Health Insurance Service Sharing Service, https://nhiss.nhis.or.kr/bd/ay/bdaya001iv.do;jsessionid=3xOUDsw6a2QK3ySYMpQ89I3lkGUasr6famrJXqsPHnXUv5ZSQHwSrTY3jZiuZLPh.primrose2_servlet_engine1), informed consent was exempted (IRB No. E-1508-002-689).

Conflict-of-interest statement: The authors declare that they have no competing interests.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author from email of DW Shin (dwshin.snuh@gmail.com). The presented data are anonymized and risk of identification is low. As the data is owned to the National Health Insurance, institutional approval must precede before providing the dataset.

Manuscript source: Unsolicited manuscript

Correspondence to: Dong Wook Shin, MD, DrPH, MBA, Department of Family Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, South Korea. dwshin.snuh@gmail.com
Telephone: +82-2-20720847
Fax: +82-2-7663276

Received: August 4, 2016
Peer-review started: August 5, 2016
First decision: September 12, 2016
Revised: October 13, 2016
Accepted: October 30, 2016
Article in press: October 31, 2016
Published online: December 28, 2016

Abstract

AIM

To assess the prevalence of possible risk factors of upper gastrointestinal bleeding (UGIB) and their age-group specific trend among the general population and osteoarthritis patients.

METHODS

We utilized data from the National Health Insurance

Service that included claims data and results of the national health check-up program. Comorbid conditions (peptic ulcer, diabetes, liver disease, chronic renal failure, and gastroesophageal reflux disease), concomitant drugs (aspirin, clopidogrel, cilostazol, non-steroidal anti-inflammatory drugs, steroid, anticoagulants, and SSRI), personal habits (smoking, and alcohol consumption) were considered as possible UGIB risk factors. We randomly imputed the prevalence of infection in the data considering the age-specific prevalence of *Helicobacter pylori* (*H. pylori*) infection in Korea. The prevalence of various UGIB risk factors and the age-group specific trend of the prevalence were identified. Prevalence was compared between osteoarthritis patients and others.

RESULTS

A total of 801926 subjects (93855 osteoarthritis patients) aged 20 and above were included. The prevalence of individual and concurrent multiple risk factors became higher as the age increased. The prevalence of each comorbid condition and concomitant drug were higher in osteoarthritis patients. Thirty-five point zero two percent of the overall population and 68.50% of osteoarthritis patients had at least one or more risk factors of UGIB. The prevalence of individual and concurrent multiple risk factors in younger age groups were also substantial. Furthermore, when personal habits (smoking, and alcohol consumption) and *H. pylori* infection were included, the prevalence of concurrent multiple risk factors increased greatly even in younger age groups.

CONCLUSION

Prevalence of UGIB risk factors was high in elderly population, but was also considerable in younger population. Patient with osteoarthritis was at higher UGIB risk than those without osteoarthritis. Physicians should consider individualized risk assessment regardless of age when prescribing drugs or performing procedures that may increase the risk of UGIB, and take necessary measures to reduce modifiable risk factors such as *H. pylori* eradication or lifestyle counseling.

Key words: Upper gastrointestinal bleeding; Prevalence; Risk factor; General population; Osteoarthritis

Core tip: This study identified the prevalence of various upper gastrointestinal bleeding (UGIB) risk factors and the age-group specific trend of the prevalence in general population and osteoarthritis patients using large population representative data. Considering the age-group specific trend of the prevalence of UGIB risk factors, physicians should consider individualized risk assessment regardless of age when prescribing drugs or performing procedures that predispose to UGIB. Additionally, subjects with high risk should control modifiable UGIB risk factors.

Kim SH, Yun JM, Chang CB, Piao H, Yu SJ, Shin DW. Prevalence of upper gastrointestinal bleeding risk factors among the general population and osteoarthritis patients. *World J Gastroenterol* 2016; 22(48): 10643-10652 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10643.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10643

INTRODUCTION

Despite advances in medical science, upper gastrointestinal bleeding (UGIB) is still a high risk condition with high morbidity and mortality[1]. Rotondano *et al*[2] reported that the overall mortality rate of UGIB was approximately 5%. Although its incidence is decreasing worldwide, a substantial amount of patients are still suffering from UGIB[3]. In the United States, the incidence of UGIB was approximately 60.6 per 100000 in 2009[4]. Furthermore, the proportion of aspirin- or non-steroidal anti-inflammatory drugs (NSAIDs)-related UGIB is also increasing[5]. This has led to incurrence of substantial costs[6].

The high mortality and economic burden of UGIB have raised concerns regarding risk factors for the disease. In general, UGIB occurs more frequently in the male sex and advancing age[7,8]. Additionally, many underlying diseases, drugs, and unhealthy lifestyle (*e.g.,* smoking) are proven risk factors of UGIB[3,8-27]. Also, the increasing trend of NSAIDS-induced UGIB has also led to research focusing on arthritis patients, who are potential long-term consumers of these predisposing drugs[28-31].

While several studies have identified significant risk factors of UGIB, many focused on assessing the relative risks of each factor in a specific population group only, not the general population. To the best of our knowledge, to date, no large general-population-based studies have investigated the epidemiology of UGIB risk factors.

In this study, we assessed the prevalence of possible risk factors of UGIB and their age-group specific trend among the general population, and compared the prevalence between patients with osteoarthritis and others.

MATERIALS AND METHODS

Data source

This study utilized data from the National Health Insurance Service (NHIS) (NHIS-2015-2-099), compiled by the NHIS. The National Health Insurance in South Korea covers approximately 98% of the population, ensuring generalization of data[32]. The original claims

data included 90% of the population in South Korea. Using a randomized stratified sampling method, the NHIS provided 2% samples of cohorts of the claims data from 2002 to 2013. Detailed explanation of the data structure is provided elsewhere[33]. To investigate recent epidemiology, we used the claims data of outpatient visits in 2013. In addition, to take into account subjects' personal habits, we used results from the national health check-up program cohorts in 2013, which included lifestyle and medical information, as well as biochemical markers. NHIS provides both claims data and results of national health check-up programs through an online review system (https://nhiss.nhis.or.kr/bd/ab/bdaba000eng.do).

This study was approved by the institutional review board of Seoul National University Hospital (IRB No. E-1508-002-689).

Risk factors of UGIB

From a literature review of previous studies, we identified the possible risk factors of UGIB. We subdivided risk factors into 3 categories: (1) comorbid conditions; (2) concomitant drugs; and (3) personal habit. Comorbid conditions included peptic ulcer[8,10,16], diabetes mellitus[15], liver disease[14], chronic renal failure[3,17,18], and gastroesophageal reflux disease (GERD)[3]. Concomitant drugs included oral aspirin[3,8,11,15,21], other antiplatelet agents including cilostazol and clopidogrel[3,11], NSAIDs[3,8,10,11,16,29], steroids[3,19], anticoagulants including warfarin, rivaroxaban, dabigatran, and apixaban[3,10,11], and selective serotonin reuptake inhibitors[3,23,24]. As selective cyclooxygenase (COX)-2 inhibitors had been prescribed to reduce the gastrointestinal complications, these drugs were not included as concomitant drugs[31,34,35]. Personal habits included smoking[8,10,11], and alcohol consumption[8,10,11].

Helicobacter pylori (*H. pylori*) infection was also considered as a possible independent risk factor[3,8,10,13,16].

Definition of variables

We defined cases of comorbid conditions based on the ICD-10 codes: "K25.X", "K26.X", "K27.X", and "K28.X" for peptic ulcer; "E08.X", "E09.X", "E10.X", "E11.X", "E12.X", "E13.X", and "E14.X" for diabetes; "K70.X", "K71.X", "K72.X", "K73.X", "K74.X", "K75.X", "K76.X", and "K77.X" for liver disease; "N18.X" for chronic renal failure; "K21.X", and "K221" for GERD. Cases of comorbid conditions were defined if any of the above-mentioned relevant ICD-10 codes was the main diagnosis (for which the patient primarily presented with), or the first additional diagnosis (that the patient was already being treated for or was diagnosed at the same visit as the main diagnosis). Subjects with "M15.X" to "M19.X" ICD-10 codes as the main or the first additional diagnosis were defined as patients with osteoarthritis.

Although short-term use of some drugs could increase the risk of UGIB[36], we aimed to identify longer-term users of predisposing drugs, especially those with the potential to consume these drugs for life. Therefore, we only included subjects who had been prescribed drugs for at least 60 d as cases of concomitant drug users.

Smoking was subdivided into 2 categories: (1) non- or former smoker; and (2) current smoker. Problematic alcohol consumption was defined as follows: men who drink more than 4 standard drinks a day or 14 standard drinks a week; women who drink more than 3 standard drinks a day or 7 standard drinks a week; and subjects aged 65 or more who drink more than 1 standard drink a day or 7 standard drinks a week. One standard drink contains approximately 14 g of alcohol[37].

Statistical analysis

Subjects aged 20 and above were included in our analysis. First, we calculated the prevalence of each risk factor within the general population and it was compared between osteoarthritis patients and others. To identify subjects with concurrent multiple risk factors, we categorized subjects into 3 categories: (1) those with 1 or more risk factors; (2) those with 2 or more risk factors; and (3) those with 3 or more risk factors or more. As patients with osteoarthritis potentially consume NSAIDs[31], we additionally calculated the prevalence after excluding NSAIDs as a concomitant drug for osteoarthritis patients. Additionally, age-group specific prevalence of individual and concurrent multiple risk factors were calculated. Since the data did not provide information about *H. pylori* infection, we formulated a statistical method to consider this infection. Considering the age-specific prevalence of *H. pylori* infection in Korea from a representative large cohort study, we randomly imputed the prevalence of infection in the data[38]. The reported prevalence of *H. pylori* infection was 26.4, 42.1, 52.6, 61.4, 61.6, and 58.6 % for subjects in their 20 s, 30 s, 40 s, 50 s, 60 s, and 70 s (or more), respectively. Following random imputation, prevalence of concurrent multiple risk factors were calculated.

As mentioned above, we included personal habits as risk factors of UGIB. However, information regarding subjects' personal habits was only available in those who participated in the national health check-up program in 2013. Subgroup analysis was performed to take into account the lifestyle factors in these subjects.

To address the possible differences of the definition of concomitant drug user, sensitivity analysis was performed. Subjects who have been prescribed the drugs for at least 30 d were considered as cases of concomitant drug users.

All statistical analyses were conducted using the STATA software version 14.0 (StataCorp., TX).

RESULTS

A total of 801926 subjects from the general population

Table 1 Prevalence of gastrointestinal bleeding risk factors

	Overall Population (n = 801926)	Without Osteoarthritis (n = 708107)	Osteoarthritis Patients (n = 93855)
Comorbid conditions			
Peptic ulcer	9.15%	7.85%	19.00%
Diabetes	8.33%	7.04%	18.12%
Chronic liver disease	5.76%	5.28%	9.32%
Chronic renal failure	0.49%	0.44%	0.83%
Gastroesophageal reflux disease	14.30%	12.67%	26.60%
Concomitant drugs[1]			
Aspirin	6.63%	5.41%	15.86%
Clopidogrel	1.78%	1.43%	4.44%
Cilostazol	0.66%	0.51%	1.76%
NSAIDs	5.99%	3.02%	28.42%
Steroid	1.28%	0.93%	3.94%
Anticoagulants[2]	0.18%	0.16%	0.36%
SSRI	0.44%	0.37%	1.00%
No. of risk factors			
⩾ 1	35.02%	30.58%	68.50%
⩾ 2	13.87%	10.66%	38.04%
⩾ 3	4.52%	2.97%	16.25%
No. of risk factors (Excluding concomitant NSAIDs Use)			
⩾ 1	33.10%	29.49%	60.40%
⩾ 2	11.81%	9.63%	28.26%
⩾ 3	3.22%	2.38%	9.60%
No. of risk factors (After *H. pylori* prevalence imputation)			
⩾ 1	64.57%	62.16%	82.76%
⩾ 2	23.26%	20.08%	47.19%
⩾ 3	8.12%	6.47%	20.60%

[1]Subjects who were prescribed 60 d or more in 2013; [2]Warfarin, rivaroxaban, dabigatran, and apixaban were included. All P values from the χ^2 test between subjects without osteoarthritis and arthritis patients are below 0.001. NSAIDs: Non-steroidal anti-inflammatory drugs; SSRI: Selective serotonin reuptake inhibitor.

aged 20 or more (93855 were patients with osteoarthritis) were included in the analysis. Out of this, 233879 subjects participated in the national health check-up program in 2013. The age- and sex-specific distributions of the study population are provided in online only supplementary Table 1.

Table 1 shows the overall prevalence of individual and concurrent multiple risk factors. GERD was the most frequent comorbid condition associated with UGIB in the overall population, subjects without osteoarthritis, and patients with osteoarthritis (14.10%, 12.67%, and 26.60%, respectively). Although aspirin was the most frequently used predisposing drug in the overall population (6.63%), NSAIDs had the highest prevalence in osteoarthritis patients (28.42%). The prevalence of each comorbid condition and concomitant drug usage were also higher in these patients compared to the general population. We found that 35.02% of the overall population and 68.50% of osteoarthritis patients had at least one or more risk factors of UGIB. More than 16% of subjects with osteoarthritis had 3 or more concurrent risk factors. Even when NSAIDs were excluded for prevalence calculation of concurrent multiple risk factors, the prevalence of osteoarthritis patients with at least one or more risk factors decreased by only 8%. When *H. pylori* infection was considered, the prevalence of concurrent multiple risk factors increased approximately 2-fold in the overall population. The increase in the prevalence of osteoarthritis patients was also substantial. All P values for each risk factor from the chi square test between subjects without osteoarthritis and osteoarthritis patients were below 0.001.

Figure 1 shows the age-group specific trend of prevalence with at least one risk factor. Prevalence of UGIB risk factors increases with age, with the highest value seen among the 70-79 year-old age group. The age-group specific prevalence of UGIB risk factors among osteoarthritis patients is provided in online only supplementary Table 2.

The increased prevalence of UGIB risk factors with age was consistently noted in the overall population, peaking among subjects in their 70 s (Table 2). For subjects who participated in the national health check-up program, the prevalence of multiple risk factors increased naturally as 2 more risk factors (smoking, and alcohol consumption) were considered (Figure 2). The number of smokers and alcohol consumers generally decreased with age. As the criteria for alcohol consumption changed at the age of 65, the prevalence of problematic drinking increased at the age-group (see online only supplementary Table 3).

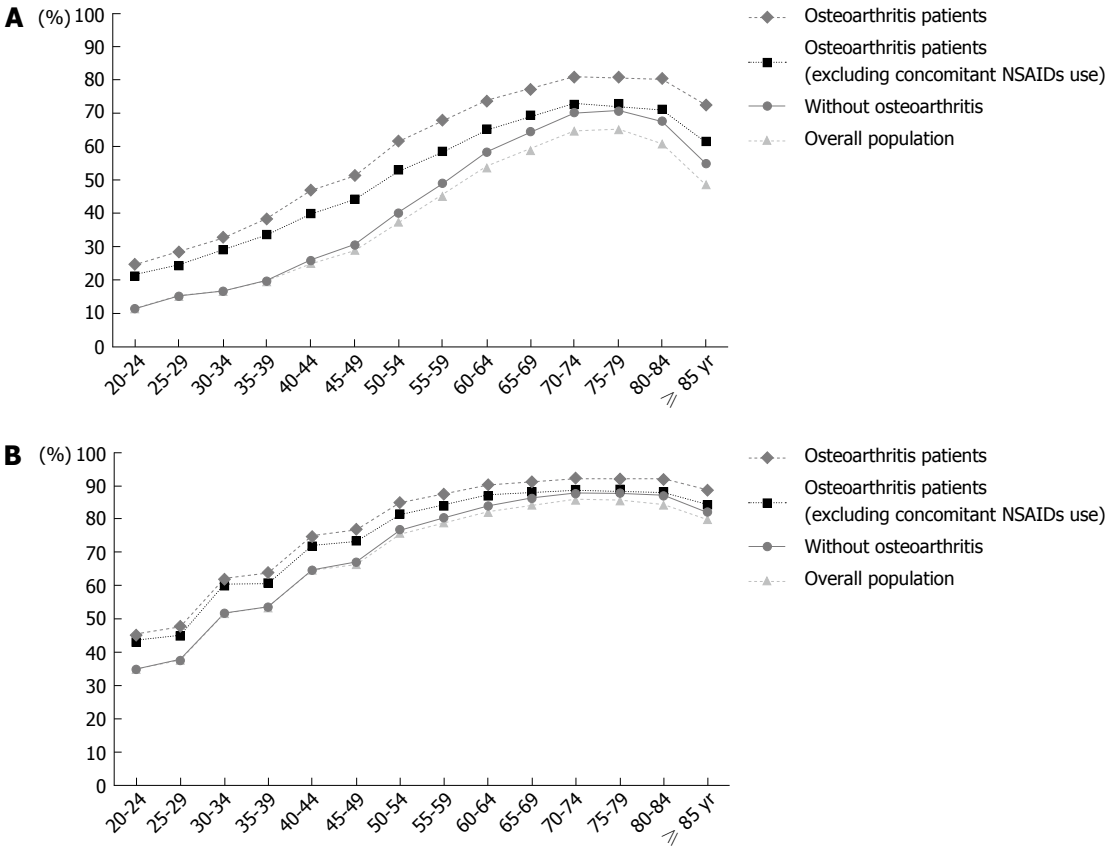

Figure 1 Age-group specific prevalence of any upper gastrointestinal bleeding risk factors. A: Without *Helicobacter pylori* (*H. pylori*) prevalence imputation; B: With *H. pylori* prevalence imputation; NSAIDs: Non-steroidal anti-inflammatory drugs.

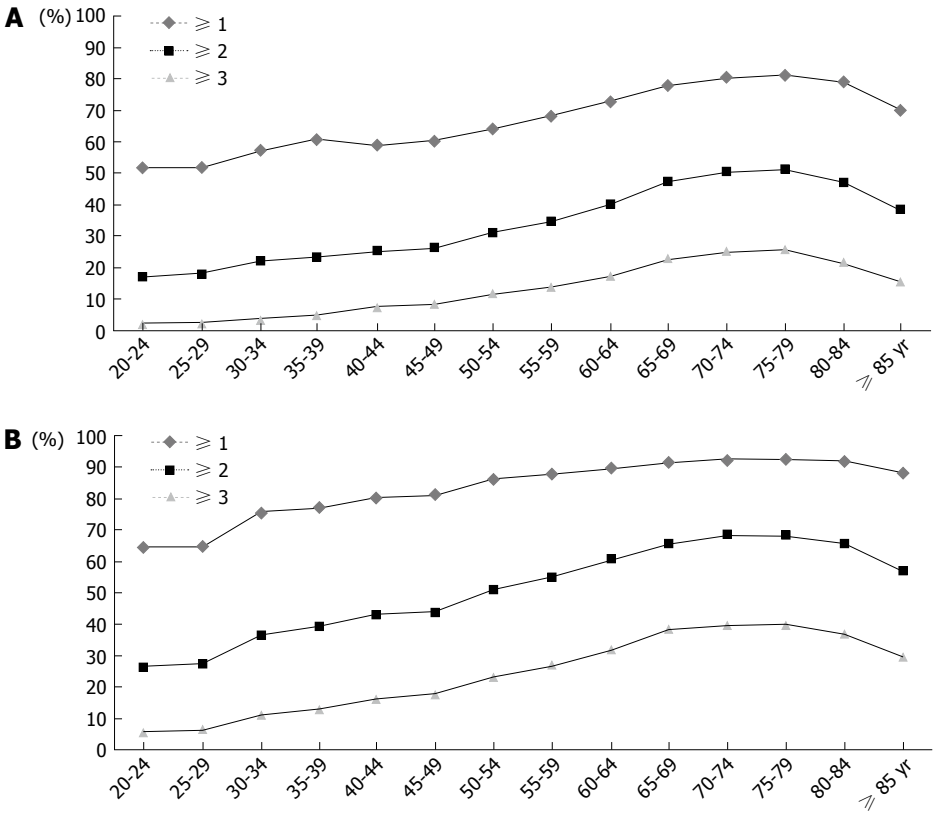

Figure 2 Age-Group Specific Prevalence of Multiple Risk Factors in Subjects Performed Health Check-up. A: Without *Helicobacter pylori* (*H. pylori*) prevalence imputation; B: With *H. pylori* prevalence imputation. Smoking and problematic alcohol drinking were included as risk factors additionally.

Table 2 Age-group specific prevalence of gastrointestinal bleeding risk factors in overall population

	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	⩾ 85 yr
Comorbid conditions														
Peptic ulcer	3.84%	4.80%	4.92%	5.49%	7.61%	8.47%	10.87%	12.45%	14.20%	15.49%	17.50%	16.08%	13.72%	9.95%
Diabetes	0.31%	0.49%	0.96%	1.76%	3.27%	5.68%	8.78%	13.17%	18.18%	22.04%	25.48%	25.96%	23.55%	16.13%
Chronic liver disease	2.17%	2.99%	3.74%	4.40%	4.76%	5.99%	7.55%	8.68%	9.09%	9.03%	8.01%	6.91%	5.65%	3.96%
Chronic renal failure	0.03%	0.06%	0.09%	0.14%	0.23%	0.28%	0.39%	0.59%	0.88%	1.13%	1.66%	1.84%	1.96%	1.66%
Gastroesophageal Reflux disease	6.02%	8.34%	8.88%	9.92%	13.22%	13.63%	17.18%	19.11%	22.11%	22.85%	23.93%	20.94%	17.29%	11.43%
Concomitant drugs[1]														
Aspirin	0.03%	0.08%	0.13%	0.42%	1.15%	2.65%	5.66%	10.21%	15.55%	19.84%	24.26%	25.57%	25.15%	19.66%
Clopidogrel	0.01%	0.01%	0.03%	0.09%	0.27%	0.56%	1.10%	2.13%	3.66%	5.18%	7.23%	8.59%	9.41%	7.19%
Cilostazol	0.00%	0.00%	0.01%	0.03%	0.11%	0.23%	0.48%	0.86%	1.36%	1.98%	2.59%	3.09%	2.96%	2.17%
NSAIDs	0.48%	0.66%	1.00%	1.65%	2.28%	3.58%	6.19%	8.77%	11.81%	15.27%	18.31%	19.83%	19.57%	14.99%
Steroid	0.27%	0.35%	0.44%	0.59%	0.72%	0.91%	1.23%	1.64%	2.18%	2.99%	3.60%	3.80%	3.48%	2.50%
Anticoagulants[2]	0.01%	0.01%	0.03%	0.04%	0.06%	0.09%	0.15%	0.23%	0.32%	0.47%	0.68%	0.88%	0.68%	0.46%
SSRI	0.16%	0.17%	0.15%	0.21%	0.24%	0.29%	0.38%	0.41%	0.59%	0.92%	1.23%	1.57%	1.88%	1.41%
No. of risk factors														
⩾ 1	11.53%	15.26%	16.72%	19.63%	25.88%	30.45%	40.22%	49.00%	58.39%	64.43%	70.27%	70.69%	67.59%	54.93%
⩾ 2	1.66%	2.42%	3.23%	4.36%	6.59%	9.35%	14.79%	20.87%	28.01%	33.92%	39.74%	40.03%	36.67%	25.34%
⩾ 3	0.16%	0.25%	0.39%	0.67%	1.22%	2.10%	3.92%	6.44%	10.06%	13.44%	17.08%	16.97%	15.05%	8.48%
No. of risk factors (After *H. pylori* prevalence imputation)														
⩾ 1	34.84%	37.72%	51.60%	53.61%	64.84%	67.08%	76.84%	80.34%	83.99%	86.35%	87.86%	87.91%	86.93%	82.20%
⩾ 2	4.24%	5.79%	8.93%	10.81%	16.85%	20.48%	30.34%	38.05%	46.72%	52.90%	57.54%	57.88%	54.60%	42.92%
⩾ 3	0.54%	0.83%	1.60%	2.26%	4.04%	5.87%	10.63%	15.35%	21.22%	26.20%	30.43%	30.36%	27.95%	18.31%

[1]Subjects who were prescribed 60 d or more in 2013; [2]Warfarin, rivaroxaban, dabigatran, and apixaban were included. NSAIDs: Non-steroidal anti-inflammatory drugs; SSRI: Selective serotonin reuptake inhibitor.

In the sensitivity analysis, although the prevalence of each concomitant drug usage was slightly decreased, the prevalence did not vary much from that of the main analysis (Table 3).

DISCUSSION

To the best of our knowledge, this is the first study to investigate the prevalence of individual and concurrent multiple UGIB risk factors in the general population and patients with osteoarthritis. We also identified the trend of age-group specific prevalence of UGIB risk factors. Large sample size with systematic sampling method ensures the generalization of the results. Moreover, the unique dataset from NHIS enabled us to address multiple personal habits such as smoking, alcohol consumption, as well as comorbid diseases and drugs.

Our findings showed that age was associated with increased prevalence of UGIB risk factors, consistent with previous studies[7,8]. Naturally, the prevalence of individual and concurrent multiple risk factors became higher as the age increased. However, the prevalence of UGIB risks in younger subjects was also substantial. In the overall population, more than 14% of subjects aged 50-54 years old already had 2 or more risk factors. When *H. pylori* infection was considered, the prevalence of 3 or more risk factors is 10% in this age group. Furthermore, approximately 50% of subjects aged 30-34 years old had at least one risk factor. This is even more

Table 3 Sensitivity analysis for prevalence of gastrointestinal bleeding risk factors in overall population (%)

	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	⩾ 85 yr
Comorbid conditions														
Peptic ulcer	3.84%	4.80%	4.92%	5.49%	7.61%	8.47%	10.87%	12.45%	14.20%	15.49%	17.50%	16.08%	13.72%	9.95%
Diabetes	0.31%	0.49%	0.96%	1.76%	3.27%	5.68%	8.78%	13.17%	18.18%	22.04%	25.48%	25.96%	23.55%	16.13%
Chronic liver disease	2.17%	2.99%	3.74%	4.40%	4.76%	5.99%	7.55%	8.68%	9.09%	9.03%	8.01%	6.91%	5.65%	3.96%
Chronic renal failure	0.03%	0.06%	0.09%	0.14%	0.23%	0.28%	0.39%	0.59%	0.88%	1.13%	1.66%	1.84%	1.96%	1.66%
Gastroesophageal reflux disease	6.02%	8.34%	8.88%	9.92%	13.22%	13.63%	17.18%	19.11%	22.11%	22.85%	23.93%	20.94%	17.29%	11.43%
Concomitant drugs[1]														
Aspirin	0.04%	0.09%	0.18%	0.50%	1.26%	2.84%	5.97%	10.67%	16.17%	20.60%	25.24%	26.59%	26.35%	20.75%
Clopidogrel	0.01%	0.01%	0.04%	0.10%	0.28%	0.59%	1.17%	2.25%	3.83%	5.47%	7.58%	9.03%	10.00%	7.75%
Cilostazol	0.00%	0.00%	0.02%	0.04%	0.13%	0.25%	0.54%	0.96%	1.48%	2.15%	2.83%	3.39%	3.21%	2.45%
NSAIDs	2.34%	3.31%	4.56%	6.29%	7.35%	9.96%	14.69%	18.52%	23.02%	27.50%	31.07%	31.68%	30.34%	22.76%
Steroid	0.88%	1.07%	1.28%	1.62%	1.88%	2.21%	2.91%	3.50%	4.43%	5.54%	6.59%	6.72%	6.07%	4.58%
Anticoagulants[2]	0.01%	0.01%	0.03%	0.04%	0.07%	0.10%	0.16%	0.25%	0.34%	0.53%	0.73%	0.96%	0.73%	0.50%
SSRI	0.21%	0.22%	0.21%	0.27%	0.32%	0.38%	0.50%	0.55%	0.74%	1.13%	1.53%	1.89%	2.13%	1.70%
No. of risk factors (without *H. pylori* prevalence imputation)														
⩾ 1	13.32%	17.55%	19.61%	23.22%	29.38%	34.49%	44.88%	53.68%	62.87%	68.98%	74.57%	74.65%	71.74%	58.61%
⩾ 2	2.25%	3.29%	4.42%	5.97%	8.57%	11.89%	18.42%	24.98%	33.02%	39.09%	45.26%	45.36%	41.69%	29.63%
⩾ 3	0.29%	0.48%	0.74%	1.18%	1.99%	3.20%	5.72%	8.93%	13.23%	17.29%	21.32%	21.25%	18.75%	11.07%
No. of risk factors (after *H. pylori* prevalence imputation)														
⩾ 1	36.21%	39.37%	53.24%	55.64%	66.45%	68.97%	78.69%	82.11%	85.68%	88.13%	89.66%	89.52%	88.55%	83.72%
⩾ 2	5.12%	7.08%	10.87%	13.30%	19.66%	23.81%	34.58%	42.48%	51.41%	57.73%	62.40%	62.59%	59.14%	46.84%
⩾ 3	0.78%	1.21%	2.32%	3.24%	5.45%	7.75%	13.52%	18.90%	25.56%	30.79%	35.35%	35.09%	32.49%	21.95%

[1]Subjects who were prescribed 60 d or more in 2013; [2]Warfarin, rivaroxaban, dabigatran, and apixaban were included. NSAIDs: Non-steroidal anti-inflammatory drugs; SSRI: Selective serotonin reuptake inhibitor.

evident when we included personal habits as risk factors.

Patients with osteoarthritis have a higher prevalence of concurrent multiple risk factors compared to the subjects without, even after excluding NSAIDs. When NSAIDs were excluded, the difference of age-group specific prevalence of having at least one risk factor between the subjects without osteoarthritis and osteoarthritis patients became smaller. However, this difference of prevalence became larger as age decreases, suggesting that young osteoarthritis patients have more underlying risk factors than the subjects without osteoarthritis.

Our findings have important clinical implications: First, elderly subjects are at high risk for UGIB by not only due to their age, but also multiple comorbid conditions and concomitant drug usage. Furthermore, increasing age is associated with UGIB occurrence, recurrence, and mortality[8]. Physicians with elderly patients should, therefore, identify measures to reduce the occurrence of UGIB in this population.

Second, our study revealed that a considerable portion of young adults has concurrent multiple risk factors of UGIB. When *H. pylori* infection was considered, more than 10% of the general population aged 35-39 had 2 or more UGIB risk factors. When we took into account subjects' personal habits, more than 60% of those aged 20-24 factors, physicians should consider individualized risk assessment for UGIB of already had at least one UGIB risk factor. Given that osteoarthritis patients are likely

to consume NSAIDs and possess higher prevalence of UGIB risk in NSAIDs prescription, regardless of patients' age[30]. Selective COX-2 inhibitors or concurrent prescription of proton pump inhibitors or misoprostol may be a good option for osteoarthritis patients with high risk of UGIB[28,34,35,39,40].

Finally, identifying and controlling modifiable risk factors is of great importance. The majority of subjects who already consume the above-mentioned drugs are those who require them long-term, possibly lifelong. In addition, most of the comorbid conditions related to UGIB are intractable chronic disease. Therefore, these high-risk subjects should control any modifiable risk factors of UGIB, such as smoking, heavy drinking, and *H. pylori* infection[27,35]. Also, further prospective studies are needed to address the issue of other lifestyle modification and UGIB prevention.

This study has several limitations. First, we did not include other risk factors apart from the ones studied. For instance, drug-drug interaction and drug dosage (both of which increase the risk of UGIB) were not considered[19,25]. Furthermore, some drugs may cause UGIB even with short-term use[36]. Additionally, we used imputation of *H. pylori* infection prevalence from a cohort study[38]. This might affect the prevalence of individual and concurrent multiple risk factors of UGIB. More detailed information about individual-level risks may give rise to a different prevalence. However, the difference in prevalence between the main and sensitivity analysis was not high, supporting the reliability of the results. Finally, ICD-10 codes based definition of osteoarthritis may not meet the specific diagnostic criteria. However, claims with such osteoarthritis diagnosis are usually made with clinical features which are consistent with symptoms and signs of osteoarthritis, and are accompanied by prescription of NSAIDS, which increases the risk of UGIB. In addition, claims data has its own strengths in terms of large sample size, representativeness, and generalizability to the real world setting.

We investigated the prevalence of various risk factors of UGIB in the general population and osteoarthritis patients. Physicians should consider individualized risk assessment regardless of age when prescribing drugs or performing procedures that may increase the risk of UGIB, and take necessary measures to reduce modifiable risk factors such as *H. pylori* eradication or lifestyle counseling.

COMMENTS

Background

Despite advances in medical science, upper gastrointestinal bleeding (UGIB) is still a high risk condition with high morbidity and mortality. Although its incidence is decreasing worldwide, a substantial amount of patients are still suffering from UGIB. Furthermore, the proportion of aspirin- or non-steroidal anti-inflammatory drugs-related UGIB is also increasing.

Research frontiers

While several studies have identified significant risk factors of UGIB, many focused on assessing the relative risks of each factor in a specific population group only, not the general population. To the best of our knowledge, to date, no large general-population-based studies have investigated the epidemiology of UGIB risk factors. The research hotspot is to assess the prevalence of various UGIB risk factors among the general population, and compared the prevalence between patients with osteoarthritis and others.

Innovations and breakthroughs

This is the first study to investigate the prevalence of individual and concurrent multiple UGIB risk factors in the general population and patients with osteoarthritis. The authors also identified the trend of age-group specific prevalence of UGIB risk factors. The prevalence of individual and concurrent multiple risk factors became higher as the age increased. However, the prevalence of UGIB risks in younger subjects was also substantial. In addition, patients with osteoarthritis have a higher prevalence of concurrent multiple risk factors compared to the general population.

Applications

Present study has important clinical implications. First, elderly subjects are at high risk for UGIB by not only due to their age, but also multiple comorbid conditions and concomitant drug usage. Second, physicians should always bear in mind the possibility of UGIB, regardless of age. Finally, identifying and controlling modifiable risk factors is of great importance.

Peer-review

In the presented article the prevalence of possible risk factors of UGIB among general population and patients with osteoarthritis were assessed. The prevalence of risk factors were higher in patients with osteoarthritis compared to general population

REFERENCES

1 **Hearnshaw SA**, Logan RF, Lowe D, Travis SP, Murphy MF, Palmer KR. Acute upper gastrointestinal bleeding in the UK: patient characteristics, diagnoses and outcomes in the 2007 UK audit. *Gut* 2011; **60**: 1327-1335 [PMID: 21490373 DOI: 10.1136/gut.2010.228437]

2 **Rotondano G**. Epidemiology and diagnosis of acute nonvariceal upper gastrointestinal bleeding. *Gastroenterol Clin North Am* 2014; **43**: 643-663 [PMID: 25440917 DOI: 10.1016/j.gtc.2014.08.001]

3 **Tielleman T**, Bujanda D, Cryer B. Epidemiology and Risk Factors for Upper Gastrointestinal Bleeding. *Gastrointest Endosc Clin N Am* 2015; **25**: 415-428 [PMID: 26142028 DOI: 10.1016/j.giec.2015.02.010]

4 **Laine L**, Yang H, Chang SC, Datto C. Trends for incidence of hospitalization and death due to GI complications in the United States from 2001 to 2009. *Am J Gastroenterol* 2012; **107**: 1190-1195; quiz 1196 [PMID: 22688850 DOI: 10.1038/ajg.2012.168]

5 **Ohmann C**, Imhof M, Ruppert C, Janzik U, Vogt C, Frieling T, Becker K, Neumann F, Faust S, Heiler K, Haas K, Jurisch R, Wenzel EG, Normann S, Bachmann O, Delgadillo J, Seidel F, Franke C, Lüthen R, Yang Q, Reinhold C. Time-trends in the epidemiology of peptic ulcer bleeding. *Scand J Gastroenterol* 2005; **40**: 914-920 [PMID: 16165708 DOI: 10.1080/00365520510015809]

6 **Laine L**, Peterson WL. Bleeding peptic ulcer. *N Engl J Med* 1994; **331**: 717-727 [PMID: 8058080 DOI: 10.1056/NEJM199409153311107]

7 **Longstreth GF**. Epidemiology of hospitalization for acute upper gastrointestinal hemorrhage: a population-based study. *Am J Gastroenterol* 1995; **90**: 206-210 [PMID: 7847286]

8 **Lau JY**, Sung J, Hill C, Henderson C, Howden CW, Metz DC. Systematic review of the epidemiology of complicated peptic ulcer disease: incidence, recurrence, risk factors and mortality. *Digestion* 2011; **84**: 102-113 [PMID: 21494041 DOI: 10.1159/000323958]

9 **Piper JM**, Ray WA, Daugherty JR, Griffin MR. Corticosteroid use

and peptic ulcer disease: role of nonsteroidal anti-inflammatory drugs. *Ann Intern Med* 1991; **114**: 735-740 [PMID: 2012355]
10 **Wolfe MM**, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1999; **340**: 1888-1899 [PMID: 10369853 DOI: 10.1056/NEJM199906173402407]
11 **Lanas Á**, Carrera-Lasfuentes P, Arguedas Y, García S, Bujanda L, Calvet X, Ponce J, Perez-Aísa Á, Castro M, Muñoz M, Sostres C, García-Rodríguez LA. Risk of upper and lower gastrointestinal bleeding in patients taking nonsteroidal anti-inflammatory drugs, antiplatelet agents, or anticoagulants. *Clin Gastroenterol Hepatol* 2015; **13**: 906-912.e2 [PMID: 25460554 DOI: 10.1016/j.cgh.2014.11.007]
12 **Massó González EL**, Patrignani P, Tacconelli S, García Rodríguez LA. Variability among nonsteroidal antiinflammatory drugs in risk of upper gastrointestinal bleeding. *Arthritis Rheum* 2010; **62**: 1592-1601 [PMID: 20178131 DOI: 10.1002/art.27412]
13 **Iijima K**, Kanno T, Koike T, Shimosegawa T. Helicobacter pylori-negative, non-steroidal anti-inflammatory drug: negative idiopathic ulcers in Asia. *World J Gastroenterol* 2014; **20**: 706-713 [PMID: 24574744 DOI: 10.3748/wjg.v20.i3.706]
14 **Luo JC**, Leu HB, Hou MC, Huang CC, Lin HC, Lee FY, Chang FY, Chan WL, Lin SJ, Chen JW. Cirrhotic patients at increased risk of peptic ulcer bleeding: a nationwide population-based cohort study. *Aliment Pharmacol Ther* 2012; **36**: 542-550 [PMID: 22817655 DOI: 10.1111/j.1365-2036.2012.05225.x]
15 **De Berardis G**, Lucisano G, D'Ettorre A, Pellegrini F, Lepore V, Tognoni G, Nicolucci A. Association of aspirin use with major bleeding in patients with and without diabetes. *JAMA* 2012; **307**: 2286-2294 [PMID: 22706834 DOI: 10.1001/jama.2012.5034]
16 **Sostres C**, Lanas A. Epidemiology and demographics of upper gastrointestinal bleeding: prevalence, incidence, and mortality. *Gastrointest Endosc Clin N Am* 2011; **21**: 567-581 [PMID: 21944411 DOI: 10.1016/j.giec.2011.07.004]
17 **Yang JY**, Lee TC, Montez-Rath ME, Paik J, Chertow GM, Desai M, Winkelmayer WC. Trends in acute nonvariceal upper gastrointestinal bleeding in dialysis patients. *J Am Soc Nephrol* 2012; **23**: 495-506 [PMID: 22266666 DOI: 10.1681/ASN.2011070658]
18 **Kalman RS**, Pedrosa MC. Evidence-based review of gastrointestinal bleeding in the chronic kidney disease patient. *Semin Dial* 2015; **28**: 68-74 [PMID: 25215610 DOI: 10.1111/sdi.12301]
19 **Gutthann SP**, García Rodríguez LA, Raiford DS. Individual nonsteroidal antiinflammatory drugs and other risk factors for upper gastrointestinal bleeding and perforation. *Epidemiology* 1997; **8**: 18-24 [PMID: 9116088]
20 **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]
21 **Valkhoff VE**, Sturkenboom MC, Kuipers EJ. Risk factors for gastrointestinal bleeding associated with low-dose aspirin. *Best Pract Res Clin Gastroenterol* 2012; **26**: 125-140 [PMID: 22542151 DOI: 10.1016/j.bpg.2012.01.011]
22 **García Rodríguez LA**, Jick H. Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs. *Lancet* 1994; **343**: 769-772 [PMID: 7907735]
23 **Anglin R**, Yuan Y, Moayyedi P, Tse F, Armstrong D, Leontiadis GI. Risk of upper gastrointestinal bleeding with selective serotonin reuptake inhibitors with or without concurrent nonsteroidal anti-inflammatory use: a systematic review and meta-analysis. *Am J Gastroenterol* 2014; **109**: 811-819 [PMID: 24777151 DOI: 10.1038/ajg.2014.82]
24 **Paton C**, Ferrier IN. SSRIs and gastrointestinal bleeding. *BMJ* 2005; **331**: 529-530 [PMID: 16150746 DOI: 10.1136/bmj.331.7516.529]
25 **Vonbach P**, Reich R, Möll F, Krähenbühl S, Ballmer PE, Meier CR. Risk factors for gastrointestinal bleeding: a hospital-based case-control study. *Swiss Med Wkly* 2007; **137**: 705-710 [PMID: 18197486]
26 **Peters HP**, De Vries WR, Vanberge-Henegouwen GP, Akkermans LM. Potential benefits and hazards of physical activity and exercise on the gastrointestinal tract. *Gut* 2001; **48**: 435-439 [PMID: 11171839]
27 **Pahor M**, Guralnik JM, Salive ME, Chrischilles EA, Brown SL, Wallace RB. Physical activity and risk of severe gastrointestinal hemorrhage in older persons. *JAMA* 1994; **272**: 595-599 [PMID: 8057514]
28 **Nasef SA**, Shaaban AA, Mould-Quevedo J, Ismail TA. The cost-effectiveness of celecoxib versus non-steroidal anti-inflammatory drugs plus proton-pump inhibitors in the treatment of osteoarthritis in Saudi Arabia. *Health Econ Rev* 2015; **5**: 53 [PMID: 26061682 DOI: 10.1186/s13561-015-0053-7]
29 **Laine L**, Harper S, Simon T, Bath R, Johanson J, Schwartz H, Stern S, Quan H, Bolognese J. A randomized trial comparing the effect of rofecoxib, a cyclooxygenase 2-specific inhibitor, with that of ibuprofen on the gastroduodenal mucosa of patients with osteoarthritis. Rofecoxib Osteoarthritis Endoscopy Study Group. *Gastroenterology* 1999; **117**: 776-783 [PMID: 10500058]
30 **Lee Bh**, Shin BJ, Kim DJ, Lee JC, Suk KS, Park YS, Kim KW, Cho KJ, Shin KY, Koh MS, Moon SH. Gastrointestinal Risk Assessment in the Patients Taking Nonsteroidal Anti-inflammarory Drugs for Lumbar Spinal Disease. *J Korean Society Spine Surg* 2011; **18**: 239 [DOI: 10.4184/jkss.2011.18.4.239]
31 **Lee SH**, Han CD, Yang IH, Ha CW. Prescription pattern of NSAIDs and the prevalence of NSAID-induced gastrointestinal risk factors of orthopaedic patients in clinical practice in Korea. *J Korean Med Sci* 2011; **26**: 561-567 [PMID: 21468265 DOI: 10.3346/jkms.2011.26.4.561]
32 **ChangBae C**, SoonYang K, JunYoung L, SangYi L. Republic of Korea. Health system review. *Health Systems Transit* 2009; **11**: 1-184
33 **Kim SH**, Cho BL, Shin DW, Hwang SS, Lee H, Ahn EM, Yun JM, Chung YH, Nam YS. The Effect of Asthma Clinical Guideline for Adults on Inhaled Corticosteroids PrescriptionTrend: A Quasi-Experimental Study. *J Korean Med Sci* 2015; **30**: 1048-1054 [PMID: 26240481 DOI: 10.3346/jkms.2015.30.8.1048]
34 **Lanza FL**, Chan FK, Quigley EM. Guidelines for prevention of NSAID-related ulcer complications. *Am J Gastroenterol* 2009; **104**: 728-738 [PMID: 19240698]
35 **Brooks J**, Warburton R, Beales IL. Prevention of upper gastrointestinal haemorrhage: current controversies and clinical guidance. *Ther Adv Chronic Dis* 2013; **4**: 206-222 [PMID: 23997925 DOI: 10.1177/2040622313492188]
36 **Chang CH**, Chen HC, Lin JW, Kuo CW, Shau WY, Lai MS. Risk of hospitalization for upper gastrointestinal adverse events associated with nonsteroidal anti-inflammatory drugs: a nationwide case-crossover study in Taiwan. *Pharmacoepidemiol Drug Saf* 2011; **20**: 763-771 [PMID: 21618340 DOI: 10.1002/pds.2140]
37 **Enoch MA**, Goldman D. Problem drinking and alcoholism: diagnosis and treatment. *Am Fam Physician* 2002; **65**: 441-448 [PMID: 11858627]
38 **Lim SH**, Kwon JW, Kim N, Kim GH, Kang JM, Park MJ, Yim JY, Kim HU, Baik GH, Seo GS, Shin JE, Joo YE, Kim JS, Jung HC. Prevalence and risk factors of Helicobacter pylori infection in Korea: nationwide multicenter study over 13 years. *BMC Gastroenterol* 2013; **13**: 104 [PMID: 23800201 DOI: 10.1186/1471-230X-13-104]
39 **Yuan JQ**, Tsoi KK, Yang M, Wang JY, Threapleton DE, Yang ZY, Zou B, Mao C, Tang JL, Chan FK. Systematic review with network meta-analysis: comparative effectiveness and safety of strategies for preventing NSAID-associated gastrointestinal toxicity. *Aliment Pharmacol Ther* 2016; **43**: 1262-1275 [PMID: 27121479 DOI: 10.1111/apt.13642]

40 **Brown TJ**, Hooper L, Elliott RA, Payne K, Webb R, Roberts C, Rostom A, Symmons D. A comparison of the cost-effectiveness of five strategies for the prevention of non-steroidal anti-inflammatory drug-induced gastrointestinal toxicity: a systematic review with economic modelling. *Health Technol Assess* 2006; **10**: iii-iv, xi-xiii, 1-183 [PMID: 17018227]

P- Reviewer: Garcia-Olmo D, Koch TR, Ozen H **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Zhang FF

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.v22.i48.10653

World J Gastroenterol 2016 December 28; 22(48): 10653-10662
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Variable outcome in infantile-onset inflammatory bowel disease in an Asian cohort

Way Seah Lee, Ruey Terng Ng, Koon-Wing Chan, Yu-Lung Lau

Way Seah Lee, Ruey Terng Ng, Department of Paediatrics, University Malaya Medical Center, Kuala Lumpur 59100, Malaysia

Way Seah Lee, University Malaya Paediatric and Child Health Research Group, University Malaya, Kuala Lumpur 59100, Malaysia

Koon-Wing Chan, Yu-Lung Lau, Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Author contributions: Lee WS conceived the concept and wrote the first draft; both Lee WS and Ng RT collected the clinical data and performed data analysis; Chan KW and Lau YL performed the mutational analysis; Lau YL provided critical analysis of the manuscript.

Supported by a research grant from Ministry of Higher Education, Malaysia (UM.C/625/HIR/MOHE/CHAN/13/1); Hong Kong Society for the Relief of Disabled Children (to Chan KW and Lau YL).

Institutional review board statement: The present study was reviewed and approved by the Medical Ethics Committee of University Malaya Medical Centre, Kuala Lumpur, Malaysia.

Informed consent statement: The legal guardians of all patients described in the present study gave informed written consent for mutational analysis performed for the present study.

Conflict-of-interest statement: The authors have declared that no competing interests exist.

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Way Seah Lee, Professor, Department of Paediatrics, University Malaya Medical Center, Lembah Pantai, Kuala Lumpur 59100, Malaysia. leews@um.edu.my
Telephone: +66-603-79492065
Fax: +66-603-79494704

Received: March 5, 2016
Peer-review started: March 8, 2016
First decision: April 14, 2016
Revised: August 31, 2016
Accepted: October 19, 2016
Article in press: October 19, 2016
Published online: December 28, 2016

Abstract

AIM

Infantile-onset inflammatory bowel disease (IO-IBD) with the onset of disease before 12 mo of age, is a different disease entity from childhood IBD. We aimed to describe the clinical features, outcome and role of mutation in interleukin-10 (IL-10) and interleukin-10 receptors (IL-10R) in Asian children with IO-IBD.

METHODS

All cases of IO-IBD, defined as onset of disease before 12 mo of age, seen at University Malaya Medical Center, Malaysia were reviewed. We performed mutational analysis for *IL10* and *IL10R* genes in patients with presenting clinical features of Crohn's disease (CD).

RESULTS

Six [13%; CD = 3, ulcerative colitis (UC) = 2, IBD-unclassified (IBD-U) = 1] of the 48 children (CD = 25; UC = 23) with IBD have IO-IBD. At final review [median (range) duration of follow-up: 6.5 (3.0-20) years], three patients were in remission without immunosuppression [one each for post-colostomy (IBD-U), after standard immunosuppression (CD), and after total colectomy (UC)]. Three patients were on immunosuppression:

one (UC) was in remission while two (both CD) had persistent disease. As compared with later-onset disease, IO-IBD were more likely to present with bloody diarrhea (100% *vs* 55%, *P* = 0.039) but were similar in terms of an associated autoimmune liver disease (0% *vs* 19%, *P* = 0.31), requiring biologics therapy (50% *vs* 36%, *P* = 0.40), surgery (50% *vs* 29%, *P* = 0.27), or achieving remission (50% *vs* 64%, *P* = 0.40). No mutations in either IL10 or IL10R in the three patients with CD and the only patient with IBD-U were identified.

CONCLUSION

The clinical features of IO-IBD in this Asian cohort of children who were negative for *IL-10* or *IL-10R* mutations were variable. As compared to childhood IBD with onset of disease after 12 mo of age, IO-IBD achieved remission at a similar rate.

Key words: Infantile-onset inflammatory bowel disease; Pediatric

Core tip: We described the clinical features, outcome and role of mutation in *IL-10* and *IL-10R* in Asian children with infantile-onset inflammatory bowel disease (IO-IBD). We reviewed all cases of IO-IBD, defined as onset of disease before 12 mo of age, seen at a single center in Malaysia. We conclude that the clinical features of IO-IBD in this Asian cohort of children were variable. IO-IBD achieved remission at a similar rate, were more likely to discontinue immunosuppression therapy at final review and not more likely to require biologics therapy or surgery.

Lee WS, Ng RT, Chan KW, Lau YL. Variable outcome in infantile-onset inflammatory bowel disease in an Asian cohort. *World J Gastroenterol* 2016; 22(48): 10653-10662 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10653.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10653

INTRODUCTION

Most of the patients with inflammatory bowel disease (IBD) have the onset of disease during adolescence or early adulthood[1,2]. There is a well-documented increase in the incidence of IBD with an onset of disease within the first two decades of life[3]. In childhood IBD, the disease phenotype and subsequent disease course are influenced by the age at first diagnosis[4]. In a large North American cohort of childhood IBD, those who had an onset of disease between 1 to 5 years (very early-onset) were more likely to have a mild disease at diagnosis but a more aggressive phenotype over time as compared to children who had an onset between 6 to 10 years of age[4].

The development of IBD in infancy is extremely rare[1]. Data from epidemiological studies and IBD registries, mostly from North America and Europe, suggest that less than 1% of children with IBD have an onset during the first 12 mo of life[5-9]. Crohn's disease (CD) appeared to be more common than ulcerative colitis (UC) in these studies[5-8]. However, a recent large cohort study from North America involving close to 2000 cases of childhood IBD did not identify any cases with an onset of disease < 1 year of age[4].

The current concept of the pathogenesis of IBD is that it develops in genetically susceptible hosts with an altered intestinal response to various external stimuli[10,11]. In infantile-onset (IO-) IBD, monogenic diseases causing persistent intestinal inflammation, such as Wiskott-Aldrich syndrome and hyper-IgM syndrome, are well documented[12,13]. Mutations in genes encoding the interleukin-10 (*IL10*) or interleukin-10 receptors (IL10R) subunit proteins have been discovered in patients with IO enterocolitis, usually within the first three months of life[14-18]. These infants have severe perianal disease and extra-intestinal features such as folliculitis and arthritis[14-17]. In some cases, hematopoietic stem cell transplant (HSCT) is curative in IBD secondary to IL10/IL10R deficiency[14-17]. Nevertheless, it has been shown that the majority of patients with severe infantile colitis produce and respond to IL10 normally[15], suggesting additional pathways to inflammation and the complex nature of the pathogenesis of infantile-onset inflammatory bowel disease (IO-IBD)[19].

IBD is not as common in Asians as in the Caucasians[20]. We reported the first case of IO-IBD in an Asian infant due to mutations in the IL10R by using exome sequencing[21]. Subsequently, other authors have also reported early-onset IBD due to mutations in IL-10R[22]. The aims of the present study were to describe the phenotypic characteristics and outcome of IO-colitis in a cohort of Asian children and to define the role of *IL10* and *IL10R* mutations in these patients.

MATERIALS AND METHODS

The present study was a retrospective review of all patients with childhood IBD who were seen at the Department of Paediatrics, University Malaya Medical Center (UMMC), Kuala Lumpur, Malaysia, from 1996 to 2014. During the study period, UMMC was the major referral center for pediatric IBD for entire Malaysia, serving both peninsular Malaysia and East Malaysia. The present study was funded by the High Impact Research Fund from Ministry of Higher Education, Malaysia (UM.C/625/HIR/MOHE/CHAN/13/1) and was approved by the institutional ethical committee of UMMC (UMMC 975.7). Written informed consent was given by the parents of the children for their clinical record, as well as the results of the mutational analysis to be used in the present study.

Patients

The medical records of all children younger than 18 years of age who have a diagnosis of IBD were reviewed. Patients who have the onset of the disease in the first 12 mo of age were included. Data on all children aged ⩽ 18 years of age with a diagnosis of IBD who are currently followed up at the department were also reviewed. The following patients were excluded: (1) patients with incomplete medical data; or follow-up or outcome data were incomplete; and (2) patients with an alternative diagnosis, such as infective, allergic, or iatrogenic (*i.e.,* radiation colitis or graft-*vs*-host diseases) causes of colitis.

Diagnosis

The patients were diagnosed to have CD, UC or IBD-unclassified (IBD-U) according to established clinical, biochemical, radiologic, endoscopic, and histologic criteria[23,24]. In UMMC, all patients suspected of having an IBD were investigated according to the recommendations by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition[23]. In addition, congenital or acquired immune deficiencies causing infantile colitis such as human immunodeficiency virus infection (HIV), severe combined immune deficiency (SCID), chronic granulomatous disease, hyper-IgM syndrome, and Wiskott-Aldrich syndrome were excluded by complete blood count, platelet count, peripheral blood film, HIV serology, immunoglobulins level (IgG, IgA, IgM and IgE), lymphocyte subset, nitroblue tetrazolium test, and anti-enterocyte antibody.

The diagnosis of IBD required endoscopic evaluation, including histologic assessment of mucosal pinch biopsies. All patients underwent esophagogastroduodenoscopy (EGDS) and colonoscopy. Multiple mucosal biopsies were reviewed by clinical pathologists. Stool studies were performed in all patients to exclude infectious causes of diarrheal illness. Disease location and behavior were classified according to the Paris Classification of Pediatric IBD[24]. Features which favor the diagnosis of CD are extensive endoscopic inflammation of the upper gastrointestinal tract; presence of perianal disease; normal looking rectum; presence of stenosis, cobblestoning, and linear ulceration in the ileum; and macroscopic ileitis in the presence of normally looking cecum[24]. Histological features favoring CD include microscopically normal appearing skip lesions as well as presence of well-formed granuloma remote from ruptured crypts[24].

Medical therapy

In children with CD, exclusive enteral nutrition (EEN) was the initial treatment of choice. In children who were unable to comply with EEN, or who did not respond to EEN, corticosteroid (CS; 1-2 mg/kg body weight, maximum 60 mg) was the initial immunosuppression of choice[25]. Azathioprine (Aza) at a maximum dose of 2.5 mg/kg body weight was used as steroid-sparing drug[25,26]. Monitoring of the therapeutic levels for Aza or determination of thiopurines methyltransferase genotype or phenotype were unavailable in the unit throughout the study period. The clinical response to Aza was closely observed while its side effects such as acute pancreatitis and marrow suppression were regularly monitored[26]. Infliximab (IFX) was used in children with refractory disease, fistulating CD, or in luminal CD despite optimal immunomodulatory therapy[25,26]. The IFX was administered in dose of 5 mg/kg body weight at weeks zero, two and six, followed by 8-weekly infusions[25]. This dosing regimen was adjusted according to the response of the patients, either by shortening the duration between two consecutive infusions, or an increase in the dose administered (maximum 10 mg/kg). No therapeutic level of IFX or antibodies against IFX was available within the country or the region during the study period. Adalimumab (Ada) was given to patients who developed a loss of effect to IFX. Ada was administered subcutaneously at week zero and two (160 mg and 80 mg, or 80 mg and 40 mg, for body weight ⩾ 40 kg or < 40 kg) and subsequently 40 mg every other week irrespective of body weight[27].

Data collection

The following data were collected: demographic data, clinical features, radiologic and histologic findings, medical and surgical therapies if applicable, and disease status at final review. Patients with CD were considered to have inactive disease if the Pediatric Crohn Disease Activity Index score was ⩽ 10, while patients with UC were considered to be in remission if the Pediatric Ulcerative Colitis Index was ⩽ 10[28,29].

Comparisons were made between patients with IO-IBD and patients who have an onset of disease after the first year of life (defined as later-onset disease) in their presenting features, immunosuppressive therapy and the need for surgery, as well as disease status at final review.

Mutational analysis

Blood samples were obtained from patients and their parents for mutational analysis after obtaining written informed consent. The analysis was performed at the Research Laboratory of the Department of Paediatrics and Adolescent Medicine, the University of Hong Kong. The genomic PCR was performed using the HotStarTaq® Plus PCR system (Qiagen GmbH, Germany). Between 10-100 ng of genomic DNA was amplified using the sense and antisense primers, flanking the coding regions and splice junctions, according to the manufacturer's protocol. DNA sequencing was performed on both strands using BigDye Terminator v3.1 Cycle Sequencing Kit and 3730xl DNA Analyzer (Applied Biosystems CA., United States). Homology analysis with the reference genomic sequence was

performed using the NCBI program BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The mutations screened for the purpose of the present study are shown in Supplementary material.

Statistical analysis

Data were managed with IBM SPSS statistical package version 21.0.0 for Windows. Dichotomous measures were compared by means Fisher exact test. Statistical significance was set at a P value of < 0.05.

RESULTS

During the study period, a total of 48 children with a diagnosis of IBD (CD = 25, UC = 23) were followed up at the Department of Paediatrics of UMMC. Of these, six (13%) had the onset of disease within the first year of life (IO-IBD). According to the Paris Classification for Pediatric IBD[24], two of the six patients had UC, three had CD, and one had IBD-U.

Demography

There were four males and two females. None of the patients has any first degree family members who also have IBD or other autoimmune conditions. With the exception of patient No. 1, none of the patient had any significant perinatal history. The age of onset of first symptom ranged from first week to 12 mo of life (median 5 mo; Table 1).

Phenotypic characteristics

Phenotypically, the patients can be classified into CD (n = 3), UC (n = 2), and IBD-U (n = 1) at initial presentation, but the subsequent disease course was diverse.

Clinical presentation

All the children presented initially with bloody diarrhea (Table 1). One patient was diagnosed to have postnatal cytomegalovirus (CMV) infection at birth. Oral ulcers were noted in one patient with CD while perianal disease was present in two of the three patients with CD and the infant with IBD-U.

Associated medical and autoimmune conditions

None of the patients developed other autoimmune diseases. One patient (infant No. 3) developed developmental regression at seven years of age with course tremors. No etiology can be ascertained despite extensive investigations. Investigations for immune system did not reveal the presence of immunodeficiency.

UC

Patient No. 1 developed watery, non-bloody diarrhea on third day of life (Table 1), and was started on extensively hydrolyzed and amino acid-based formulae. The diarrhea became bloody from three months onwards. When referred at our center at four months of age, there was no oral ulcer or perianal disease. During bowel rest and total parenteral nutrition, there was no diarrhea, but diarrhea promptly resumed when extensively hydrolyzed formula was introduced. A colonoscopy showed pancolitis with inflamed mucosa and friability but no ulceration. The rectum, sigmoid and descending colon were the most severely affected. Histologically, there was dense lymphoplasmacytic and eosinophilic infiltration of the colonic mucosa. A course of oral CS failed to improve his symptoms. The diarrhea gradually improved with amino acid-based formula. The stool frequency improved but was persistently blood stained.

There was a relapse of bloody diarrhea at sixteen month of age when the child was gradually weaned off from amino acid formula to a normal diet. Repeated courses of CS and a course of oral cyclosporin failed to improve his symptoms. A total colectomy was performed. Macroscopically, the entire colon was pale in appearance with prominent submucosal vascular pattern, indicating mucosal atrophy. No ulcers were noted. Histologically the mucosa was thin. There were areas of patchy inflammation, with evidence of chronic inflammation, dense lymphoplasmacytic infiltration in the mucosa, sparing the submucosa, muscular layer and serosa. There was no crypt branching, or irregularity in the shape and sizes of the crypt. There was also scant eosinophilic infiltration. The child remained symptom free after total colectomy.

CD

Patient No. 3 developed symptoms of recurrent bloody diarrhea at the age of seven months (Table 1). A diagnosis of postnatal CMV infection was made at another hospital at the age of three months due to persistent neonatal cholestasis. The IgM for CMV was positive. The jaundice subsided at the age of six months. The bloody diarrhea was intermittent in nature. At referral to our unit at two years of age, the child had failure to thrive, abdominal pain and bloody diarrhea, with multiple perianal fistulae and abscesses. EGDS showed duodenitis. Colonoscopy showed extensive ulceration with pseudopolyps formation. EEN was commenced, together with antibiotics, CS and Aza. IFX was given in view of severe perianal disease. After one year, there was improvement in general condition but minimal improvement in the perianal disease. He was noted to have three episodes of pneumonia and two episodes of herpes zoster infection when he was receiving IFX therapy. However, no recurrent infections were noted when the therapy was discontinued. In addition, investigations into an underlying immunodeficiency were negative.

A defunctioning colostomy was performed at six years of age. There was persistent perianal disease. A course of Ada (six doses) was given with no improvement. A repeat colonoscopy showed extensive

Table 1 Phenotypic characteristics, disease behavior, therapy and outcome in six Asian children with infantile-onset inflammatory bowel disease

Patients' initials	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Sex	Male	Male	Male	Male	Female	Female
Ethnicity	Chinese	Chinese	Malay	Chinese	Indian	Malay
Consanguinity	No	No	No	No	No	No
Breast feeding (duration)	No	No	No	Yes (3 mo)	Yes (2 mo)	Yes (4 mo)
Age at onset	First week	12 mo	7 mo	2 mo	6 mo	4 mo
Disease phenotype	EC→UC	UC	CD	CD	CD	IBD-U
Major symptoms at presentation	Bloody diarrhea and PR bleeding	Bloody diarrhea, anemia	Oral ulcers, bloody diarrhea, abdominal pain	Bloody diarrhea, abdominal pain	Bloody diarrhea, growth faltering	Bloody diarrhea
Perianal disease	Nil	Nil	Abscess and fistula	Abscess and fistula	Nil	Abscess and fistula
Other medical or autoimmune conditions	Nil	Nil	Nil	Nil	Nil	Nil
Recurrent infections	Nil	Nil	Congenital CMV infection,	Nil	Nil	Nil
Disease location[1]	E4S1	E4	L3L4a	L3L4a	L3	L2
Disease behavior/ severity[1]	S1	S1	B2B3p	B2B3p	B1	B1p
Histopathology	Dense lymphoplasmacytic and eosinophilic infiltration of the lamina propria involving the gastric mucosa, duodenum and colonic mucosa.	Colonic mucosa showed mild degenerative atypia, cryptitis and crypt abscesses. Lamina propria showed increase in neutrophilic and lymphoplasmacytic infiltration.	Lymphocytic infiltration of lamina propria. No granuloma or crypt abscess seen. The duodenum showed chronic inflammation.	Lymphocytic infiltration of lamina propria. No granuloma or crypt abscess seen	Mild inflammation in the lamina propria with lymphocytes and plasma cells. No granuloma or crypt abscess	First biopsy: transmural inflammation involving all layers of bowel wall, including the skeletal muscle with dense lymphoplasmacytic infiltration. No crypt abscess seen. Second biopsy: colonic mucosa inflammed but the architecture was preserved. Significant lymphoplasmacytic and neutrophilic infiltration mainly confined to the lamina propria
Other associated diseases	Nil	Nil	Developmental regression at 6 yr age	Nil	Nil	Nil
Mutational analysis	Not done	Not done	Not detected	Not detected	Not detected	Not detected
Medical therapy	CS, CsA, enteral nutrition	CS, Aza	CS, Aza, IFX × 24 doses	EN, CS, Aza, IFX × 14 doses	Aza, IFX × 3 doses	Nil
Surgery	Total colectomy at 18 mo	Nil	Ileostomy at 6 yr of age	Nil	Nil	Ileostomy; closure at 18 mo of age
Age at last follow up	21 yr	6 yr	9 yr	6 yr	13 yr	3 yr
Final clinical status	Alive, deafness due to aminoglycosides. in remission, off therapy for 18 yr	Alive, in remission; on Aza	Alive, persistent disease, on CS, Aza. Developmentally delayed.	Alive, persistent disease, on CS, Aza and IFX. Parents refused surgery	Alive, in remission; off therapy for 2 yr	Alive, in remission; no therapy

[1]According to Levine *et al*[24]. Aza: Azathioprine; CD: Crohn's disease; CMV: Cytomegalovirus; CS: Corticosteroid; CsA: Cyclosporin; EC: Eosinophilic colitis; EN: Enteral nutrition; IFX: Infliximab; PR: Per rectal; UC: Ulcerative colitis.

colonic ulceration. There was persistent and severe perianal disease. EEN (amino acid-based formula) was recommenced in addition to low dose CS (10 mg). Repeated courses of oral antibiotics (amoxicillin, doxycycline, metronidazole and vancomycin) were also given[30]. The condition improved with this regime with a reduction of the number of bloody stools. The perianal disease became quiescent with no abscess or fistula.

IBD-U

Infant #6 developed chronic bloody diarrhea at four months of age. She has been breast-fed exclusively since birth. She was given repeated courses of antibiotics even though the stool studies were negative for any pathogens. Subsequently she had several

Table 2 Comparison of disease characteristics, management and final outcome of infantile-onset inflammatory bowel disease and children with onset of disease after 12 mo of age

	Onset before 1 year, *n* (%)	Onset after 1 year, *n* (%)	*P* value
n	6	41	
Median age at diagnosis (yr)	0.44	8.34	
Duration of follow-up (yr), median (range)	6.1 (1.4-19.6)	8.3 (1.0-16.6)	
Male	4	22	
Female	2	19	
Crohn's disease	3	22	
Ulcerative colitis	2	19	
IBD-unclassified	1	0	
Initial presentation, *n* (%)			
Bloody diarrhea	6 (100)	23 (55)	0.039
Perianal disease	3 (50)	8 (19)	0.11
Extraintestinal involvement			
Autoimmune liver disease	0 (0)	8 (19)	0.31
Therapy			
Biologics-infliximab	3 (50)	15 (36)	0.40
Surgery	3 (50)	12 (29)	0.27
Disease status at final review			
Inactive disease or in clinical remission	3 (50)	27 (66)	0.40
Discontinuation of immunosuppression			
Yes	3 (50)	5 (12)	0.053
No	3 (50)	36 (88)	

IBD: Inflammatory bowel disease.

perianal abscesses and fistulas requiring incision and drainage. She was seen at another hospital at five months of age, where a limited sigmoidoscopy showed extensive, deep linear ulceration up to the sigmoid colon. A sigmoidal colostomy was performed. Histological examination of the bowel tissue obtained during the operation showed transmural inflammation involving the muscular layer and the serosa. After the colostomy, the child improved with weight gain and a complete cessation of diarrhea. No immunosuppression treatment was started.

When seen at our hospital at seven months of age, the perianal area was quiescent with a skin tag. No abscess was noted. An EGDS was normal. A colonoscopy showed multiple flat nodules with relatively normal mucosa over the rectum. The remaining colon was pale with loss of normal vascular pattern. There were no pseudopolyps, mucosal ulcerations or friability. Histologically, the colonic mucosa was inflamed. The tubular glands were devoid of significant architectural distortion. The lamina propria showed marked infiltration by lymphoplasmacytic cells with the formation of lymphoid follicles and mild neutrophilic infiltrates. There were occasional cryptitis, but no crypt abscess or granuloma was noted.

She received nutritional supplement. No immunosuppressive therapy was started. A repeat EGDS and colonoscopy at 14 mo of age were entirely normal macroscopically. Histologically the colonic architecture was well preserved with minimal lymphoplasmacytic infiltration. A closure of the colostomy was performed at 15 mo of age. Postoperatively she remained well and asymptomatic. The clinical presentation, colonoscopic appearance and histologic features were classically that of CD, but the subsequent course, *i.e.*, sustained remission without immunosuppression made a diagnosis of CD unlikely. Hence a diagnosis of IBD-U was made.

Medical and surgical therapies

Five of the six patients had immunosuppressive therapies consisted of CS, Aza, cyclosporin and IFX. In addition, one patient (infant No. 1) who had an initial diagnosis of allergic colitis also had elemental diet. All the three patients with CD were given IFX (see above).

Three patients required surgery: one had total colectomy, one had colostomy and another had ileostomy.

Final disease status

At final review (median duration of follow up: 5.5 years; range 2.0-20 years), all patients survived. Of these, three patients (50%) were in complete remission, two patients with CD had inactive disease with no immunosuppression while one patient with UC was in remission after total colectomy (Table 1). One patient with UC was in remission with Aza. Two patients have active disease despite adequate immunosuppressive therapies at a recommended dose.

Comparison with patients with later-onset disease

A comparison was made between the IO-IBD described in the present study and other patients with later-onset IBD (onset of disease after 12 mo of age) who were followed up at the department (Table 2). As compared to patients with later-onset disease, children with IO-IBD were more likely to have bloody diarrhea at presentation (100% *vs* 55%, $P = 0.039$) but were not more likely to have an associated autoimmune liver disease (0% *vs* 19%, $P = 0.31$), require the use of biologics (50% *vs* 36%, $P = 0.40$), or surgery (50% *vs* 29%, $P = 0.27$), or achieving remission at final review (50% *vs* 64%, $P = 0.40$). However, there was a trend for patients with IO-IBD to discontinue immunosuppressive therapy (50% *vs* 88%, $P = 0.053$).

Comparison with Caucasian patients

A comparison was made between patients described in the present study and those reported in the literature (Table 3). Three other studies, all from European centers, described IO-IBD[8,9,14]. Generally patients with IO-IBD required aggressive immunosuppression. Between 19% and 33% of the patients had surgery. However, prolonged remission, some without immunosuppression, was achieved in 67% to 100% of patients.

Mutational analysis

Mutational analysis for coding regions together with

Table 3 Clinical characteristics, management and outcome of infantile-onset inflammatory bowel disease in selected series

	Ref.	Patients in all age group), *n* (%)	Nature of patients, *n* (%)	Age of patients at onset of disease	Duration of follow-up (yr), median (range)	Medical therapies	Surgery, *n* (%)	Disease status: remission of survivors at final review, *n* (%)	Deaths
1	Ruemmele *et al*[8]	10 (2.5)	CD = 4 (40) UC = 2 (20) IC = 4 (40)	First 12 mo	2.5 (2.5-8)	Bowel rest, PN, CS, Aza, CsA	3 (30); 2 colectomy, 1 ileostomy	10 (100); off therapy, 2 (20); ongoing therapy, 8 (80)	None
2	Cannioto *et al*[9]	16 (8.6)	CD = 6 (37.5) UC = 8 (50) Indeterminate = 2 (12.5)	First 2 yr	6 (4-22)	Aggressive multi-drug therapy, Aza, IFX, thalidomide, CsA	3 (19); 2 colectomy, 1 ileostomy	11 (100); off therapy, 4 (25); ongoing therapy, 6 (38), after BMT, 1 (6)	5 (mortality rate 31%)[1]
3	Begue *et al*[15]	13 (-)	All had pancolitis, 6 had small bowel involvement[2]	First 12 mo	-	-	4 (31)	All required immuno-suppressive therapy. Final disease status not described	Not described
4	Present study, Malaysia, 2016	6 (13)	CD = 4 (67) UC = 2 (33)	First 12 mo	5 (1.5-20)	Bowel rest, steroids, Aza, CsA, INF	3 (33); 1 colectomy, 2 ileostomies	4 (67); off therapy, 3 (50); ongoing therapy, 1 (17); not in remission, 2 (33)	None

The authors included IBD and IBD-mimicking enterocolitis; [1]Two deaths were related to infections; one death each for interstitial pneumonia, post-BMT, and giant cell hepatitis progressing to liver failure; [2]The authors did not classify the patients into either Crohn's disease (CD), ulcerative colitis (UC) or indeterminate colitis (IC). Aza: Azathioprine; BMT: Bone marrow transplantation; CS: Corticosteroid; CsA: Cyclosporin; IBD: Inflammatory bowel disease; IFX: Infliximab; PN: Parenteral nutrition.

splice junctions of *IL10* and *IL10R* were performed by genomic Sanger sequencing of the three patients with CD and one infant with IBD-U. No causative mutation was identified.

DISCUSSION

Inherited genetic defect leading to immune dysregulation, the influence of intestinal microbiome and environmental factors have all been considered playing an important role in the pathogenesis of IBD, including IO-IBD[14]. Mutations in IL10 and IL10R have been identified in a subset of infants with severe IO-IBD[13-17,20], often presenting with perianal fistulae, respond poorly to medical therapies and needing early surgical interventions[16]. HSCT has been shown to be curative in IBD secondary to IL10/IL10R deficiency[13-16].

However, clearly not every young child with IO-IBD have mutations in IL10 or IL10R[14,15]. Of the 13 infants with IO-IBD reported by Begue *et al*[15], only two (15%) were found to have a deficient IL10 signaling[14]. Instead the authors found additional compromised signaling in IL22 in a patient with absent IL10RB[14]. Similarly, Shim *et al*[22] reported that only seven (50%) of the 14 Korean infants with IO-IBD had mutations in IL10RA. Thus it is likely that IO-IBD represents a heterogeneous group of disorders with the common feature of early-onset severe colitis within the first weeks to months of life[8,9,14,22]. Mutations in *IL10* and *IL10R* may be responsible in a significant proportion of, but not all, young children with IO-IBD. Uhlig *et al*[13] have shown that many other monogenic disorders and primary immunodeficiencies, including SCID and Wiskott-Aldrich syndrome, are recognized causes of IO colitis.

In the present study on six Asian children with IO-IBD, the disease phenotype was diverse. From the initial presentation and subsequent disease course, three patients [1 UC (patient No. 2) and 2 CD (patient No. 4 and 5)] closely resembled classical UC and CD. In the remaining three cases, the disease behavior and progression significantly differed from classical CD or UC.

The only patient with IBD-U achieved sustained remission and resolution of the perianal disease after ileostomy was created at four months of age, despite no immunosuppression. The initial features were indistinguishable with that of classical CD with severe perianal abscesses and fistulas, and the presence of deep, linear, extensive ulcerations at the rectum and sigmoid colon. Severe allergic colitis was unlikely as the initial histology showed transmural involvement. There was continuing remission even after the closure of ileostomy at 16 mo of age. Mutational analysis for *IL10* and *IL10R* was negative.

A review in the literature did not reveal any cases of CD with spontaneous remission without immunosuppression or other medical therapy[31,32]. Thus even though the clinical, colonoscopic and histological features in this patient closely resembled that of CD, the child was classified as having IBD-U.

Another interesting case that merits special attention was the child with initial presentation with pancolitis, heavy eosinophilic infiltration of the colonic mucosa,

and resistance to elemental formula and even immunosuppression. This closely resembled a case of severe allergic colitis. This was followed at later stage with lymphoplasmacytic infiltration seen typically in IBD. This transformation from allergic colitis to IBD has been similarly observed by other authors[8].

Our findings are similar to those described by other authors[8,9,14]. Generally, patients with IO-IBD need aggressive therapy, often with a combination of immunosuppression[8,9,14]. Some needed biologics such as IFX[9]. Between 19% to 33% needed surgery, either ileostomy or colectomy[8,9]. Nevertheless complete remission can be achieved in a significant proportion of patients either with ongoing immunosuppression or discontinuation of therapy[8,9,14].

With the exception that children with IO-IBD were more likely to have bloody diarrhea at presentation, there were no significant differences between the IO-IBD and children with the onset of disease after one-year of age in terms of developing autoimmune liver disease, the need for biologics, risk for subsequent surgery, achieving remission at final review, or achieving remission without on-going immunosuppression.

Unlike the authors from Korea which showed that 50% of the 14 children with IO-IBD had mutations in IL10RA[19], none of the three patients with CD phenotype and the case with IBD-U in the present study had any identified mutations in *IL10* or *IL10R*. Nevertheless continuing efforts is necessary to identify such mutations in all patients with IO-IBD. However, it should be pointed out that only four of the six patients with IO-IBD had mutational analysis for *IL10* and *IL10R* performed. The remaining two patients, who both had phenotype similar to UC, had no mutational analysis. This was because IO-IBD secondary to mutations in *IL10* and *IL10R* genes usually present with CD phenotype.

The present study was conducted from a region where the incidence of IBD is much lower as compared to the West[33]. This have contributed to the small number of patients with both IO-IBD and later-onset disease reported in the present study.

We did not perform anti-*Saccharomyces* cerevisae antibody (ASCA) for the patients with early onset disease. Recently, high ASCA seropositivity rates have been found in patients with early-onset IBD[34]. The implications of finding a positive ASCA in patient with CD include oral involvement and a more severe disease[35]. However, the finding of high seropositivity rate of ASCA in early onset CD has not been reported by other authors[36].

There are several weaknesses in the present study. Firstly, the number of children with infantile colitis described was small. The present study was conducted in an area with low incidence of IBD. Over the study period of 18 years in the only referral center for pediatric IBD in Malaysia, only six cases of IO-IBD were noted. Thus it may be difficult to draw many significant conclusions from the findings of the present study.

Secondly, mutational analysis for other known causes of infantile colitis were not performed in the six cases of IO-IBD described in the present study[10]. Thus we were unable to characterize further the genetic basis of these six cases of IO-IBD described.

In addition, there may be selection bias when comparing the outcome of IO-IBD with the outcome of patients with later-onset disease. It is well known that IO-IBD is a heterogenous group of conditions and is comprised of several different disease caused different genetic mutations but characterized by early onset of disease.

However, it is well known that the incidence of IBD in Asian adults is increasing[33]. It is anticipated that the incidence of IBD in Asian children would similarly be increasing[33]. The present study will add to the body of knowledge for this rare disease in Asian children. Currently, most of the cases of IO-IBD described in the literature were from the Middle East or Caucasian population[8,9].

We conclude that IO-IBD consists of a heterogeneous group of disorders with different pathogenic mechanisms but with the common manifestation of severe colitis presenting in the first few months of life. Aggressive immunosuppression and surgery are often necessary. Nevertheless sustained remission, in some cases without immunosuppression, can be achieved in a significant proportion of patients. Continuing efforts to elucidate novel mechanisms responsible for the breaking down of intestinal integrity is necessary in this group of infants.

ACKNOWLEDGMENTS

The present research was funded by a research grant from Ministry of Higher Education, Malaysia (UM.C/625/HIR/MOHE/CHAN/13/1). None of the authors declared any potential conflict of interest. The genetics study of patients with primary immunodeficiencies was funded by the Hong Kong Society for the Relief of Disabled Children.

COMMENTS

Background

Inflammatory bowel disease (IBD) is not as common in the Asian population as in the Caucasians, although recent epidemiology studies suggest that the incidence of IBD in the Asian population is increasing. Infantile-onset inflammatory bowel disease (IO-IBD) is uncommon and is usually associated with a genetic mutation. One of the most common mutations described that is associated with IO-IBD is mutations in IL-10 and IL-10 receptor.

Research frontiers

It is important to elucidate the role of IL-10 and *IL-10* mutations in children with IO-IBD as it is usually non-responsive to conventional immunosuppressive therapy but may be amendable to stem-cell transplantation.

Innovations and breakthrough

As compared to children with IBD with an onset after the first year of life, IO-IBD achieved remission at a similar rate, were more likely to discontinue immunosuppression therapy while not more likely to require biologics therapy or surgical intervention.

Applications

Although mutations in *IL-10* and *IL-10R* were not found in the present cohort of infantile-onset inflammatory bowel disease, it is important to screen for such mutations in all cases of IO-IBD as the therapy and prognosis is different.

Terminology

IO-IBD refers to a subset of early-onset IBD with an onset before twelve months of life.

Peer-review

The manuscript is interesting and adds new knowledge in the field of IO-IBD but requires a major statistical revision (or no statistical analysis as the conclusions may be false and can not be extrapolated on the bigger group of all IO-IBD patients).

REFERENCES

1 **Kappelman MD**, Grand RJ. Does inflammatory bowel disease develop in infants? *Inflamm Bowel Dis* 2008; **14** Suppl 2: S6-S8 [PMID: 18816729 DOI: 10.1002/ibd.20544]

2 **Gower-Rousseau C**, Salomez JL, Dupas JL, Marti R, Nuttens MC, Votte A, Lemahieu M, Lemaire B, Colombel JF, Cortot A. Incidence of inflammatory bowel disease in northern France (1988-1990). *Gut* 1994; **35**: 1433-1438 [PMID: 7959201]

3 **Miralles R**, Santander H, Ide W, Bull R. Influence of mucosal mechanoreceptors on elevator muscle activity in healthy subjects. *J Prosthet Dent* 1991; **65**: 431-435 [PMID: 2056465 DOI: 10.1002/ibd.21349]

4 **Oliva-Hemker M**, Hutfless S, Al Kazzi ES, Lerer T, Mack D, LeLeiko N, Griffiths A, Cabrera J, Otley A, Rick J, Bousvaros A, Rosh J, Grossman A, Saeed S, Kay M, Carvalho R, Keljo D, Pfefferkorn M, Faubion W, Kappelman M, Sudel B, Schaefer ME, Markowitz J, Hyams JS. Clinical Presentation and Five-Year Therapeutic Management of Very Early-Onset Inflammatory Bowel Disease in a Large North American Cohort. *J Pediatr* 2015; **167**: 527-532.e1-3 [PMID: 25982142 DOI: 10.1016/j.jpeds.2015.04.045]

5 **Kugathasan S**, Judd RH, Hoffmann RG, Heikenen J, Telega G, Khan F, Weisdorf-Schindele S, San Pablo W, Perrault J, Park R, Yaffe M, Brown C, Rivera-Bennett MT, Halabi I, Martinez A, Blank E, Werlin SL, Rudolph CD, Binion DG. Epidemiologic and clinical characteristics of children with newly diagnosed inflammatory bowel disease in Wisconsin: a statewide population-based study. *J Pediatr* 2003; **143**: 525-531 [PMID: 14571234]

6 **Hildebrand H**, Finkel Y, Grahnquist L, Lindholm J, Ekbom A, Askling J. Changing pattern of paediatric inflammatory bowel disease in northern Stockholm 1990-2001. *Gut* 2003; **52**: 1432-1434 [PMID: 12970135]

7 **Heyman MB**, Kirschner BS, Gold BD, Ferry G, Baldassano R, Cohen SA, Winter HS, Fain P, King C, Smith T, El-Serag HB. Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry. *J Pediatr* 2005; **146**: 35-40 [PMID: 15644819]

8 **Ruemmele FM**, El Khoury MG, Talbotec C, Maurage C, Mougenot JF, Schmitz J, Goulet O. Characteristics of inflammatory bowel disease with onset during the first year of life. *J Pediatr Gastroenterol Nutr* 2006; **43**: 603-609 [PMID: 17130735]

9 **Cannioto Z**, Berti I, Martelossi S, Bruno I, Giurici N, Crovella S, Ventura A. IBD and IBD mimicking enterocolitis in children younger than 2 years of age. *Eur J Pediatr* 2009; **168**: 149-155 [PMID: 18546019]

10 **Uhlig HH**. Monogenic diseases associated with intestinal inflammation: implications for the understanding of inflammatory bowel disease. *Gut* 2013; **62**: 1795-1805 [PMID: 24203055 DOI: 10.1136/gutjnl-2012-303956]

11 **Glocker EO**, Frede N, Perro M, Sebire N, Elawad M, Shah N, Grimbacher B. Infant colitis--it's in the genes. *Lancet* 2010; **376**: 1272 [PMID: 20934598 DOI: 10.1016/S0140-6736(10)61008-2]

12 **Soubiran JM**. [Decubitus ulcer. General conclusions]. *Soins* 1979; **24**: 43 [PMID: 256647 DOI: 10.1097/DCR.0000000000000274]

13 **Uhlig HH**, Schwerd T, Koletzko S, Shah N, Kammermeier J, Elkadri A, Ouahed J, Wilson DC, Travis SP, Turner D, Klein C, Snapper SB, Muise AM. The diagnostic approach to monogenic very early onset inflammatory bowel disease. *Gastroenterology* 2014; **147**: 990-1007.e3 [PMID: 25058236 DOI: 10.1053/j.gastro.2014.07.023]

14 **Glocker EO**, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, Perro M, Diestelhorst J, Allroth A, Murugan D, Hätscher N, Pfeifer D, Sykora KW, Sauer M, Kreipe H, Lacher M, Nustede R, Woellner C, Baumann U, Salzer U, Koletzko S, Shah N, Segal AW, Sauerbrey A, Buderus S, Snapper SB, Grimbacher B, Klein C. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N Engl J Med* 2009; **361**: 2033-2045 [PMID: 19890111 DOI: 10.1056/NEJMoa0907206]

15 **Begue B**, Verdier J, Rieux-Laucat F, Goulet O, Morali A, Canioni D, Hugot JP, Daussy C, Verkarre V, Pigneur B, Fischer A, Klein C, Cerf-Bensussan N, Ruemmele FM. Defective IL10 signaling defining a subgroup of patients with inflammatory bowel disease. *Am J Gastroenterol* 2011; **106**: 1544-1555 [PMID: 21519361 DOI: 10.1038/ajg.2011.112]

16 **Kotlarz D**, Beier R, Murugan D, Diestelhorst J, Jensen O, Boztug K, Pfeifer D, Kreipe H, Pfister ED, Baumann U, Puchalka J, Bohne J, Egritas O, Dalgic B, Kolho KL, Sauerbrey A, Buderus S, Güngör T, Enninger A, Koda YK, Guariso G, Weiss B, Corbacioglu S, Socha P, Uslu N, Metin A, Wahbeh GT, Husain K, Ramadan D, Al-Herz W, Grimbacher B, Sauer M, Sykora KW, Koletzko S, Klein C. Loss of interleukin-10 signaling and infantile inflammatory bowel disease: implications for diagnosis and therapy.*Gastroenterology* 2012; **143**: 347-355 [PMID: 22549091 DOI: 10.1053/j.gastro.2012.04.045]

17 **Pigneur B**, Escher J, Elawad M, Lima R, Buderus S, Kierkus J, Guariso G, Canioni D, Lambot K, Talbotec C, Shah N, Begue B, Rieux-Laucat F, Goulet O, Cerf-Bensussan N, Neven B, Ruemmele FM. Phenotypic characterization of very early-onset IBD due to mutations in the IL10, IL10 receptor alpha or beta gene: a survey of the Genius Working Group. *Inflamm Bowel Dis* 2013; **19**: 2820-2828 [PMID: 24216686 DOI: 10.1097/01.MIB.0000435439.22484.d3]

18 **Dinwiddie DL**, Bracken JM, Bass JA, Christenson K, Soden SE, Saunders CJ, Miller NA, Singh V, Zwick DL, Roberts CC, Dalal J, Kingsmore SF. Molecular diagnosis of infantile onset inflammatory bowel disease by exome sequencing. *Genomics* 2013; **102**: 442-447 [PMID: 24001973 DOI: 10.1016/j.ygeno.2013.08.008]

19 **Engelhardt KR**, Grimbacher B. IL-10 in humans: lessons from the gut, IL-10/IL-10 receptor deficiencies, and IL-10 polymorphisms. *Curr Top Microbiol Immunol* 2014; **380**: 1-18 [PMID: 25004811 DOI: 10.1007/978-3-662-43492-5_1]

20 **Prideaux L**, Kamm MA, De Cruz PP, Chan FK, Ng SC. Inflammatory bowel disease in Asia: a systematic review. *J Gastroenterol Hepatol* 2012; **27**: 1266-1280 [PMID: 22497584 DOI: 10.1111/j.1440-1746.2012.07150.x]

21 **Mao H**, Yang W, Lee PP, Ho MH, Yang J, Zeng S, Chong CY, Lee TL, Tu W, Lau YL. Exome sequencing identifies novel compound heterozygous mutations of IL-10 receptor 1 in neonatal-onset Crohn's disease. *Genes Immun* 2012; **13**: 437-442 [PMID: 22476154 DOI: 10.1038/gene.2012.8]

22 **Shim JO**, Seo JK. Very early-onset inflammatory bowel disease (IBD) in infancy is a different disease entity from adult-onset IBD; one form of interleukin-10 receptor mutations. *J Hum Genet* 2014; **59**: 337-341 [PMID: 24785691 DOI: 10.1038/jhg.2014.32]

23 **Levine A**, Koletzko S, Turner D, Escher JC, Cucchiara S, de Ridder L, Kolho KL, Veres G, Russell RK, Paerregaard A, Buderus S, Greer ML, Dias JA, Veereman-Wauters G, Lionetti P, Sladek M, Martin de Carpi J, Staiano A, Ruemmele FM, Wilson DC. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr* 2014; **58**: 795-806 [PMID: 24231644 DOI: 10.1097/MPG.0000000000000239]

24 **Levine A**, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, Fell J, Ruemmele FM, Walters T, Sherlock M, Dubinsky M, Hyams JS. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011; **17**: 1314-1321 [PMID: 21560194 DOI: 10.1002/ibd.21493]

25 **Kammermeier J**, Morris MA, Garrick V, Furman M, Rodrigues A, Russell RK. Management of Crohn's disease. *Arch Dis Child* 2016; **101**: 475-480 [PMID: 26553907 DOI: 10.1136/archdischild-2014-307217]

26 **Nolte T**. Stfm launches interprofessional leading change fellowship. *Ann Fam Med* 2015; **13**: 593 [PMID: 26553903 DOI: 10.1136/archdischild-2014-307218]

27 **Hyams JS**, Griffiths A, Markowitz J, Baldassano RN, Faubion WA, Colletti RB, Dubinsky M, Kierkus J, Rosh J, Wang Y, Huang B, Bittle B, Marshall M, Lazar A. Safety and efficacy of adalimumab for moderate to severe Crohn's disease in children. *Gastroenterology* 2012; **143**: 365-374.e2 [PMID: 22562021 DOI: 10.1053/j.gastro.2012.04.046]

28 **Hyams JS**, Ferry GD, Mandel FS, Gryboski JD, Kibort PM, Kirschner BS, Griffiths AM, Katz AJ, Grand RJ, Boyle JT. Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr* 1991; **12**: 439-447 [PMID: 1678008]

29 **Turner D**, Hyams J, Markowitz J, Lerer T, Mack DR, Evans J, Pfefferkorn M, Rosh J, Kay M, Crandall W, Keljo D, Otley AR, Kugathasan S, Carvalho R, Oliva-Hemker M, Langton C, Mamula P, Bousvaros A, LeLeiko N, Griffiths AM. Appraisal of the pediatric ulcerative colitis activity index (PUCAI). *Inflamm Bowel Dis* 2009; **15**: 1218-1223 [PMID: 19161178 DOI: 10.1002/ibd.20867]

30 **Turner D**, Levine A, Kolho KL, Shaoul R, Ledder O. Combination of oral antibiotics may be effective in severe pediatric ulcerative colitis: a preliminary report. *J Crohns Colitis* 2014; **8**: 1464-1470 [PMID: 24958064 DOI: 10.1016/j.crohns.2014.05.010]

31 **Hoption Cann SA**, van Netten JP. Spontaneous remission of Crohn's disease following a febrile infection: case report and literature review. *BMC Gastroenterol* 2011; **11**: 57 [PMID: 21592411 DOI: 10.1186/1471-230X-11-57]

32 **Short SS**, Dubinsky MC, Rabizadeh S, Farrior S, Berel D, Frykman PK. Distinct phenotypes of children with perianal perforating Crohn's disease. *J Pediatr Surg* 2013; **48**: 1301-1305 [PMID: 23845622 DOI: 10.1016/j.jpedsurg.2013.03.027]

33 **Ng SC**, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, Wong TC, Leung VK, Tsang SW, Yu HH, Li MF, Ng KK, Kamm MA, Studd C, Bell S, Leong R, de Silva HJ, Kasturiratne A, Mufeena MN, Ling KL, Ooi CJ, Tan PS, Ong D, Goh KL, Hilmi I, Pisespongsa P, Manatsathit S, Rerknimitr R, Aniwan S, Wang YF, Ouyang Q, Zeng Z, Zhu Z, Chen MH, Hu PJ, Wu K, Wang X, Simadibrata M, Abdullah M, Wu JC, Sung JJ, Chan FK. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. *Gastroenterology* 2013; **145**: 158-165.e2 [PMID: 23583432 DOI: 10.1053/j.gastro.2013.04.007]

34 **Nisihara RM**, de Carvalho WB, Utiyama SR, Amarante H, Baptista ML. Diagnostic role and clinical association of ASCA and ANCA in Brazilian patients with inflammatory bowel disease. *Dig Dis Sci* 2010; **55**: 2309-2315 [PMID: 19826949 DOI: 10.1007/s10620-009-0998-7]

35 **Bueno de Mesquita M**, Ferrante M, Henckaerts L, Joossens M, Janssens V, Hlavaty T, Pierik M, Joossens S, Van Schuerbeek N, Van Assche G, Rutgeerts P, Vermeire S, Hoffman I. Clustering of (auto)immune diseases with early-onset and complicated inflammatory bowel disease. *Eur J Pediatr* 2009; **168**: 575-583 [PMID: 18670786 DOI: 10.1007/s00431-008-0798-7]

36 **Mamula P**, Telega GW, Markowitz JE, Brown KA, Russo PA, Piccoli DA, Baldassano RN. Inflammatory bowel disease in children 5 years of age and younger. *Am J Gastroenterol* 2002; **97**: 2005-2010 [PMID: 12190168 DOI: 10.1111/j.1572-0241.2002.05915.x]

P- Reviewer: Koh SJ, Takagi T, Waszczuk K **S- Editor**: Yu J
L- Editor: A **E- Editor**: Zhang FF

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.v22.i48.10663

World J Gastroenterol 2016 December 28; 22(48): 10663-10672
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Prospective Study

Risk factors of admission for acute colonic diverticulitis in a population-based cohort study: The North Trondelag Health Study, Norway

Aras Jamal Talabani, Stian Lydersen, Eivind Ness-Jensen, Birger Henning Endreseth, Tom-Harald Edna

Aras Jamal Talabani, Tom-Harald Edna, Department of Surgery, Levanger Hospital, North Trondelag Hospital Trust, 7600 Levanger, Norway

Stian Lydersen, Regional Centre for Child and Youth Mental Health and Child Welfare - Central Norway, Faculty of Medicine, Norwegian University of Science and Technology, 7491 Trondheim, Norway

Eivind Ness-Jensen, HUNT Research Centre, Department of Public Health and General Practice, Faculty of Medicine, Norwegian University of Science and Technology, 7491 Levanger, Norway

Eivind Ness-Jensen, Upper Gastrointestinal Surgery, Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, SE - 171 76 Stockholm, Sweden

Eivind Ness-Jensen, Clinic of Medicine, St. Olav's Hospital, Trondheim University Hospital, 7006 Trondheim, Norway

Birger Henning Endreseth, Clinic of Surgery, St. Olav's Hospital, Trondheim University Hospital, 7006 Trondheim, Norway

Birger Henning Endreseth, Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, 7491 Trondheim, Norway

Tom-Harald Edna, Unit for Applied Clinical Research, Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, 7491 Trondheim, Norway

Author contributions: Jamal Talabani A contributed to conception of the study, interpretation of data, manuscript preparation; Lydersen S contributed to statistical analyses and interpretation of data, critically reviewed the manuscript; Ness-Jensen E contributed conception and design of the study, to interpretation of data, drafting the article and revising it critically for important intellectual content; Endreseth BH made substantial contributions to the conception of the study, interpretation of data, making critical revisions related to important intellectual content of the manuscript; Edna TH made substantial contributions to conception and design of the study, acquisition of data, analysis and interpretation of data; drafting the article; all authors read and approved the final manuscript.

Supported by Institute of Cancer Research and Molecular Medicine, The Medical Faculty, Norwegian University of Science and Technology, Trondheim, Norway; and the Department of Research, Levanger Hospital, Levanger.

Institutional review board statement: The present study is approved by the Regional Committee for Health and Research Ethics (2011/1782/REK midt).

Informed consent statement: The participants in HUNT2 gave written informed consent for medical research, including future linkage to patient records at the hospitals.

Conflict-of-interest statement: The authors declare the absence of conflict of interests.

Data sharing statement: Data from the HUNT Databank may be available after individual application from researchers to the Databank, see https://hunt-db.medisin.ntnu.no/hunt-db/.

Manuscript source: Invited manuscript

Correspondence to: Tom-Harald Edna, MD, PhD, Professor, Surgeon, Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Postboks 8905, 7491 Trondheim,
Norway. tom-harald.edna@ntnu.no
Telephone: +47-74-098000

Fax: +47-74-098901

Received: August 3, 2016
Peer-review started: August 6, 2016
First decision: September 28, 2016
Revised: October 20, 2016
Accepted: November 23, 2016
Article in press: November 28, 2016
Published online: December 28, 2016

Core tip: We sought to determine what health related factors were together associated with later admission for acute colonic diverticulitis. The factors were derived from the North Trondelag Health Study (HUNT2) in Norway, the HUNT2 study, performed during 1995-1997. The study had 42570 participants who used Levanger Hospital as their primary hospital. They were observed until 2012. Following HUNT2, the participants contributed 611492 person-years of follow-up. In all, 358 cases had been admitted with acute colonic diverticulitis. In a multivariable analysis, increasing age and increasing Body Mass Index were associated with increased risk of admission for acute colonic diverticulitis in both gender. In females, cigarette smoking likewise increased the risk of admission. In males, breathlessness, a HUNT variable associated with Chronic Obstructive Pulmonary Disease, increased the risk of admission. On the other hand, physical activity, constipation and type of bread eaten showed no association with admission for acute colonic diverticulitis.

Jamal Talabani A, Lydersen S, Ness-Jensen E, Endreseth BH, Edna TH. Risk factors of admission for acute colonic diverticulitis in a population-based cohort study: The North Trondelag Health Study, Norway. *World J Gastroenterol* 2016; 22(48): 10663-10672 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10663.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10663

Abstract

AIM

To assess risk factors of hospital admission for acute colonic diverticulitis.

METHODS

The study was conducted as part of the second wave of the population-based North Trondelag Health Study (HUNT2), performed in North Trondelag County, Norway, 1995 to 1997. The study consisted of 42570 participants (65.1% from HUNT2) who were followed up from 1998 to 2012. Of these, 22436 (52.7%) were females. The cases were defined as those 358 participants admitted with acute colonic diverticulitis during follow-up. The remaining participants were used as controls. Univariable and multivariable Cox regression analyses was used for each sex separately after multiple imputation to calculate HR.

RESULTS

Multivariable Cox regression analyses showed that increasing age increased the risk of admission for acute colonic diverticulitis: Comparing with ages < 50 years, females with age 50-70 years had HR = 3.42, $P < 0.001$ and age > 70 years, HR = 6.19, $P < 0.001$. In males the corresponding values were HR = 1.85, $P = 0.004$ and 2.56, $P < 0.001$. In patients with obesity (body mass index $\geqslant$ 30) the HR = 2.06, $P < 0.001$ in females and HR = 2.58, $P < 0.001$ in males. In females, present (HR = 2.11, $P < 0.001$) or previous (HR = 1.65, $P = 0.007$) cigarette smoking increased the risk of admission. In males, breathlessness (HR = 2.57, $P < 0.001$) and living in rural areas (HR = 1.74, $P = 0.007$) increased the risk. Level of education, physical activity, constipation and type of bread eaten showed no association with admission for acute colonic diverticulitis.

CONCLUSION

The risk of hospital admission for acute colonic diverticulitis increased with increasing age, in obese individuals, in ever cigarette smoking females and in males living in rural areas.

Key words: Acute colonic diverticulitis; North Trondelag Health Study; Risk factors; Multivariable Cox regression analysis; Multiple imputation

INTRODUCTION

Diverticular disease of the colon is highly prevalent in Western populations and adds to the already rising expenditures in healthcare systems[1]. The prevalence of diverticular disease is age dependent and increases from 5% in the age group 30-39 years to 60% among those older than 80 years of age[2].

Acute diverticulitis is the most common complication of colonic diverticulosis[3], with increasing incidence and admission rates in recent years[4,5]. Acute diverticulitis may also recur in 9% to 23% of patients[6].

The heritability of diverticular disease has been estimated at 40% in a large Swedish twin study[7], and a number of lifestyle related risk factors have been attributed to acute colonic diverticulitis. Obesity, reduced physical activity, tobacco smoking and reduced dietary fiber intake have all been associated with increased risk of acute colonic diverticulitis, but few studies have been able to assess all these factors together[8-12].

The aim of the present study was to assess risk factors of hospital admission for acute colonic diverticulitis in a prospective population-based cohort study.

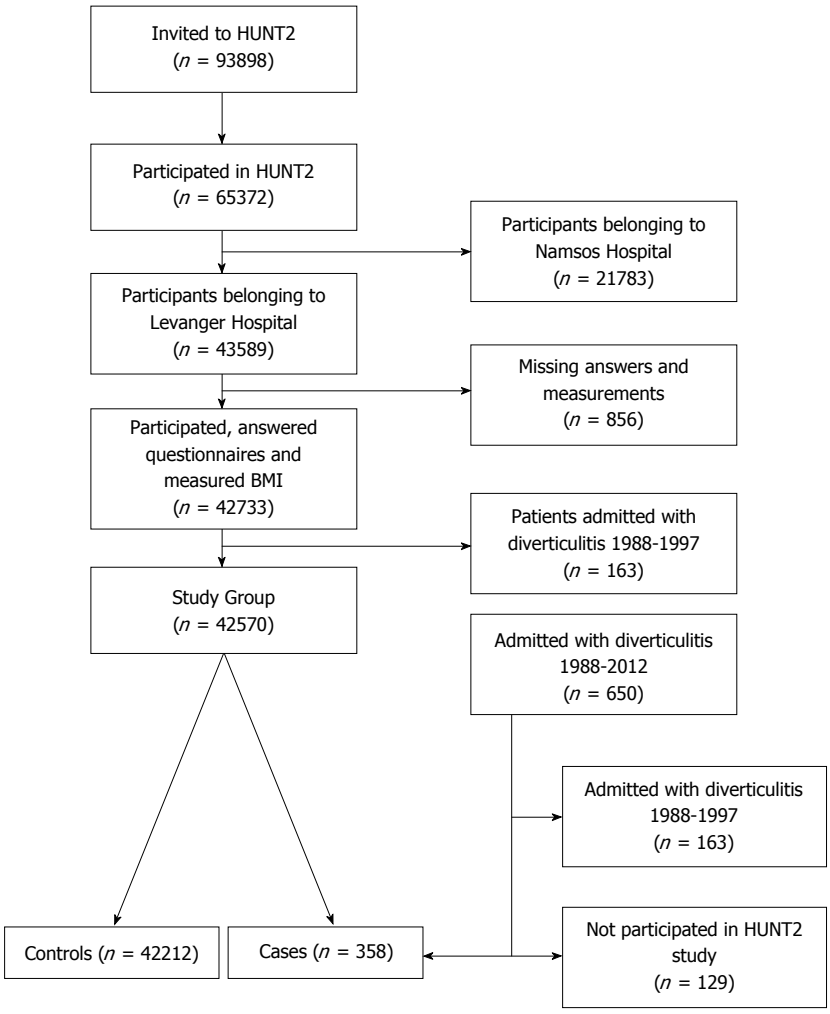

Figure 1 Flow diagram for participants and patient inclusions. HUNT2: North Trondelag Health Study; BMI: Body mass index.

MATERIALS AND METHODS

Study population

During 1995 to 1997, all residents in North Trondelag County, Norway, aged 20 years and older, were invited to participate in the second wave of the North Trondelag Health Study (HUNT2), a survey consisting of written questionnaires on health related topics, physical examinations and blood sampling[13]. In the present study, the population of the ten municipalities who used Levanger Hospital as the primary hospital was included, representing 73% of the population in North Trondelag County. The great majority are ethnic whites.

During 2012 and 2013 we retrospectively searched for all patients who had been admitted to Levanger Hospital following HUNT2, from 1998 to 2012, with the diagnosis acute colonic diverticulitis. The population of North Trondelag is served by two hospitals, Levanger Hospital is the largest and serving 10 municipalities. All treatment is given free of charge for the population. The patients were identified in the hospital patient administrative system, using the discharge codes for colonic diverticular disease in international classification of diseases ICD-9 and ICD-10. All the records were reviewed to ensure a higher probability of a correct diagnosis of acute colonic diverticulitis. All 358 patients who had been admitted with acute colonic diverticulitis and had participated in HUNT2 were included in the present study as cases, and the remaining HUNT2 participants from the same ten municipalities were used as controls. Figure 1 shows a flowchart of participants and patient inclusions.

A computed tomography (CT) scan had been performed during the hospital stay in 161 patients (45%) and later in 52 (14.5%) additional patients during outpatient follow-up. Colonoscopy had been performed in 139 (38.8%) patients. During the early years of the study (mainly between 1998 and 2006), a barium enema had been done in 120 (33.5%) patients to diagnose diverticula in the colon. One or more of these examinations had been performed in 331 patients (92.5%).

Risk factors

A full description of the questionnaires and measurements in HUNT2 is given at http://www.ntnu.

Table 1 Percentage missing values in different variables

Variable	Females	Males
Hard physical activity	39	28
Cigarette smoking	7	5
Breathlessness	16	10
Constipation	12	9
Type of bread	13	16
Highest educational level	6	5

edu/hunt/data/que. From HUNT2 we gathered data on sex, age, body mass index (BMI) and self-reported data on physical activity, cigarette smoking, duration of breathlessness, duration of constipation, main type of bread eaten, level of education and type of living area. Qualified personnel completed the standardized measurements of height and weight. Individuals were categorized as normal weight (with BMI < 25.0 kg/m^2), overweight (BMI 25-29.9) or obese (BMI $\geqslant$ 30), based on the World Health Organization's classification[14]. Physical activity was categorized as hard physical activity less than or at least one hour per week. Cigarette smoking was reported as never, previous or present daily smoking. Both variables, "To what extent have you had problems with breathlessness the last 12 mo" and "To what extent have you had problems with constipation the last 12 mo", were answered by the respondents as "not at all", "slightly" or "very much". Main type of bread eaten was categorized as "only fine" [white bread, white multigrain or whole meal (medium ground)], "only coarse" [multigrain, whole meal (coarsely ground)] or crisp bread, or "mixed" (mix of coarse and fine bread). Highest educational level was classified into primary school level or higher than primary school level. Living area was categorized as urban or rural area.

Statistical analysis

The primary study endpoint was the first admission for acute colonic diverticulitis at Levanger Hospital following participation in HUNT2, until end of study December 31, 2012. The observation time was measured from date of participation in HUNT2 to date of admission for acute colonic diverticulitis. All HUNT2 participants belonging to Levanger Hospital not admitted, were treated as controls. Their observation time was measured from date of participation in HUNT2 until end of study, December 31, 2012, date of death, or moving out of North Trondelag County, whichever occurred first.

Females and males were analysed separately. Six of the analysed risk factors had a large number of missing values; 54% of females and 46% of males had one or more missing values (Table 1).

To reduce bias and avoid loss of sample size, we used multiple imputation to handle these missing data. We imputed 100 data sets, as recommended by Carpenter and Kenward[15].

A Cox proportional hazards model was used for multivariable analysis of risk factors of admission for acute colonic diverticulitis, providing hazard ratios (HRs) with 95%CIs. Two-sided *P*-values < 0.05 were considered significant.

The analyses were performed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, United States) and StatXact 9 (Cytel Inc., Cambridge, MA, United States).

Statistical review of the study was performed by one of the authors, professor in medical statistics, Stian Lydersen.

Ethical approval

The participants in HUNT2 gave written informed consent for medical research, including future linkage to patient records at the hospitals. The present study is approved by the Regional Committee for Health and Research Ethics (2011/1782/REK midt).

RESULTS

In HUNT2, 65372 persons attended (69.5% response rate). Of these, 42570 persons had Levanger Hospital as their primary hospital and were included in the present study. Following HUNT2, the participants contributed 611492 person-years of follow-up, and 358 cases were admitted with acute colonic diverticulitis (Figure 1). The remaining HUNT2 participants belonging to Levanger Hospital were used as controls, excluding the 129 persons who had been admitted with acute colonic diverticulitis during 1988 and 1997 (preceding HUNT2).

Baseline characteristics

In the included cohort, 22436 (52.7%) were females. The mean age at baseline was 50.0 (SD 17.2) and 49.5 (SD 16.7) years for females and males, respectively. During follow-up, 358 participants were admitted to Levanger Hospital with acute colonic diverticulitis, 233 (65.1%) were females, and the mean age at admission was 69.3 (SD 13.2) and 63.9 (SD 14.1) years for females and males, respectively. Among the patients who had been admitted with acute colonic diverticulitis, but did not participate in HUNT2 (excluded from the present study), 54 (42%) were females, and the mean age at admission was 62.6 years (SD 16.0) for females, and 52.7 years (SD 16.6) for males.

Mean time from participation in HUNT2 to admission for acute colonic diverticulitis was 9.48 (SD 4.5) and 9.16 (SD 4.4) years for females and males, respectively.

Table 2 shows the sex-specific distribution of baseline characteristics of cases and controls. In both sexes, the age and BMI were higher and frequency of physical activity was lower among cases than controls. In females, more cases smoked cigarettes daily than controls. In both sexes, cases were more affected with breathlessness and constipation than controls. In both sexes, coarse type of bread was eaten more frequently in cases than controls. In both sexes, cases had lower educational level than controls. Among males, cases lived more often in a rural area than controls.

Table 2 Baseline characteristics of the study population *n* (%)

Characteristic	Acute colonic diverticulitis 1998-2012			
	Females		Males	
	No	Yes	No	Yes
Study population	22203	233	20009	125
Age in years at inclusion				
Mean ± SD	49.9 (17.2)	59.8 (13.8)	49.5 (16.7)	54.7 (14.4)
Age groups (yr)				
< 50	11892 (99.5)	57 (0.5)	10787 (99.6)	45 (0.4)
50-70	6708 (98.4)	112 (1.6)	6307 (99.1)	58 (0.9)
> 70	3603 (98.3)	64 (1.7)	2915 (99.3)	22 (0.7)
BMI (kg/m^2)				
< 25	9987 (99.3)	68 (0.7)	6983 (99.6)	26 (0.4)
25-29.9	8206 (98.9)	89 (1.1)	10146 (99.4)	64 (0.6)
⩾ 30	4010 (98.1)	76 (1.9)	2880 (98.8)	35 (1.2)
Hard physical activity (h/wk)				
< 1	9346 (99.0)	91 (1.0)	8368 (99.3)	58 (0.7)
⩾ 1	4286 (99.6)	19 (0.4)	6105 (99.4)	36 (0.6)
Missing	8571 (98.6)	123 (1.4)	5536 (99.4)	31 (0.6)
Smoking cigarettes				
Never	10134 (99.2)	81 (0.8)	7337 (99.6)	31 (0.4)
Previous	4137 (98.8)	51 (1.2)	6175 (99.2)	52 (0.8)
Daily	6295 (98.8)	75 (1.2)	5491 (99.4)	33 (0.6)
Missing	1637 (98.4)	26 (1.6)	1006 (99.1)	9 (0.9)
Problems with breathlessness the last 12 mo				
Not at all	17030 (99.1)	155 (0.9)	16585 (99.5)	87 (0.5)
Slightly	1434 (98.8)	17 (1.2)	1262 (98.5)	19 (1.5)
Very much	185 (98.4)	3 (1.6)	189 (98.4)	3 (1.6)
Missing	3554 (98.4)	58 (1.6)	1973 (99.2)	16 (0.8)
Problems with constipation the last 12 mo				
Not at all	13664 (99.1)	125 (0.9)	15690 (99.4)	90 (0.6)
Slightly	4640 (99.0)	48 (1.0)	2201 (99.4)	14 (0.6)
Very much	1213 (98.3)	21 (1.7)	285 (99.0)	3 (1.0)
Missing	2686 (98.6)	39 (1.4)	1833 (99.0)	18 (1.0)
Type of bread				
Only fine	2670 (99.0)	28 (1.0)	3798 (99.5)	20 (0.5)
Mixed	3158 (99.0)	33 (1.0)	4062 (99.4)	23 (0.6)
Only coarse	13440 (98.9)	147 (1.1)	9018 (99.3)	62 (0.7)
Missing	2935 (99.2)	25 (0.8)	3131 (99.4)	20 (0.6)
Highest educational level				
Primary school	8146 (98.7)	110 (1.3)	5519 (99.2)	46 (0.8)
Above primary school	12687 (99.3)	95 (0.7)	13536 (99.5)	73 (0.5)
Missing	1370 (97.3)	28 (2.7)	954 (99.4)	6 (0.6)
Living area				
Urban	18225 (98.9)	195 (1.1)	16425 (99.4)	91 (0.6)
Rural	3978 (99.1)	38 (0.9)	3584 (99.1)	34 (0.9)

Data given as *n* (%) unless otherwise stated.

Risk factors for admission for acute colonic diverticulitis

Tables 3 and 4 shows the results of univariable and multivariable Cox regression analyses for each sex separately after the multiple imputation. In the multivariable analysis, increasing age was associated with increased risk of admission for acute colonic diverticulitis in both sexes (HR = 3.42, 95%CI: 2.40-4.86, $P < 0.001$ for ages 50-70 years and HR = 6.19, 95%CI: 4.02-9.54, $P < 0.001$ for ages > 70 years in females). In males the corresponding values were HR = 1.85, 95% CI: 1.22 to 7.80, $P = 0.004$ and 2.56, 95%CI: 1.45-4.52, $P < 0.001$. Obesity was associated with increased risk of admission for acute colonic diverticulitis in both sexes (HR = 2.06, 95%CI: 1.46-2.91, $P < 0.001$ in females and HR = 2.58, 95%CI: 1.53-4.34, $P < 0.001$ in males). In females, both present and former daily cigarette smoking was associated with increased risk of admission for acute colonic diverticulitis (HR = 2.11, 95%CI: 1.51-2.94, $P < 0.001$ for daily smokers; HR = 1.65, 95%CI: 1.15-2.36, $P = 0.007$ for former smokers). In males, an increased risk of admission for acute colonic diverticulitis was observed in persons who reported slight problem with breathlessness during the last 12 mo (HR = 2.57, 95%CI: 1.55-4.28, $P < 0.001$) and in persons living in a rural area (HR = 1.74, 95%CI: 1.17-2.58, $P = 0.007$).

Level of education, hard physical activity, constipation and type of bread eaten showed no association with the risk of admission for acute colonic diverticulitis.

Table 3 Univariable Cox regression analysis of risk factors relating to admission for acute diverticulitis, after multiple imputation for missing values

Characteristic	Females		Males	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Age in years (yr)				
< 50	1 (ref)		1 (ref)	
50-70	3.55 (2.58-4.89)	< 0.001	2.36 (1.60- 3.48)	< 0.001
> 70	5.88 (4.10-8.44)	< 0.001	3.46 (2.06-5.79)	< 0.001
BMI (kg/m^2)				
< 25	1 (ref)		1 (ref)	
25-29.9	1.59 (1.16-2.18)	0.004	1.62 (1.03-2.56)	0.040
⩾ 30	2.86 (2.06-3.97)	< 0.001	3.20 (1.93-5.31)	< 0.001
Hard physical activity (h/wk)				
< 1	1 (ref)		1 (ref)	
⩾ 1	0.46 (0.27- 0.77)	0.003	0.85 (0.56-1.29)	0.45
Smoking cigarettes				
Never daily	1 (ref)		1 (ref)	
Formerly	1.45 (1.04-2.01)	0.027	2.12 (1.36- 3.13)	0.001
Present daily	1.36 (1.01-1.83)	0.043	1.49 (0.91- 2.44)	0.11
Problems with breathlessness the last 12 mo				
Not at all	1 (ref)		1 (ref)	
Slightly	1.44 (0.87-2.36)	0.15	3.09 (1.88- 5.08)	< 0.001
Very much	2.32 (0.76- 7.12)	0.14	3.54 (1.13-11.2)	0.031
Problems with constipation the last 12 mo				
Not at all	1 (ref)		1 (ref)	
Slightly	1.14 (0.82-1.60)	0.44	1.25 (0.72-1.19)	0.43
Very much	2.02 (1.29-3.18)	0.002	2.35 (0.76-7.24)	0.14
Type of bread				
Only fine	1 (ref)		1 (ref)	
Mixed	0.97 (0.58-1.61)	0.90	1.03 (0.57-1.86)	0.93
Only coarse	1.01 (0.67-1.51)	0.96	1.26 (0.76-1.08)	0.38
Highest educational level				
Primary school	1 (ref)		1 (ref)	
Higher than primary school	0.49 (0.37-0.64)	< 0.001	0.57 (0.39-0.82)	0.002
Living area				
Urban	1 (ref)		1 (ref)	
Rural	0.92 (0.65-1.30)	0.63	1.80 (1.21-2.67)	0.004

BMI: Body mass index.

We also carried out these analyses separately for each of the three age groups. Results were substantially the same (data not shown).

In another analysis, we excluded the variable breathlessness, and there were only insignificant changes in the HRs for all of the remaining variables.

DISCUSSION

Main findings

The main finding of this prospective population-based cohort study was that obese individuals had twice the risk of admission for acute colonic diverticulitis compared to normal weight individuals. This association was found in both females and males. Moreover, previous and present daily cigarette smoking also increased the risk of admission for acute colonic diverticulitis in females. There was no association between hard physical activity, constipation or eating bread with fine or coarse grains and risk of admission for acute colonic diverticulitis.

Relation to other reports

The present study demonstrated an increased risk of admission for acute colonic diverticulitis by increasing age in both females and males. This is consistent with findings reported by other studies[4,16].

There was an increased risk of admission for acute colonic diverticulitis in obese persons (BMI ⩾ 30). This is in agreement with other population-based studies. In two prospective cohort studies of males[17,18], the risk of acute colonic diverticulitis was 1.6 and 3.2 times higher among overweight individuals (BMI 25-29.9) and 1.8 and 4.4 times higher among obese individuals (BMI ⩾ 30), compared with those of normal weight (BMI 18.5-4.9). In a prospective cohort study of females, the risk was 1.3 times higher for overweight and obese individuals[19]. A weakness with these studies was the sex-specific nature of the cohorts.

Previous studies found that physical activity, in general, decreased the risk of acute colonic diverticulitis[12,19]. However, the present study found no association between hard physical activity for at least

Table 4 Multivariable Cox regression analysis of risk factors relating to admission for acute diverticulitis, after multiple imputation for missing values

Characteristic	Females		Males	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Age in years (yr)				
< 50	1 (ref)		1 (ref)	
50-70	3.42 (2.40-4.86)	< 0.001	1.85 (1.22-7.80)	0.004
> 70	6.19 (4.02-9.54)	< 0.001	2.56 (1.45-4.52)	0.001
BMI (kg/m^2)				
< 25	1 (ref)		1 (ref)	
25-29.9	1.25 (0.90-1.73)	0.18	1.46 (0.92-2.32)	0.11
⩾ 30	2.06 (1.46-2.91)	< 0.001	2.58 (1.53-4.34)	< 0.001
Hard physical activity (h/wk)				
< 1	1 (ref)		1 (ref)	
⩾ 1	0.67 (0.39-1.15)	0.15	1.03 (0.67-1.57)	0.90
Smoking cigarettes				
Never daily	1 (ref)		1 (ref)	
Formerly	1.65 (1.15-2.36)	0.007	1.45 (0.91-2.31)	0.12
Present daily	2.11 (1.51-2.94)	< 0.001	1.38 (0.84-2.29)	0.21
Problems with breathlessness the last 12 mo				
Not at all	1 (ref)		1 (ref)	
Slightly	1.12 (0.68-1.86)	0.66	2.57 (1.55-4.28)	< 0.001
Very much	1.43 (0.45-4.59)	0.55	2.48 (0.74-8.39)	0.14
Problems with constipation the last 12 mo				
Not at all	1 (ref)		1 (ref)	
Slightly	1.05 (0.75-1.48)	0.77	0.94 (0.53-1.66)	0.82
Very much	1.54 (0.95-2.49)	0.078	1.41 (0.43-4.68)	0.57
Type of bread				
Only fine	1 (ref)		1 (ref)	
Mixed	1.08 (0.65-1.79)	0.77	1.06 (0.58-1.93)	0.85
Only coarse	0.93 (0.62-1.41)	0.74	1.24 (0.74-2.07)	0.41
Highest educational level				
Primary school	1 (ref)		1 (ref)	
Higher than primary school	1.13 (0.82-1.54)	0.46	0.90 (0.61-1.33)	0.60
Living area				
Urban	1 (ref)		1 (ref)	
Rural	0.92 (0.65-1.30)	0.63	1.80 (1.21-2.67)	0.004

one hour per week and admission for the disease after adjustments. Cigarette smoking has been associated with increased severity of acute colonic diverticulitis[10]. In the present study, daily smoking increased the risk of admission for the disease by 2.2-fold in females. Previous studies found that smoking increased the risk of acute colonic diverticulitis by 25%-60% in both sexes[20-22] and increased the risk of complicated diverticulitis by 2.7 to 3.6-fold[10,22]. However, drawbacks of these studies were small number of cases[10], sex-specific cohorts[20,21] or lack of adjustments for dietary and other lifestyle factors like physical activity[22].

A previous long term study from this area of patients admitted with acute colonic diverticulitis, revealed an increased long term mortality by chronic obstructive pulmonary disease (COPD) of 10% (95%CI: 6.0-16) in[23]. In comparison, COPD was the cause of death in 4.2% (95%CI: 4.0-4.4) in the total population from the same area. This suggested a link between COPD and acute colonic diverticulitis, which was also found in another study[24]. In the present study, we wanted to elucidate the association between COPD and diverticulitis in another way. Duration of breathlessness was chosen as a proxy variable for COPD from HUNT2. The results of the present study showed that shortness of breath was associated with increased risk of admission for the disease. Few studies have previously studied the possible relationship between COPD and diverticulitis, although one other study, found that complicated diverticulitis was associated with pulmonary symptoms and problems in 23% of the cases[25].

In the present study, constipation was not associated with increased risk of admission for acute colonic diverticulitis. Similar, a recent multicenter study found no association between constipation and left-sided colonic diverticulosis[26], while in another study, constipation was considered a symptom of, rather than a direct risk factor for, acute colonic diverticulitis[27].

High dietary fiber intake is traditionally thought to be associated with decreased risk of diverticular disease[28,29], although high quality evidence is lacking[30,31]. In the present study, type of bread, whether fine or coarse, had no effect on the risk of admission for acute colonic diverticulitis. This is consistent with other studies, which found no association between cereals and acute colonic diverticulitis[21,32,33].

In the present study, males living in rural areas

had 80% increased risk of admission for acute colonic diverticulitis. In two previous studies, the risk of acute colonic diverticulitis was slightly elevated in both sexes living in rural areas[16,34].

Biological mechanisms

Aging showed increased risk of admission for acute colonic diverticulitis. Colonic diverticulosis can be considered a degenerative disease in which increasing age leads to weakness of supporting connective tissue and subsequent increase in intraluminal pressure and later increased risk of colonic diverticulitis. However, the exact mechanism of increased risk of diverticulitis in humans with aging is still unclear[35].

Obesity is shown to be a risk factor for diverticular disease and its complications[8,11,19,35]. One likely mechanism for development of acute colonic diverticulitis is that increased fat deposition in the mesentery act pro-inflammatory with activation of macrophages within the adipose tissue and subsequent increase in TNF-α. Translocation of luminal bacteria from the intestine to the systemic circulation due to impaired barrier function may also play a role in this pathogenesis[35].

Cigarette smoking causes changes in the colonic wall structure in patients with diverticular disease that are similar to changes found in blood vessels caused by smoking in other organs. In addition, smoking is thought to affect colonic motility and intraluminal pressure[36].

There is a possible coexistence between COPD and acute colonic diverticulitis. While smoking is a known risk factor for both diseases, it is unclear whether COPD is a risk factor or comorbidity in this context. Both inflammatory bowel disease and COPD share many similarities in inflammatory pathogenesis[37], but the mechanism behind the association between breathlessness and risk of acute colonic diverticulitis is poorly understood.

Strengths and weaknesses

This study has a population-based design with a large sample size and high participation rate. This diminishes selection bias, strengthens the study power, reduces the risk of chance findings and facilitates subgroup analyses. The objective and uniform measurement of height and weight by qualified personnel minimized the risk of differential misclassification of BMI[38]. The prospective nature and use of standardized questionnaires on risk factors limits the potential for recall bias.

The population of North Trondelag County is stable, ethnic white, homogenous and representative of the Norwegian population, with the exceptions of a slightly lower average income and absence of a large city[39].

The wide range of exposure data collected in HUNT2 made it possible to adjust for a number of potential confounding factors, like symptoms of constipation and breathlessness, educational level and living area, which is seldom done in analyses of acute colonic diverticulitis. We used multiple imputation to account for missing data with the resulting more accurate HRs and more precise CIs and significance tests.

Many patients with acute colonic diverticulitis have vague symptoms, does not seek the doctor, and recover without antibiotics. Only part of all patients with this disease will be admitted to hospital. We are not aware of any change in admission policy for acute colonic diverticulitis during the study period. In addition to using the ICD-codes set by the doctor in charge at discharge, every hospital record was reviewed to ensure a higher probability of a correct diagnosis and prevent misclassifications[4]. Still, the diagnosis of acute colonic diverticulitis may have been incorrect in a minority of patients with known diverticulosis, who presented with pain in the lower abdomen, fever and an elevated CRP, without a CT scan.

CT scan is considered to be gold standard for the diagnosis of acute colonic diverticulitis. During the earlier years of this study, CT scan was used more infrequently. Even with the nowadays widespread availability of CT scans in many countries, we think it would be unadvisable due to radiation hazard, to perform CT scans in every patient with suspected uncomplicated, acute colonic diverticulitis.

Another limitation of the study is the 30% of non-participants in HUNT2. Non-participants, who were later admitted with acute colonic diverticulitis, were younger than participants in HUNT2: 7 and 9 years younger for females and males, respectively. Therefore, the study results may not be directly generalizable to a younger population. Some subjective variables that we used in the analysis, like symptom of breathlessness and type of bread eaten, could have led to information bias due to their questionnaires' nature. Another limitation is possible biased estimates due to residual confounding, as there are always unknown confounders that are unaccounted for in such studies.

The results of the present study apply to patients with colonic diverticulitis that was severe enough to require admission to hospital. The conclusions cannot be transformed without reservations to patients with diverticulitis not admitted to hospital.

In conclusion, this large prospective population-based cohort study showed that increasing age was associated with increased risk of admission for acute colonic diverticulitis in both sexes. Obese individuals had twice the risk of admission for acute colonic diverticulitis compared to those with normal weight. Previous or present cigarette smoking increased the risk of admission for acute colonic diverticulitis in females. There was no association between constipation or eating bread with fine or coarse grains and admission for acute colonic diverticulitis.

ACKNOWLEDGMENTS

The North Trondelag Health Study (HUNT) is performed through collaboration between HUNT Research Centre

(Faculty of Medicine, Norwegian University of Science and Technology, NTNU), North Trondelag County Council, Central Norway Health Authority, and the Norwegian Institute of Public Health. We want to thank clinicians and other employees at North Trondelag Hospital Trust for their support and for contributing to data collection in this research project.

COMMENTS

Background

Admission rates for acute colonic diverticulitis is increasing in the Western world. What health related factors are independently associated with diverticulitis?

Research frontiers

During the North-Trondelag Health Study in Norway, the HUNT2 study, performed during 1995-1997, a number of health related variables were collected. The present investigation studied which factors were independently associated with later admission for acute colonic diverticulitis. The participants contributed 611492 person-years of follow-up.

Innovations and breakthroughs

This large study demonstrated the independent association between increasing age and increasing body mass index and risk of admission for acute colonic diverticulitis in both gender. In females, cigarette smoking likewise increased the risk of admission. In males, breathlessness, a HUNT variable associated with chronic obstructive pulmonary disease, increased the risk of admission. On the other hand, physical activity, constipation and type of bread eaten showed no independent association with admission for acute colonic diverticulitis.

Applications

The results of this study may give further strength to argue for changes in lifestyle factors in a matter to possibly avoid acute colonic diverticulitis.

Peer-review

The study of a large population provides a useful overview of the various risk factors and their clinical impact.

REFERENCES

1 **Papagrigoriadis S**, Debrah S, Koreli A, Husain A. Impact of diverticular disease on hospital costs and activity. *Colorectal Dis* 2004; **6**: 81-84 [PMID: 15008903 DOI: 10.1111/j.1463-1318.2012.03000.x]

2 **Peppas G**, Bliziotis IA, Oikonomaki D, Falagas ME. Outcomes after medical and surgical treatment of diverticulitis: a systematic review of the available evidence. *J Gastroenterol Hepatol* 2007; **22**: 1360-1368 [PMID: 17716342 DOI: 10.1111/j.1440-1746.2007.05118.x]

3 **Rafferty J**, Shellito P, Hyman NH, Buie WD. Practice parameters for sigmoid diverticulitis. *Dis Colon Rectum* 2006; **49**: 939-944 [PMID: 16741596 DOI: 10.1007/s10350-006-0578-2]

4 **Jamal Talabani A**, Lydersen S, Endreseth BH, Edna TH. Major increase in admission- and incidence rates of acute colonic diverticulitis. *Int J Colorectal Dis* 2014; **29**: 937-945 [PMID: 24802814 DOI: 10.1007/s00384-014-1888-9]

5 **Nguyen GC**, Sam J, Anand N. Epidemiological trends and geographic variation in hospital admissions for diverticulitis in the United States. *World J Gastroenterol* 2011; **17**: 1600-1605 [PMID: 21472127 DOI: 10.3748/wjg.v17.i12.1600]

6 **Andeweg CS**, Mulder IM, Felt-Bersma RJ, Verbon A, van der Wilt GJ, van Goor H, Lange JF, Stoker J, Boermeester MA, Bleichrodt RP. Guidelines of diagnostics and treatment of acute left-sided colonic diverticulitis. *Dig Surg* 2013; **30**: 278-292 [PMID: 23969324 DOI: 10.1159/000354035]

7 **Granlund J**, Svensson T, Olén O, Hjern F, Pedersen NL, Magnusson PK, Schmidt PT. The genetic influence on diverticular disease--a twin study. *Aliment Pharmacol Ther* 2012; **35**: 1103-1107 [PMID: 22432696 DOI: 10.1111/j.1365-2036.2012.05069.x]

8 **Dobbins C**, Defontgalland D, Duthie G, Wattchow DA. The relationship of obesity to the complications of diverticular disease. *Colorectal Dis* 2006; **8**: 37-40 [PMID: 16519636 DOI: 10.1111/j.1463-1318.2005.00847.x]

9 **Papagrigoriadis S**, Macey L, Bourantas N, Rennie JA. Smoking may be associated with complications in diverticular disease. *Br J Surg* 1999; **86**: 923-926 [PMID: 10417566 DOI: 10.1046/j.1365-2168.1999.01177.x]

10 **Park NS**, Jeen YT, Choi HS, Kim ES, Kim YJ, Keum B, Seo YS, Chun HJ, Lee HS, Um SH, Kim CD, Ryu HS. Risk factors for severe diverticulitis in computed tomography-confirmed acute diverticulitis in Korea. *Gut Liver* 2013; **7**: 443-449 [PMID: 23898385 DOI: 10.5009/gnl.2013.7.4.443]

11 **Rodríguez-Wong U**, Cruz-Rubin C, Pinto-Angulo VM, García Álvarez J. [Obesity and complicated diverticular disease of the colon]. *Cir Cir* 2015; **83**: 292-296 [PMID: 26116035 DOI: 10.1016/j.circir.2015.02.003]

12 **Strate LL**, Liu YL, Aldoori WH, Giovannucci EL. Physical activity decreases diverticular complications. *Am J Gastroenterol* 2009; **104**: 1221-1230 [PMID: 19367267 DOI: 10.1038/ajg.2009.121]

13 **Krokstad S**, Langhammer A, Hveem K, Holmen TL, Midthjell K, Stene TR, Bratberg G, Heggland J, Holmen J. Cohort Profile: the HUNT Study, Norway. *Int J Epidemiol* 2013; **42**: 968-977 [PMID: 22879362 DOI: 10.1093/ije/dys095]

14 **WHO**. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 2000; **894**: i-xii, 1-253 [PMID: 11234459]

15 **Carpenter JR**, Kenward MG. Multiple Imputation and its Application. 1st ed. Chichester: John Wiley & Sons; 2013

16 **Hjern F**, Johansson C, Mellgren A, Baxter NN, Hjern A. Diverticular disease and migration--the influence of acculturation to a Western lifestyle on diverticular disease. *Aliment Pharmacol Ther* 2006; **23**: 797-805 [PMID: 16556182 DOI: 10.1111/j.1365-2036.2006.02805.x]

17 **Rosemar A**, Angerås U, Rosengren A. Body mass index and diverticular disease: a 28-year follow-up study in men. *Dis Colon Rectum* 2008; **51**: 450-455 [PMID: 18157570 DOI: 10.1007/s10350-007-9172-5]

18 **Strate LL**, Liu YL, Aldoori WH, Syngal S, Giovannucci EL. Obesity increases the risks of diverticulitis and diverticular bleeding. *Gastroenterology* 2009; **136**: 115-122.e1 [PMID: 18996378 DOI: 10.1053/j.gastro.2008.09.025]

19 **Hjern F**, Wolk A, Håkansson N. Obesity, physical inactivity, and colonic diverticular disease requiring hospitalization in women: a prospective cohort study. *Am J Gastroenterol* 2012; **107**: 296-302 [PMID: 22008890 DOI: 10.1038/ajg.2011.352]

20 **Hjern F**, Wolk A, Håkansson N. Smoking and the risk of diverticular disease in women. *Br J Surg* 2011; **98**: 997-1002 [PMID: 21462366 DOI: 10.1002/bjs.7477]

21 **Aldoori WH**, Giovannucci EL, Rimm EB, Wing AL, Trichopoulos DV, Willett WC. A prospective study of alcohol, smoking, caffeine, and the risk of symptomatic diverticular disease in men. *Ann Epidemiol* 1995; **5**: 221-228 [PMID: 7606311 DOI: 10.1016/1047-2797(94)00109-7]

22 **Humes DJ**, Ludvigsson JF, Jarvholm B. Smoking and the Risk of Hospitalization for Symptomatic Diverticular Disease: A Population-Based Cohort Study from Sweden. *Dis Colon Rectum* 2016; **59**: 110-114 [PMID: 26734968 DOI: 10.1097/DCR.0000000000000515]

23 **Edna TH**, Jamal Talabani A, Lydersen S, Endreseth BH. Survival after acute colon diverticulitis treated in hospital. *Int J Colorectal Dis* 2014; **29**: 1361-1367 [PMID: 24986140 DOI: 10.1007/s00384-014-1946-3]

24 **Lorimer JW**, Doumit G. Comorbidity is a major determinant of severity in acute diverticulitis. *Am J Surg* 2007; **193**: 681-685 [PMID: 17512276 DOI: 10.1016/j.amjsurg.2006.10.019]

25 **Chapman J**, Davies M, Wolff B, Dozois E, Tessier D, Harrington J, Larson D. Complicated diverticulitis: is it time to rethink the

rules? *Ann Surg* 2005; **242**: 576-581; discussion 581-583 [PMID: 16192818 DOI: 10.1097/01.sla.0000184843.89836.35]
26 **Yamada E**, Inamori M, Watanabe S, Sato T, Tagri M, Uchida E, Tanida E, Izumi M, Takeshita K, Fujisawa N, Komatsu K, Hamanaka J, Kanesaki A, Matsuhashi N, Nakajima A. Constipation is not associated with colonic diverticula: a multicenter study in Japan. *Neurogastroenterol Motil* 2015; **27**: 333-338 [PMID: 25469640 DOI: 10.1111/nmo.12478]
27 **Humes DJ**, Spiller RC. Review article: The pathogenesis and management of acute colonic diverticulitis. *Aliment Pharmacol Ther* 2014; **39**: 359-370 [PMID: 24387341 DOI: 10.1111/apt.12596]
28 **Aldoori W**, Ryan-Harshman M. Preventing diverticular disease. Review of recent evidence on high-fibre diets. *Can Fam Physician* 2002; **48**: 1632-1637 [PMID: 12449547]
29 **Crowe FL**, Balkwill A, Cairns BJ, Appleby PN, Green J, Reeves GK, Key TJ, Beral V. Source of dietary fibre and diverticular disease incidence: a prospective study of UK women. *Gut* 2014; **63**: 1450-1456 [PMID: 24385599 DOI: 10.1136/gutjnl-2013-304644]
30 **Strate LL**. Lifestyle factors and the course of diverticular disease. *Dig Dis* 2012; **30**: 35-45 [PMID: 22572683 DOI: 10.1159/000335707]
31 **Ünlü C**, Daniels L, Vrouenraets BC, Boermeester MA. A systematic review of high-fibre dietary therapy in diverticular disease. *Int J Colorectal Dis* 2012; **27**: 419-427 [PMID: 21922199 DOI: 10.1007/s00384-011-1308-3]
32 **Ornstein MH**, Littlewood ER, Baird IM, Fowler J, North WR, Cox AG. Are fibre supplements really necessary in diverticular disease of the colon? A controlled clinical trial. *Br Med J* (Clin Res Ed) 1981; **282**: 1353-1356 [PMID: 6263396]
33 **Strate LL**, Liu YL, Syngal S, Aldoori WH, Giovannucci EL. Nut, corn, and popcorn consumption and the incidence of diverticular disease. *JAMA* 2008; **300**: 907-914 [PMID: 18728264 DOI: 10.1001/jama.300.8.907]
34 **Maguire LH**, Song M, Strate LL, Giovannucci EL, Chan AT. Association of geographic and seasonal variation with diverticulitis admissions. *JAMA Surg* 2015; **150**: 74-77 [PMID: 25409019 DOI: 10.1001/jamasurg.2014.2049]
35 **Spiller RC**. Changing views on diverticular disease: impact of aging, obesity, diet, and microbiota. *Neurogastroenterol Motil* 2015; **27**: 305-312 [PMID: 25703217 DOI: 10.1111/nmo.12526]
36 **Milner P**, Crowe R, Kamm MA, Lennard-Jones JE, Burnstock G. Vasoactive intestinal polypeptide levels in sigmoid colon in idiopathic constipation and diverticular disease. *Gastroenterology* 1990; **99**: 666-675 [PMID: 1696228 DOI: 10.1016/0016-5085(90)90953-X]
37 **Keely S**, Talley NJ, Hansbro PM. Pulmonary-intestinal cross-talk in mucosal inflammatory disease. *Mucosal Immunol* 2012; **5**: 7-18 [PMID: 22089028 DOI: 10.1038/mi.2011.55]
38 **Palta M**, Prineas RJ, Berman R, Hannan P. Comparison of self-reported and measured height and weight. *Am J Epidemiol* 1982; **115**: 223-230 [PMID: 7058781]
39 **Statistics Norway**. Statistical Yearbook of Norway. Oslo, Kongsvinger: Statistics Norway; 2014

P- Reviewer: Asteria CR, Lakatos PL **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Liu WX

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.v22.i48.10673

World J Gastroenterol 2016 December 28; 22(48): 10673-10679
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Randomized Controlled Trial

Effects of premedication with Pronase for endoscopic ultrasound of the stomach: A randomized controlled trial

Guo-Xin Wang, Xiang Liu, Sheng Wang, Nan Ge, Jin-Tao Guo, Si-Yu Sun

Guo-Xin Wang, Xiang Liu, Sheng Wang, Nan Ge, Jin-Tao Guo, Si-Yu Sun, Endoscopy Center, Shengjing Hospital of China Medical University, Shenyang 110004, Liaoning Province, China

Author contributions: Wang GX and Sun SY were involved in the study conception and design; Wang GX drafted the article and analyzed and interpreted the data; Liu X performed critical revision of the article for important intellectual content and collected data; Wang S performed the endoscopic procedure and statistical analysis; Ge N and Guo JT conducted the EUS imaging evaluation; all authors provided approval of the final article.

Supported by the National Natural Science Foundation of China, No. 81470908.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of Shengjing Hospital of China Medical University.

Clinical trial registration statement: The trial was registered in the Chinese Clinical Trial Registry (www.chictr.org.cn). The registration identification number is ChiCTR-DPD-15006240.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: None of the authors have any competing interests or financial ties to disclose.

Data sharing statement: The technical appendix, statistical code, and data set are available from the corresponding author.

Manuscript source: Unsolicited manuscript

Correspondence to: Si-Yu Sun, MD, PhD, Professor, Endoscopy Center, Shengjing Hospital of China Medical University, No. 36 Sanhao Street, Shenyang 110004, Liaoning Province, China. sun-siyu@163.com
Telephone: +86-024-9661526111

Received: October 13, 2016
Peer-review started: October 14, 2016
First decision: November 14, 2016
Revised: November 22, 2016
Accepted: November 23, 2016
Article in press: November 28, 2016
Published online: December 28, 2016

Abstract

AIM

To analyze the effects of premedication with Pronase for endoscopic ultrasound (EUS) examination of the stomach.

METHODS

This was a prospective, randomized and controlled clinical study. All patients were randomly assigned to either the Pronase group or placebo group. The pretreatment solution was a mixed solution of 20000 U of Pronase and 60 mL sodium bicarbonate solution in the Pronase group, while an equal amount of sodium bicarbonate solution was administered to the placebo group. All operators, image evaluators and experimental recorders in EUS did not participate in the preparation and allocation of pretreatment solution. Two blinded investigators assessed the obscurity scores for the EUS images according to the size of artifacts (including ultrasound images of the gastric cavity and the gastric wall). Differences in imaging quality, the duration of examination and the usage of physiological saline during the examination process between the Pronase group and the control group were compared.

RESULTS

No differences existed in patient demographics between the two groups. For the gastric cavity, the Pronase group had significantly lower mean obscurity scores than the placebo group (1.0476 ± 0.77 *vs* 1.6129 ± 0.96, respectively, P = 0.000). The mean obscurity scores for the gastric mucosal surface were significantly lower in the Pronase group than the placebo group (1.2063 ± 0.90 *vs* 1.7581 ± 0.84, respectively, P = 0.001). The average EUS procedure duration for the Pronase group was 11.60 ± 3.32 min, which was significantly shorter than that of the placebo group (13.13 ± 3.81 min, P = 0.007). Less saline was used in the Pronase group than the placebo group, and the difference was significant (417.94 ± 121.38 mL *vs* 467.42 ± 104.52 mL, respectively, P = 0.016).

CONCLUSION

The group that had Pronase premedication prior to the EUS examination had clearer images than the placebo group. With Pronase premedication, the examination time was shorter, and the amount of saline used during the EUS examination was less.

Key words: Artifacts; Randomized controlled trial; Endosonography; Pronase; Stomach

Core tip: Previous studies have confirmed that Pronase can improve the quality of endoscopic ultrasound (EUS) images. Based on previous findings, this study hypothesized that Pronase could further shorten the duration of examination and reduce the usage of physiological saline during EUS examination through improving the quality of EUS images. Moreover, this study verified this hypothesis. This study found that for EUS examination, preoperative application of Pronase could provide clearer ultrasound images, shorten the duration of EUS examination, and reduce the intraoperative usage of physiological saline.

Wang GX, Liu X, Wang S, Ge N, Guo JT, Sun SY. Effects of premedication with Pronase for endoscopic ultrasound of the stomach: A randomized controlled trial. *World J Gastroenterol* 2016; 22(48): 10673-10679 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10673.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10673

INTRODUCTION

Endoscopic ultrasound (EUS) is an important tool to diagnosis benign and malignant diseases of the gastrointestinal tract and pancreaticobiliary system[1-4]. Previous studies demonstrated its superiority in evaluating the staging of early gastric carcinoma and gastric submucosal tumors, as compared with standard diagnostic modalities such as computed tomography, conventional ultrasonography, and magnetic resonance imaging[5-14]. Gastric mucus is one of the most frequent sources of artifacts during an EUS[15,16]. The vague image will influence the EUS procedure and increase the inspection time. Low-quality EUS images could lead to the misdiagnosis of small lesions and misinterpretation of the invasion depth in early gastric cancer[17,18]. To flush the mucosa and eliminate artifacts, more saline would need to be injected into the stomach, which is associated with a more uncomfortable examination and an increased risk of aspiration.

Pronase, separated and extracted from the culture filtrate of *Streptomyces griseus*[19-21], is a kind of proteolytic enzyme that can disrupt the mucous gel layer on the surface of the stomach[22], and it has been used to prepare digestive and anti-inflammatory enzymes. Fujii *et al*[23] first found that during chromoendoscopy and conventional endoscopy procedures, premedication with Pronase could improve endoscopic visualization. Over the last 10 years, it has become common practice to provide patients a pretreatment solution containing dimethylpolysiloxane and Pronase before endoscopy. Sakai *et al*[15] found that pretreatment with Pronase could reduce artifacts during an EUS examination. Han *et al*[24] also found that premedication with a mixture containing bicarbonate and Pronase seemed to reduce hyperechoic artifacts secondary to the gastric wall and lumen.

Herein, we presumed that decreasing the number of artifacts would shorten the EUS examination, leading to a decrease in the amount of saline solution irrigated during the procedure. To further address this hypothesis, we conducted this study to analyze the effects of Pronase on EUS imaging and EUS duration time, as well as the saline volume irrigated during EUS.

MATERIALS AND METHODS

Patients

This prospective, randomized and controlled single-center study was conducted at the Endoscopic Center of Shengjing Hospital of China Medical University. The eligibility and exclusion criteria are listed in Table 1. At least 102 patients were needed to acquire 90% statistical power based on a previous study performed by Han *et al*[24] in 2011. All patients provided written informed consent before the procedure. The Institutional Review Board of China Medical University approved this study based on the Helsinki Declaration. The trial was registered in the Chinese Clinical Trial Registry (ChiCTR-DPD-15006240).

Randomization and endoscopic procedures

The local clinical trials unit performed computerized individual randomization. Included patients were

Table 1 Eligibility and exclusion criteria for this study

Eligibility criteria	
1	Patients who required an EUS examination because of gastric diseases
2	Patients aged 18-70 yr
Exclusion criteria	
1	Patients with contraindications to endoscopy
2	Patients allergic to the pharmaceutical ingredients
3	Patients with gastric bleeding or suspected gastric bleeding
4	Patients with blood coagulation dysfunction
5	Patients with severe psychological diseases such as depression, anxiety, hypochondria and hysteria
6	Patients with severe cardiac dysfunction (NYHA cardiac function classification ⩾ class Ⅲ)
7	Patients with abnormal hepatic function (serum ALT and AST levels of ⩾ 4 times the upper normal limit)
8	Patients with renal dysfunction (serum Cr level of ⩾ 2 times the upper normal limit)
9	Patients with moderate to severe ventilatory dysfunction
10	Diabetic patients with unsatisfactory glycemic control
11	Hypertensive patients with unsatisfactory blood pressure control
12	Pregnant women or women who are breastfeeding

ALT: Alanine aminotransferase; AST: Aspartate transaminase; Cr: Creatinine; EUS: Endoscopic ultrasonography; NYHA: New York Heart Association.

Table 2 Scoring of the gastric cavity obscurity grade

Score	Number of high-echo spots
0	No or few
1	Low
2	Moderate
3	High

Table 3 Scoring of the gastric wall surface in endoscopic ultrasonography imaging

Score	Artifacts
0	Notable, affecting the diagnosis
1	Moderate
2	Negligible
3	None, clear wall interface

randomly assigned to either the Pronase or placebo group with a computer-generated random allocation sequence. In the placebo group, the premedication solution contained a 1 g sodium bicarbonate solution; in the Pronase group, the premedication solution contained a 1 g sodium bicarbonate solution and 20000 U of Pronase. All the solutions were placed in a paper cup of the same color. In both groups, 60 mL of the premedication solution was administrated about 10-30 min before the EUS examination, as a previous report recommended[25]. The study investigators were not involved in the preparation of the premedication solution. All patients underwent EUS examinations using both radial and linear-array systems. All procedures were carried out by one endosonography.

Measurements

As previously reported[15,24], for EUS imaging, the gastric cavity obscurity grade is scored from 0 to 3 (Figure 1), according to the number of high-echo spots, as shown in Table 2. EUS imaging of the gastric wall surface was similarly scored (Figure 2), as shown in Table 3. All EUS images were assigned to two experienced endoscopists, who scored the images and were blind to the procedure at the time of scoring. We recorded the duration of the EUS procedure for all patients. EUS duration was measured from the time the endoscope was inserted into the mouth to the time the endoscope was withdrawn from the mouth. One investigator recorded the volume of saline solution irrigated during the EUS procedure to determine whether premedication with Pronase decreased the amount of saline used.

Statistical analysis

The demographic characteristics were assessed using a Pearson χ^2 test or one-way analysis of variance. The obscurity scores for the two groups were assessed using a rank sum test with Mann-Whitney *U* comparisons and the Student's *t*-test. The mean obscurity scores for the gastric cavity and gastric mucosal surface, the EUS procedure duration and the volume of saline were expressed as mean ± SD and compared using the Student's *t*-test. A *P* value of < 0.05 was considered significant.

RESULTS

From May 2015 to July 2015, 125 patients were enrolled in the study and allocated equally to either the Pronase group (63 patients) or placebo group (62 patients). There were no differences in age ($P = 0.319$), sex ($P = 0.611$), location ($P = 0.532$), or EUS methods ($P = 0.391$) between groups, as shown in Table 4.

The obscurity scores for the gastric cavity and gastric mucosal surface were compared between the two groups (Table 5). The Pronase group had significantly lower obscurity scores for the gastric cavity and gastric mucosal surface than the placebo group ($P < 0.05$).

Table 6 compares the mean obscurity scores for the gastric cavity and gastric mucosal surface, and

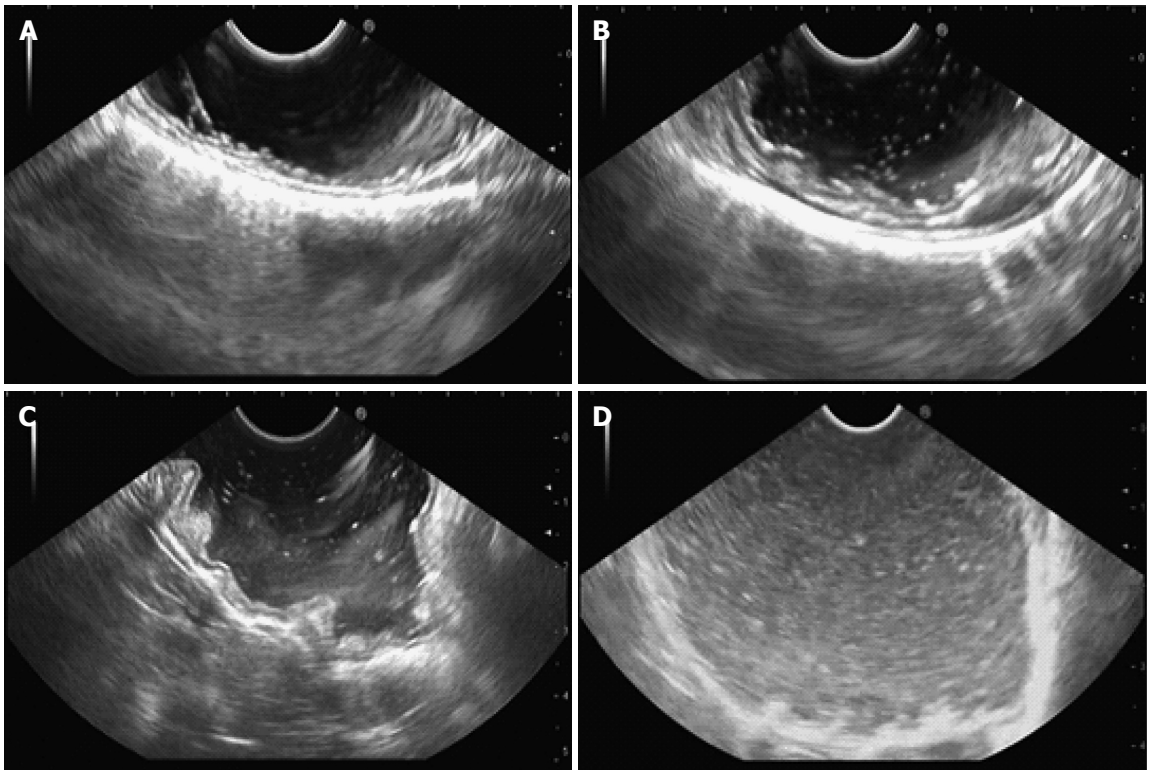

Figure 1 Scoring of the gastric cavity obscurity grade depending on the numbers of high-echo spots. A: Score 0, few or no high-echo spots in the gastric cavity; B: Score 1, low number of high-echo spots; C: Score 2, moderate number of high-echo spots; and D: Score 3, high number of high-echo spots.

Table 4 Demographic characteristics of enrolled patients

	Pronase group	Placebo group	Value	*P* value
Number of patients	63	62		
Age, mean ± SD	55.78 ± 12.37	53.47 ± 13.41	$t = 1.001$	0.319
Sex				
Male	22	19	$\chi^2 = 0.259$	0.611
Female	41	43		
Location				
Fundus	14	9	$\chi^2 = 1.264$	0.532
Corpus	26	29		
Antrum	23	24		
Methods				
Radial EUS	48	43	$\chi^2 = 0.737$	0.391
Linear-array EUS	15	19		

EUS: Endoscopic ultrasonography

EUS procedure means, as well as the mean volume of saline irrigated during the EUS procedure. As for the gastric cavity, the mean obscurity scores in the Pronase and placebo groups were 1.0476 ± 0.77 and 1.6129 ± 0.96, respectively. Additionally, for the gastric cavity, the Pronase group had significantly lower mean obscurity scores than the placebo group ($P = 0.000$). As for the gastric mucosal surface, the mean obscurity scores in the Pronase and placebo groups were 1.2063 ± 0.90 and 1.7581 ± 0.84, respectively. Further, for the gastric cavity, the Pronase group had significantly higher mean obscurity scores than the placebo group ($P = 0.001$).

The average EUS procedure time for the Pronase group was 11.60 ± 3.32 min, which was significantly shorter than the placebo group (13.13 ± 3.81 min, $P = 0.007$). The mean saline volumes were 417.94 ± 121.38 mL and 467.42 ± 104.52 mL in the Pronase group and placebo group, respectively. The amount of saline used for the Pronase group was less than that of the placebo group, and the difference was significant ($P = 0.016$).

DISCUSSION

EUS is now increasingly available and plays a significant role in the diagnosis and intervention of gastrointestinal and pancreaticobiliary diseases[1,26-29]. Artifacts secondary to gastric mucus can potentially interfere with visibility during EUS scanning of the stomach. Bubbles and foams may lead to blurred layers and borders and the possible diagnosis of a lesion that does not exist[16,30-32]. Before EUS, premedication played a major role in ensuring satisfactory visualization of the gastric cavity and wall[22,23,33].

Pronase, which can eliminate gastric mucus as a mucolytic enzyme, can further improve diagnosis of gastric diseases using radiographic imaging techniques[34]. A randomized study conducted by Fujii *et al*[23] demonstrated that premedication with Pronase not only substantially enhanced visibility before and after methylene blue spraying, but also reduced the duration of chromoendoscopy examination. In 2002, Kuo *et al*[22] also found that premedication with Pronase provided

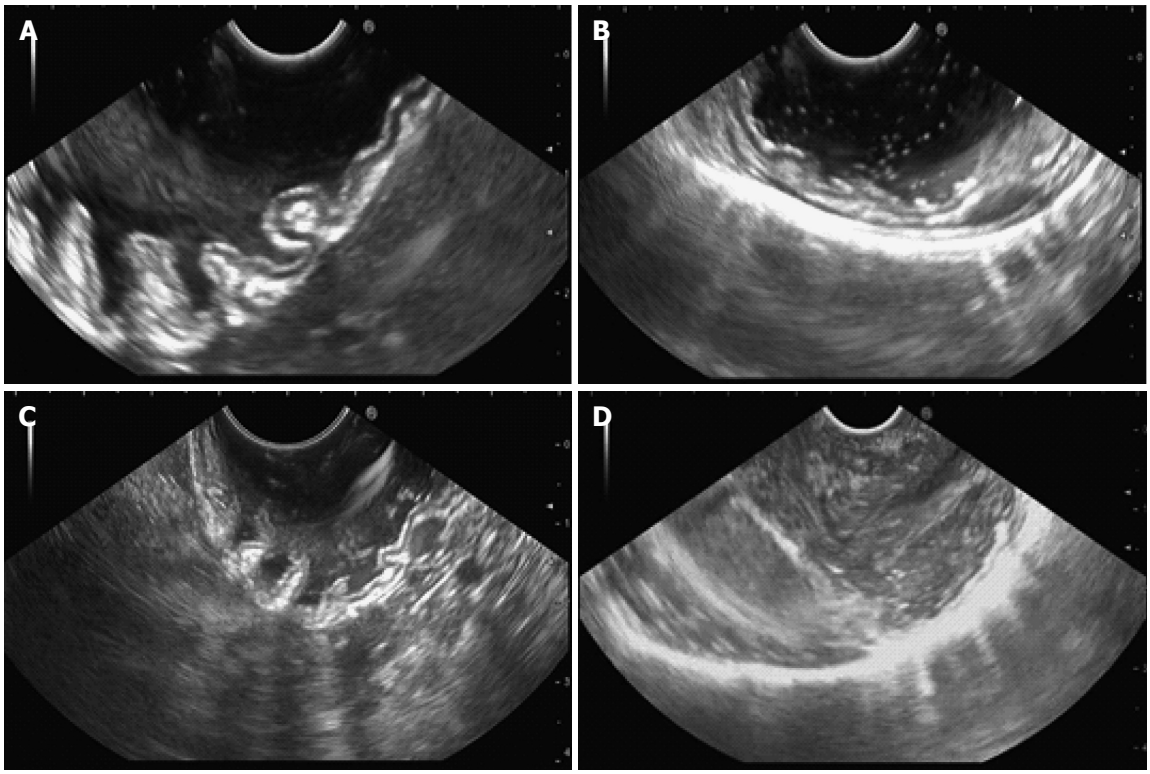

Figure 2 Scoring of the gastric wall surface depending on the amount of adherent mucus. A: Score 0, no artifacts, clear wall interface; B: Score 1, minimal artifacts, negligible; C: Score 2, moderate artifacts; and D: Score 3, significant artifacts affecting diagnostic judgment.

Table 5 Endoscopic ultrasonography obscurity scores for the gastric cavity and mucosal surface

	Pronase group	Placebo group	Value	*P* value
Gastric cavity obscurity scores during EUS				
3	14	8	Z = -3.428	0.001
2	35	21		
1	11	20		
0	3	13		
Gastric mucosal surface obscurity scores during EUS				
3	11	7	Z = -3.861	0.000
2	37	10		
1	6	36		
0	9	9		

EUS: Endoscopic ultrasonography.

Table 6 Mean endoscopic ultrasonography obscurity scores for the gastric cavity and mucosal surface

	Pronase group	Placebo group	Value	*P* value
Mean gastric cavity obscurity scores	1.0476 ± 0.77	1.6129 ± 0.96	*t* = -3.617	0.000
Mean gastric mucosal surface obscurity scores	1.2063 ± 0.90	1.7581 ± 0.84	*t* = -3.534	0.001
Duration of EUS, mean ± SD	11.60 ± 3.32	13.13 ± 3.81	*t* = -2.387	0.018
Volume of saline, mean ± SD	417.94 ± 121.38	467.42 ± 104.52	*t* = -2.441	0.016

the clearest endoscopic visibility. In 2003, Sakai *et al*[15] reported the first Pronase trial and suggested that premedication with Pronase reduced artifacts during endoscopic ultrasonography. In 2011, Han *et al*[24] found that premedication with bicarbonate mixed with Pronase decreased the number of hyperechoic artifacts secondary to the stomach wall and lumen during EUS.

As for the gastric mucosal surface and gastric cavity, we found that the Pronase group had significantly lower obscurity scores than the placebo group. The average time for the EUS examination was significantly shorter for the Pronase group than the placebo group. The amount of saline irrigated was significantly less for the Pronase group than the placebo group. The Pronase

premedication solution provided clearer images of the patients according to the endosonographer, which may facilitate EUS examination and shorten the procedure duration. Meanwhile, a clearer image may lead to less saline usage during the EUS examination.

Woo *et al*[25] found that the administration of Pronase, sodium bicarbonate, and dimethylpolysiloxane 30 min before gastroduodenoscopy helped improve endoscopic visualization remarkably, and the best visibility was achieved with the Pronase administration 10 min to 30 min before the gastrointestinal endoscopic procedure. In this study, we recommended that patients take the premedication solution 10 min to 30 min before the EUS procedure.

In conclusion, for EUS, the group that was administered Pronase premedication had clearer images than the placebo group. With Pronase premedication, the examination time was shorter, and the amount of saline used during the EUS procedure was less.

COMMENTS

Background

Previous studies have confirmed that Pronase can improve the quality of endoscopic ultrasonography (EUS) images. Based on previous findings, this study hypothesized that Pronase could further shorten the duration of examination and reduce the usage of physiological saline during EUS examination through improving the quality of EUS images.

Research frontiers

A few human studies have suggested that premedication with Pronase could improve endoscopic visualization. This study found that for EUS examination, preoperative application of Pronase could provide clearer ultrasound images, shorten the duration of EUS examination, and reduce the intraoperative usage of physiological saline.

Innovations and breakthrough

This study aimed to analyze and evaluate the effect of pretreatment with Pronase on imaging quality, the duration of examination and the usage of physiological saline during the examination process in gastric endoscopic ultrasound.

Applications

With Pronase premedication, the EUS examination time was shorter and the amount of saline used during the EUS procedure was less.

Peer-review

This is an interesting study on the use of Pronase premedication during EUS examination. The authors analyzed the effects of premedication with Pronase for EUS examination of the stomach. Two blinded investigators assessed the obscurity scores for the EUS images.

REFERENCES

1 **Byrne MF**, Jowell PS. Gastrointestinal imaging: endoscopic ultrasound. *Gastroenterology* 2002; **122**: 1631-1648 [PMID: 12016428 DOI: 10.1053/gast.2002.33576]

2 **Levy MJ**, Heimbach JK, Gores GJ. Endoscopic ultrasound staging of cholangiocarcinoma. *Curr Opin Gastroenterol* 2012; **28**: 244-252 [PMID: 22274618 DOI: 10.1097/MOG.0b013e32835005bc]

3 **Yoshinaga S**, Hilmi IN, Kwek BE, Hara K, Goda K. Current status of endoscopic ultrasound for the upper gastrointestinal tract in Asia. *Dig Endosc* 2015; **27** Suppl 1: 2-10 [PMID: 25537645 DOI: 10.1111/den.12422]

4 **Lennon AM**, Penman ID. Endoscopic ultrasound in cancer staging. *Br Med Bull* 2007; **84**: 81-98 [PMID: 18096635 DOI: 10.1093/bmb/ldm033]

5 **Rösch T**, Braig C, Gain T, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Staging of pancreatic and ampullary carcinoma by endoscopic ultrasonography. Comparison with conventional sonography, computed tomography, and angiography. *Gastroenterology* 1992; **102**: 188-199 [PMID: 1727753]

6 **Snady H**, Cooperman A, Siegel J. Endoscopic ultrasonography compared with computed tomography with ERCP in patients with obstructive jaundice or small peri-pancreatic mass. *Gastrointest Endosc* 1992; **38**: 27-34 [PMID: 1612375 DOI: 10.1016/S0016-5107(92)70326-5]

7 **Boyce GA**, Sivak MV, Rösch T, Classen M, Fleischer DE, Boyce HW, Lightdale CJ, Botet JF, Hawes RH, Lehman GA. Evaluation of submucosal upper gastrointestinal tract lesions by endoscopic ultrasound. *Gastrointest Endosc* 1991; **37**: 449-454 [PMID: 1916167 DOI: 10.1016/S0016-5107(05)80300-1]

8 **Adler DG**, Diehl DL. Missed lesions in endoscopic ultrasound. *Endosc Ultrasound* 2015; **4**: 165-167 [PMID: 26374573 DOI: 10.4103/2303-9027.162993]

9 **Altonbary AY**, Deiab AG, Negm EH, El Sorogy MM, Elkashef WF. Endoscopic ultrasound of isolated gastric corrosive stricture mimicking linitis plastica. *Endosc Ultrasound* 2015; **4**: 66-68 [PMID: 25789288 DOI: 10.4103/2303-9027.151365]

10 **Bhutani MS**, Arora A. New developments in endoscopic ultrasound-guided therapies. *Endosc Ultrasound* 2015; **4**: 304-311 [PMID: 26643698 DOI: 10.4103/2303-9027.170419]

11 **Razavi SM**, Khodadost M, Sohrabi M, Keshavarzi A, Zamani F, Rakhshani N, Ameli M, Sadeghi R, Hatami K, Ajdarkosh H, Golmahi Z, Ranjbaran M. Accuracy of endoscopic ultrasonography for determination of tumor invasion depth in gastric cancer. *Asian Pac J Cancer Prev* 2015; **16**: 3141-3145 [PMID: 25921111 DOI: 10.7314/APJCP.2015.16.8.3141]

12 **Alkaade S**, Chahla E, Levy M. Role of endoscopic ultrasound-guided fine-needle aspiration cytology, viscosity, and carcinoembryonic antigen in pancreatic cyst fluid. *Endosc Ultrasound* 2015; **4**: 299-303 [PMID: 26643697 DOI: 10.4103/2303-9027.170417]

13 **Hata S**, Arai M, Suzuki T, Maruoka D, Matsumura T, Nakagawa T, Katsuno T, Imazeki F, Yokosuka O. Clinical significance of endoscopic ultrasound for gastric submucosal tumors. *Clin Res Hepatol Gastroenterol* 2013; **37**: 207-212 [PMID: 22633196 DOI: 10.1016/j.clinre.2012.04.004]

14 **Gotoda T**, Yamamoto H, Soetikno RM. Endoscopic submucosal dissection of early gastric cancer. *J Gastroenterol* 2006; **41**: 929-942 [PMID: 17096062 DOI: 10.1007/s00535-006-1954-3]

15 **Sakai N**, Tatsuta M, Iishi H, Nakaizumi A. Pre-medication with pronase reduces artefacts during endoscopic ultrasonography. *Aliment Pharmacol Ther* 2003; **18**: 327-332 [PMID: 12895217 DOI: 10.1046/j.1365-2036.2003.01582.x]

16 **Yiengpruksawan A**, Lightdale CJ, Gerdes H, Botet JF. Mucolytic-antifoam solution for reduction of artifacts during endoscopic ultrasonography: a randomized controlled trial. *Gastrointest Endosc* 1991; **37**: 543-546 [PMID: 1657680 DOI: 10.1016/S0016-5107(91)70825-0]

17 **Yamamoto S**, Nishida T, Kato M, Inoue T, Hayashi Y, Kondo J, Akasaka T, Yamada T, Shinzaki S, Iijima H, Tsujii M, Takehara T. Evaluation of endoscopic ultrasound image quality is necessary in endosonographic assessment of early gastric cancer invasion depth. *Gastroenterol Res Pract* 2012; **2012**: 194530 [PMID: 23024651 DOI: 10.1155/2012/194530]

18 **Rana SS**, Sharma V, Sharma R, Gunjan D, Dhalaria L, Gupta R, Bhasin DK. Gastric gastrointestinal stromal tumor mimicking cystic tumor of the pancreas: Diagnosed by endoscopic ultrasound-fine-needle aspiration. *Endosc Ultrasound* 2015; **4**: 351-352 [PMID: 26643707 DOI: 10.4103/2303-9027.170452]

19 **Tarentino AL**, Maley F. Purification and properties of an endo-beta-N-acetylglucosaminidase from Streptomyces griseus. *J Biol*

Chem 1974; **249**: 811-817 [PMID: 4204552]
20 **Ambler RP**. The purification and amino acid composition of pseudomonas cytochrome C-551. *Biochem J* 1963; **89**: 341-349 [PMID: 14084621 DOI: 10.1042/bj0890349]
21 **Narahashi Y**, Shibuya K, Yanagita M. Studies on proteolytic enzymes (pronase) of Streptomyces griseus K-1. II. Separation of exo- and endopeptidases of pronase. *J Biochem* 1968; **64**: 427-437 [PMID: 5707831]
22 **Kuo CH**, Sheu BS, Kao AW, Wu CH, Chuang CH. A defoaming agent should be used with pronase premedication to improve visibility in upper gastrointestinal endoscopy. *Endoscopy* 2002; **34**: 531-534 [PMID: 12170403]
23 **Fujii T**, Iishi H, Tatsuta M, Hirasawa R, Uedo N, Hifumi K, Omori M. Effectiveness of premedication with pronase for improving visibility during gastroendoscopy: a randomized controlled trial. *Gastrointest Endosc* 1998; **47**: 382-387 [PMID: 9609431 DOI: 10.1016/S0016-5107(98)70223-8]
24 **Han JP**, Hong SJ, Moon JH, Lee GH, Byun JM, Kim HJ, Choi HJ, Ko BM, Lee MS. Benefit of pronase in image quality during EUS. *Gastrointest Endosc* 2011; **74**: 1230-1237 [PMID: 21963063 DOI: 10.1016/j.gie.2011.07.044]
25 **Woo JG**, Kim TO, Kim HJ, Shin BC, Seo EH, Heo NY, Park J, Park SH, Yang SY, Moon YS, Lee NY. Determination of the optimal time for premedication with pronase, dimethylpolysiloxane, and sodium bicarbonate for upper gastrointestinal endoscopy. *J Clin Gastroenterol* 2013; **47**: 389-392 [PMID: 23442831 DOI: 10.1097/MCG.0b013e3182758944]
26 **Bhutani MS**. Role of endoscopic ultrasound for pancreatic cystic lesions: Past, present, and future! *Endosc Ultrasound* 2015; **4**: 273-275 [PMID: 26643692 DOI: 10.4103/2303-9027.170400]
27 **Lu X**, Zhang S, Ma C, Peng C, Lv Y, Zou X. The diagnostic value of EUS in pancreatic cystic neoplasms compared with CT and MRI. *Endosc Ultrasound* 2015; **4**: 324-329 [PMID: 26643701 DOI: 10.4103/2303-9027.170425]
28 **Yoon WJ**, Brugge WR. The safety of endoscopic ultrasound-guided fine-needle aspiration of pancreatic cystic lesions. *Endosc Ultrasound* 2015; **4**: 289-292 [PMID: 26643695 DOI: 10.4103/2303-9027.170408]
29 **Wilcox CM**, Varadarajulu S, Eloubeidi M. Role of endoscopic evaluation in idiopathic pancreatitis: a systematic review. *Gastrointest Endosc* 2006; **63**: 1037-1045 [PMID: 16733122 DOI: 10.1016/j.gie.2006.02.024]
30 **Hussain N**, Hawes RH. Principles of endosonography and imaging. *Gastrointest Endosc Clin N Am* 2005; **15**: 1-12, vii [PMID: 15555948 DOI: 10.1016/j.giec.2004.07.010]
31 **Kwee RM**, Kwee TC. Imaging in local staging of gastric cancer: a systematic review. *J Clin Oncol* 2007; **25**: 2107-2116 [PMID: 17513817 DOI: 10.1200/JCO.2006.09.5224]
32 **Polkowski M**, Larghi A, Weynand B, Boustière C, Giovannini M, Pujol B, Dumonceau JM. Learning, techniques, and complications of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline. *Endoscopy* 2012; **44**: 190-206 [PMID: 22180307 DOI: 10.1055/s-0031-1291543]
33 **Everett SM**, White KL, Schorah CJ, Calvert RJ, Skinner C, Miller D, Axon AT. In vivo DNA damage in gastric epithelial cells. *Mutat Res* 2000; **468**: 73-85 [PMID: 10863159 DOI: 10.1016/S1383-5718(00)00041-3]
34 **Chang CC**, Chen SH, Lin CP, Hsieh CR, Lou HY, Suk FM, Pan S, Wu MS, Chen JN, Chen YF. Premedication with pronase or N-acetylcysteine improves visibility during gastroendoscopy: an endoscopist-blinded, prospective, randomized study. *World J Gastroenterol* 2007; **13**: 444-447 [PMID: 17230616 DOI: 10.3748/wjg.v13.i3.444]

P- Reviewer: Chamberlain MC, Faloppi L, Jones G
S- Editor: Gong ZM **L- Editor**: Filipodia **E- Editor**: Zhang FF

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.v22.i48.10680

World J Gastroenterol 2016 December 28; 22(48): 10680-10686
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Efficiency of olaparib in colorectal cancer patients with an alteration of the homologous repair protein

Francois Ghiringhelli, Corentin Richard, Sandy Chevrier, Frédérique Végran, Romain Boidot

Francois Ghiringhelli, Department of Medical Oncology, Center GF Leclerc, 21000 Dijon, France

Francois Ghiringhelli, Corentin Richard, Sandy Chevrier, Frédérique Végran, Romain Boidot, Platform of Transfer in Oncology, Center GF Leclerc, 21000 Dijon, France

Francois Ghiringhelli, Frederique Vegran, Romain Boidot, INSERM UMR866, 21000 Dijon, France

Francois Ghiringhelli, Corentin Richard, University of Bourgogne Franche Comte, 21000 Dijon, France

Author contributions: Ghiringhelli F and Boidot R designed the report; Richard C, Chevrier S and Végran F performed the genetic analyses; Ghiringhelli F collected the patient's clinical data; Ghiringhelli F and Boidot R analyzed the data and wrote the paper.

Institutional review board statement: This study was approved by the local institutional review board.

Informed consent statement: Patients gave their written consent to authorize genetic analyses.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

Correspondence to: Francois Ghiringhelli, MD, PhD, Professor, Department of Medical Oncology, Center GF Leclerc, 21000 Dijon, France. fghiringhelli@cgfl.fr
Telephone: +33-380393371
Fax: +33-380393434

Received: July 7, 2016
Peer-review started: July 11, 2016
First decision: August 22, 2016
Revised: August 26, 2016
Accepted: September 28, 2016
Article in press: September 28, 2016
Published online: December 28, 2016

Abstract

Precision medicine is defined by the administration of drugs based on the tumor's particular genetic characteristics. It is developing quickly in the field of cancer therapy. For example, *KRAS*, *NRAS* and *BRAF* genetic testing demonstrates its efficiency for precision medicine in colorectal cancer (CRC). Besides for these well-known mutations, the purpose of performing larger genetic testing in this pathology is unknown. Recent reports have shown that using the poly ADP ribose polymerase (PARP) inhibitor olaparib in patients with homologous repair enzyme deficiency gave positive clinical results in breast, ovarian and prostate cancers. We have reported here the cases of 2 patients with multi-treated metastatic CRC who underwent somatic and constitutional exome analyses. The analyses revealed a loss of function mutation in a homologous repair enzyme resulting in the loss of heterozygosity for both patients (Check2 for the first patient and RAD51C for the second one). Both patients were treated with off-label usage of olaparib. While the first patient showed clinical benefit, reduction of carcinoembryonic antigen tumor marker and radiologic response, the second patient quickly presented a progression of the tumor. Additional genetic analyses revealed a frameshift truncating mutation of the *TP53BP1* gene in the patient who progressed. Interestingly, deficiency in TP53BP1 was previously described to confer resistance to olaparib in mice breast cancer models. Our findings suggest that exome analysis may be a helpful tool to

highlight targetable mutations in CRC and that olaparib may be efficient in patients with a homologous repair deficiency.

Key words: Colorectal cancer; Exome analysis; Genetic aberrations; Homologous repair deficiency; Precision medicine

Core tip: The role of genetic profiling in metastatic colorectal cancer for precision medicine is currently under investigation. This case report underlines for the first time, that olaparib may have some clinical efficiency in patients with homologous repair deficiency in colorectal tumor.

Ghiringhelli F, Richard C, Chevrier S, Végran F, Boidot R. Efficiency of olaparib in colorectal cancer patients with an alteration of the homologous repair protein. *World J Gastroenterol* 2016; 22(48): 10680-10686 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10680.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10680

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide with about 1 million new cases and over 500000 deaths every year[1]. Approximately 30% of patients with CRC develop an overt metastatic disease. Meanwhile, 40% to 50% of newly diagnosed patients already present a metastatic disease. For patients with non-operable metastatic CRC (mCRC), there are no curative options, but the use of palliative systemic chemotherapy dramatically enhances response rates, progression-free survival (PFS) and overall survival (OS)[2-4]. Currently, few drugs have demonstrated effectiveness in the treatment of metastatic colorectal disease and patients are treated based on the use of three cytotoxic chemotherapies: fluoropyrimidine, oxaliplatin and irinotecan, associated with targeted therapies [anti-EGFR (panitumumab and cetuximab) or anti-VEGF (bevacizumab) monoclonal antibodies].

In consequence, at the end of their treatment sequence, many patients still have a good performance status and would still be able to undergo treatment; unfortunately, we do not have any approved and efficient therapies to offer. Cancer cells are characterized by multiple genomic alterations. This finding has led to the development of molecularly targeted agents that inhibit mutated proteins. Such drugs are theoretically more specific for cancer cells bearing specific mutations. While such targeted agents have followed clinical development based on tumor location, most genetic molecular alterations exist across tumor types and histologies. This observation raises the possibility of shifting towards the administration of drugs based on molecular pattern rather than on histological status. Improvements in genetic sequencing have made possible the identification of multiple genomic molecular alterations in a timeframe compatible with clinical practice. Many retrospective and prospective trials have shown the usage and efficiency of off-label molecularly targeting agents. We report here the usage of exome testing in 2 patients with metastatic CRC and show for the first time that homologous repair deficiency could be targeted by poly ADP ribose polymerase (PARP) inhibitor in this pathology.

Sample preparation

Formalin-fixed paraffin-embedded (FFPE) tumors were characterized by a pathologist to determine the tumor cell content and sent to the molecular biology platform for DNA extraction. Tumor cell content was 85% for Case 1 and 60% for Case 2. Seven 15-μm slices were extracted using the Maxwell 16 FFPE Plus LEV DNA purification kit (Promega) according to the manufacturer's protocol. Corresponding normal DNA was extracted from 500 μL ethylenediaminetetraacetic acid blood samples with the Maxwell 16 Blood DNA Purification System (Promega) according to the manufacturer's instructions. DNA quality was assessed by spectrophotometry with absorbance at 230, 260 and 280 nm. DNA was quantified using a fluorimetric assay with a Qubit device.

Two-hundred nanogram of genomic DNA from normal and cancer cells were fragmented with a Covaris device to obtain fragments around 180-200 bp. Then, libraries were constructed and captured by using SureSelect Human All Exon v5 kit (Agilent), following the manufacturer's protocol. Paired-end (2 × 151 bases) sequencing was performed on a NextSeq500 device (Illumina). Obtained sequences were aligned and annotated with the human Hg19 genome based on the SureSelect Human All Exon v5 manifest by using Burrows-Wheeler Aligne (BWA) and Genome Analysis toolkit (GATK) algorithms. Only sequences with a read depth of 10 × and a mutation allele frequency superior to 5% were conserved for the analysis. The analysis is fostered on 137 clinically relevant genes related to biological pathways linked to cancer predisposition or available targeted therapies (Supplementary Table 1). Copy number variations were studied by using Control-FREEC software as described[5,6].

CASE REPORT

Patient 1

A 58-year-old Caucasian male initially presented with abdominal pain. A CT scan, carried out in June 2011, revealed a metastatic sigmoid cancer with the following metastatic locations: mediastinal and lomboartic lymph nodes, lungs. A lymph node biopsy was performed

Table 1 Alterations observed for Case 1 in the short list of clinically relevant genes

Gene	Nucleotide variation	Protein variation	Origin	Impact
AKT1	c.235C>A	Gln79Lys	Somatic	Activating
CCNE1	c.1022C>T	Thr341Met	Somatic	Unknown
CHEK2	c.478A>G	Arg117Gly	Constitutive	Likely pathogenic
CUL2	c.70C>T	Pro24Ser	Somatic	Unknown
ERCC6	c.4179G>A	Met1393Ile	Somatic	Unknown
PMS2	c.1531A>G	Thr511Ala	Constitutive	Benign
SMAD4	c.1091T>A	Leu364X	Somatic	Loss of function
SUFU	c.1211T>C	Met404Thr	Constitutive	Unknown

and the diagnosis of wild-type KRAS, NRAS and BRAF, moderately differentiated Lieberkühn adenocarcinoma was made. From 2011 to 2015, the patient received different chemotherapeutic regimens. In July 2015, the patient's lung metastases progressed and he started to show symptoms such as breathlessness and a permanent dry cough. As no approved chemotherapy or targeted therapy could be proposed, we performed somatic and constitutional exome analyses. We observed 479 somatic mutations. For clinical use, we analyzed a short list of 137 genes. It was interesting to see that among the 8 altered genes (Table 1), we observed a SMAD4 stop mutation, which is frequently found in CRC. An activating mutation of *AKT1* was observed (Q79K). It could be targetable by protein kinase B (AKT)/mTor inhibitors. Surprisingly, we observed a constitutive Chek2 mutation (R117G), a gene involved in the homologous repair process. This mutation is cited in the public database for conferring a predisposition to cancer. Moreover, the analysis of copy number variation showed a loss of heterozygosity in chromosome 22 (from 29091114 to 29130709) (Figure 1A). This analysis suggested a complete deletion of Check2 function in tumor cells. After discussion of the case at the molecular tumor board, the patient was proposed to receive off-label PARP inhibitor olaparib which previously showed efficiency in patients with Chek2 mutation in metastatic prostate cancer[7]. One mo after beginning the therapy by PARP inhibitor, the patient declared reduction of cough and disappearance of breathlessness. After 3 mo we observed a reduction in carcinoembryonic antigen serum level (57 ng/mL to 25 ng/mL) and a tumor size reduction upon CT scan (Figure 2). No hematological toxicity was mentioned. Patient weight increased from 62 kg to 68 kg. However, despite this response, the patient died suddenly at home 4 mo after introduction of the therapy.

Patient 2

A 49-year-old woman presented with rectal bleeding and diarrhea in March 2015. She underwent a colonoscopy with biopsies that revealed a KRAS mutated, well differentiated Lieberkühn rectal adenocarcinoma. The patient did not present any family history of CRC. The genetic testing did not show microsatellite instability. CT scan showed multiple, bilobar liver metastases. The patient was treated with FOLFIRINOX combination plus bevacizumab for 12 cycles. The CT scan showed major response in the liver and the rectum but no curative option could be proposed for the liver disease. Despite efficacy, the therapy had major toxicity with grade 3 peripheral neuropathy which precluded further usage of oxaliplatin. A therapeutic pause was proposed. The patient refused and asked for additional therapy. We performed somatic and constitutional exome analyses. We observed 905 somatic mutations. For clinical use, we analyzed a short list of 137 genes and interestingly, among the 13 altered genes (Table 2), we observed a potentially pathogenic APC mutation, which could predispose to CRC. Moreover, a *KRAS* activating mutation (G12D), commonly found in CRC, was observed. What was even more interesting is that we observed a somatic mutation of the homologous repair in RAD51C (T287A) previously reported to be associated with a loss of function[8]. Analysis of copy number variable showed a loss of heterozygosity in chromosome 17 from 56770004 to 56801461 (Figure 1B). This analysis suggested a complete deletion of RAD51C function in tumor cells. After discussion of the case at the molecular tumor board, the patient was proposed to receive off-label PARP inhibitor olaparib which previously showed efficacy in patients with RAD51 mutation in metastatic prostate cancer[7]. The patient received 3 mo of olaparib therapy without toxicity. Despite the absence of toxicity, the magnetic resonance imaging (Figure 2) showed tumor progression. Olaparib was stopped and the patient was included in a phase Ⅰ clinical trial.

As loss of TP53BP1 was previously described to be involved in PARP inhibitor resistance in homologous repair deficient breast cancer models in mice[9], we searched for the mutation of this gene in both patients. While patient 1 had a wild-type *TP53PB1* gene, patient 2 had a frameshift truncating insertion in *TP53BP1* (AG insertion at chromosomal position 17:43766919) (Figure 1C), thus suggesting a loss of function of the protein.

DISCUSSION

The use of exome sequencing in cancer has largely taken place in the setting of research studies, such

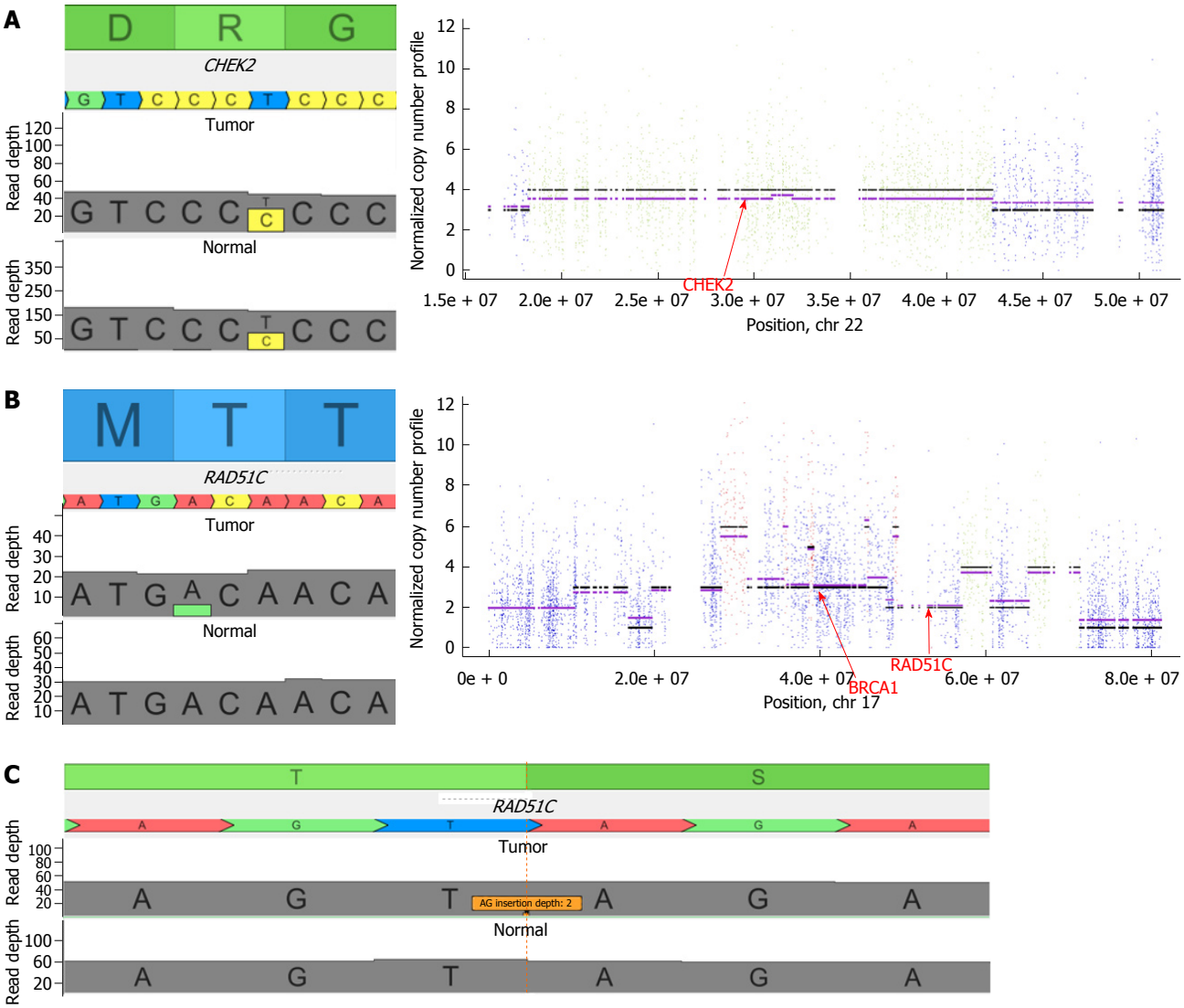

Figure 1 Genetic data of targeted alterations. A: Patient 1 presented a T>C variation in the *CHEK2* gene inducing a constitutive deleterious R117G mutation (left panel). At the chromosomal level, it appeared that chromosome 22 harbored a loss of heterozygosity inducing (right panel) a complete inactivation of CHEK2; B: Patient 2 presented a A>G variation in the *RAD51C* gene inducing a somatic loss of function T287A mutation (left panel). At the chromosomal level, it appeared that chromosome 17 harbored an important loss of heterozygosity at the RAD51C locus inducing a complete inactivation of RAD51C; C: Patient 2 also harbored an AG insertion resulting in a frameshift truncating mutation in the *TP53BP1* gene.

as The Cancer Genome Atlas. However, integration of exome sequencing into precision cancer care remains a challenging issue because samples issued from formalin-fixed tissues (*i.e.*, FFPE) are difficult to analyze (due to small size and poor quality). Furthermore, bioinformatics approaches require detection of the wide-spectrum of mutations with the capacity to identify actionable mutations at an acceptable sensitivity and it remains an issue. This case report is the first to suggest efficacy of olaparib in CRC with homologous repair deficiency and to demonstrate that exome sequencing could be used to help drug repositioning. Accordingly, single-agent olaparib as well as combinations with irinotecan were previously tested in unselected CRC that is resistant to standard therapy without any clinical efficacy[10,11]. Such data raises the hypothesis that olaparib activity also requires molecular testing of homologous repair deficiency for CRC as requested for ovarian cancer.

Homologous repair is an enzymatic complex aimed at repairing DNA double-strand breaks. CHEK2 and RAD51 play a central role in the repair of DNA double-strand breaks performed by homologous repair[12]. After the detection of DNA double-strand breaks by ATM and CHEK2, BRCA1 is phosphorylated by these proteins. Rad52 binds recombinase polymerase amplification and displaces it to allow Rad51 binding. BRCA2 binds to Rad51 until BRCA2 becomes phosphorylated, releasing Rad51 and allowing it to localize to the double strand break with Rad52. Homologous repair-mediated repair goes on; Rad51 then forms a nucleoprotein filament that invades a homologous sequence and activates strand exchange to generate a crossover between the juxtaposed DNA[13]. Several studies have shown that

Table 2 Alterations observed for Case 2 in the short list of clinically relevant genes

Gene	Nucleotide variation	Protein variation	Origin	Impact
APC	c.3949G>C	Glu1317Gln	Constitutive	Potentially Pathogenic
BRCA1	c.5180G>C	Gly1727Ala	Somatic	Unknown
BRCA1	c.3119G>A	Ser1040Asn	Constitutive	SNP
BRCA1	c.2521C>T	Arg841Trp	Somatic	Neutral
FGFR4	c.1676G>A	Arg559Gln	Somatic	Unknown
GLI2	c.1418G>A	Arg473His	Constitutive	Unknown
JAK2	c.195A>C	Glu65Asp	Constitutive	Unknown
JAK3	c.2164G>A	Val722Ile	Constitutive	Activating
KDR	c.2159G>A	Arg720Gln	Somatic	Unknown
KRAS	c.35G>A	Gly12Asp	Somatic	Activating
MET	c.2572G>A	Val858Met	Constitutive	Unknown
RAD51C	c.859A>G	Thr287Ala	Somatic	Loss of function
SLC28A1	c.568G>T	Ala190Ser	Constitutive	SNP
SLC28A1	c.1152delG	Val385Ser fsX16	Constitutive	Loss of function
THRA	c.454C>T	Arg152X	Somatic	Loss of function
UIMC1	c.43C>T	Arg15Trp	Constitutive	Unknown

SNP: Single nucleotide polymorphism.

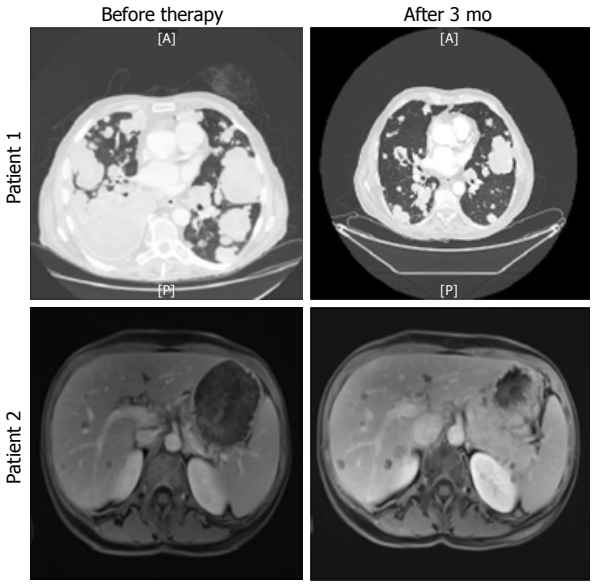

Figure 2 Computed tomography scan of patient 1 before and after 3 mo of olaparib (upper panel), and magnetic resonance imaging of liver metastasis of patient 2 before and after 3 mo of olaparib (lower panel).

the level of RAD51 protein expression is elevated in immortalized cells as well as in a wide variety of human cancer cell lines[14,15]. It is generally suggested that RAD51 overexpression results in an increased cellular resistance to radiation and some chemotherapeutic drugs, such as topoisomerase inhibitors or crosslinking agents[15-17]. In CRC, high expression of RAD51 is associated with poor prognosis[18]. Several *in vitro* studies have shown that an increase in RAD51 expression stimulates homologous repair, resulting in greater cellular resistance to treatment with crosslinking agents, etoposide or irradiation[15-17]. High numbers of RAD51 foci in tumor biopsies were also positively associated with greater chemoresistance in breast cancer patients[19]. Chek2 is also involved in the colorectal oncogenesis and protein truncating mutations in *CHEK2* have been reported to confer higher risks of cancer of the breast and the prostate but also CRC[20].

Biallelic invalidation of homologous repair enzymes are still described to be a predictive marker of response to PARP inhibitor like olaparib in different diseases such as breast, ovarian and prostate cancer[7,21-23]. Our report is the first one that researched biallelic deficiency of homologous repair enzyme in metastatic CRC patients and underlines that such events could also occur in this pathology and could be targeted.

Resistance to PARP inhibitors is a key question and determining a predictive biomarker is important for the future design of clinical trials. Three mechanisms of resistance have been described: restoration of BRCA function by additional mutations[24-26], increased *Mdr1* gene expression[27] or loss of TP53BP1[9]. Interestingly, TP53BP1 loss also induces resistance to the topoisomerase I inhibitor while tumor cells remain sensitive to DNA crosslinking agents like platinum, may thus explain the good response to FOLFIRINOX.

To conclude, we believe that this case report supports that large genetic characterization of metastatic CRC patients could be useful to find molecular hits that could be targeted by off-label targeted therapy. PARP inhibitors could be particularly useful is this context. Nevertheless, biallelic deficiency of homologous repair enzyme is a prerequisite to benefit from a PARP inhibitor therapy, but is not always associated to a PARP inhibitor response. Additional resistance pathways like TP53BP1 loss must be determined before prescribing PARP inhibitors.

COMMENTS

Case characteristics

Two case of multitreated metastatic colorectal cancer patients that benefit from

genetic testing.

Clinical diagnosis
metastatic colorectal cancer.

Laboratory diagnosis
Whole exome sequencing revealed inactivating mutation in the homologous repair gene with genetic deficiency in RAD51C or Check2.

Treatment
Patients were treated with the poly ADP ribose polymerase inhibitor olaparib.

Experiences and lessons
One out the 2 patients gained clinical benefit from olaparib usage, thus suggesting that genetic testing could also be used in colorectal cancer to predict response to olaparib.

Peer-review
This case report highlighting the importance of the exome sequencing analysis before administering targeted therapy.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

2 **Nordlinger B**, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, Jaeck D, Mirza D, Parks RW, Collette L, Praet M, Bethe U, Van Cutsem E, Scheithauer W, Gruenberger T, EORTC Gastro-Intestinal Tract Cancer Group, Cancer Research UK, Arbeitsgruppe Lebermetastasen und-tumoren in der Chirurgischen Arbeitsgemeinschaft Onkologie (ALM-CAO), Australasian Gastro-Intestinal Trials Group (AGITG), Fédération Francophone de Cancérologie Digestive (FFCD). Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. *Lancet* 2008; **371**: 1007-1016 [PMID: 18358928 DOI: 10.1016/S0140-6736(08)60455-9]

3 **Van den Eynde M**, Hendlisz A. Treatment of colorectal liver metastases: a review. *Rev Recent Clin Trials* 2009; **4**: 56-62 [PMID: 19149763 DOI: 10.2174/157488709787047558]

4 **Douillard JY**, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alakl M, Gruia G, Awad L, Rougier P. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000; **355**: 1041-1047 [PMID: 10744089 DOI: 10.1016/S0140-6736(00)02034-1]

5 **Boeva V**, Popova T, Bleakley K, Chiche P, Cappo J, Schleiermacher G, Janoueix-Lerosey I, Delattre O, Barillot E. Control-FREEC: a tool for assessing copy number and allelic content using next-generation sequencing data. *Bioinformatics* 2012; **28**: 423-425 [PMID: 22155870 DOI: 10.1093/bioinformatics/btr670]

6 **Boeva V**, Zinovyev A, Bleakley K, Vert JP, Janoueix-Lerosey I, Delattre O, Barillot E. Control-free calling of copy number alterations in deep-sequencing data using GC-content normalization. *Bioinformatics* 2011; **27**: 268-269 [PMID: 21081509 DOI: 10.1093/bioinformatics/btq635]

7 **Mateo J**, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, Nava Rodrigues D, Robinson D, Omlin A, Tunariu N, Boysen G, Porta N, Flohr P, Gillman A, Figueiredo I, Paulding C, Seed G, Jain S, Ralph C, Protheroe A, Hussain S, Jones R, Elliott T, McGovern U, Bianchini D, Goodall J, Zafeiriou Z, Williamson CT, Ferraldeschi R, Riisnaes R, Ebbs B, Fowler G, Roda D, Yuan W, Wu YM, Cao X, Brough R, Pemberton H, A'Hern R, Swain A, Kunju LP, Eeles R, Attard G, Lord CJ, Ashworth A, Rubin MA, Knudsen KE, Feng FY, Chinnaiyan AM, Hall E, de Bono JS. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *N Engl J Med* 2015; **373**: 1697-1708 [PMID: 26510020 DOI: 10.1056/NEJMoa1506859]

8 **Meindl A**, Hellebrand H, Wiek C, Erven V, Wappenschmidt B, Niederacher D, Freund M, Lichtner P, Hartmann L, Schaal H, Ramser J, Honisch E, Kubisch C, Wichmann HE, Kast K, Deissler H, Engel C, Müller-Myhsok B, Neveling K, Kiechle M, Mathew CG, Schindler D, Schmutzler RK, Hanenberg H. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. *Nat Genet* 2010; **42**: 410-414 [PMID: 20400964 DOI: 10.1038/ng.569]

9 **Jaspers JE**, Kersbergen A, Boon U, Sol W, van Deemter L, Zander SA, Drost R, Wientjens E, Ji J, Aly A, Doroshow JH, Cranston A, Martin NM, Lau A, O'Connor MJ, Ganesan S, Borst P, Jonkers J, Rottenberg S. Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. *Cancer Discov* 2013; **3**: 68-81 [PMID: 23103855 DOI: 10.1158/2159-8290.CD-12-0049]

10 **Leichman L**, Groshen S, O'Neil BH, Messersmith W, Berlin J, Chan E, Leichman CG, Cohen SJ, Cohen D, Lenz HJ, Gold P, Boman B, Fielding A, Locker G, Cason RC, Hamilton SR, Hochster HS. Phase II Study of Olaparib (AZD-2281) After Standard Systemic Therapies for Disseminated Colorectal Cancer. *Oncologist* 2016; **21**: 172-177 [PMID: 26786262 DOI: 10.1634/theoncologist.2015-0319]

11 **Chen EX**, Jonker DJ, Siu LL, McKeever K, Keller D, Wells J, Hagerman L, Seymour L. A Phase I study of olaparib and irinotecan in patients with colorectal cancer: Canadian Cancer Trials Group IND 187. *Invest New Drugs* 2016; **34**: 450-457 [PMID: 27075016 DOI: 10.1007/s10637-016-0351-x]

12 **Benson FE**, Baumann P, West SC. Synergistic actions of Rad51 and Rad52 in recombination and DNA repair. *Nature* 1998; **391**: 401-404 [PMID: 9450758 DOI: 10.1038/34937]

13 **West SC**. Molecular views of recombination proteins and their control. *Nat Rev Mol Cell Biol* 2003; **4**: 435-445 [PMID: 12778123 DOI: 10.1038/nrm1127]

14 **Raderschall E**, Stout K, Freier S, Suckow V, Schweiger S, Haaf T. Elevated levels of Rad51 recombination protein in tumor cells. *Cancer Res* 2002; **62**: 219-225 [PMID: 11782381]

15 **Hansen LT**, Lundin C, Spang-Thomsen M, Petersen LN, Helleday T. The role of RAD51 in etoposide (VP16) resistance in small cell lung cancer. *Int J Cancer* 2003; **105**: 472-479 [PMID: 12712436 DOI: 10.1002/ijc.11106]

16 **Slupianek A**, Hoser G, Majsterek I, Bronisz A, Malecki M, Blasiak J, Fishel R, Skorski T. Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G(2)/M phase, and protection from apoptosis. *Mol Cell Biol* 2002; **22**: 4189-4201 [PMID: 12024032 DOI: 10.1128/MCB.22.12.4189-4201.2002]

17 **Vispé S**, Cazaux C, Lesca C, Defais M. Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. *Nucleic Acids Res* 1998; **26**: 2859-2864 [PMID: 9611228 DOI: 10.1093/nar/26.12.2859]

18 **Tennstedt P**, Fresow R, Simon R, Marx A, Terracciano L, Petersen C, Sauter G, Dikomey E, Borgmann K. RAD51 overexpression is a negative prognostic marker for colorectal adenocarcinoma. *Int J Cancer* 2013; **132**: 2118-2126 [PMID: 23065657 DOI: 10.1002/ijc.27907]

19 **Asakawa H**, Koizumi H, Koike A, Takahashi M, Wu W, Iwase H, Fukuda M, Ohta T. Prediction of breast cancer sensitivity to neoadjuvant chemotherapy based on status of DNA damage repair proteins. *Breast Cancer Res* 2010; **12**: R17 [PMID: 20205718 DOI: 10.1186/bcr2486]

20 **Cybulski C**, Wokołorczyk D, Kładny J, Kurzawski G, Suchy J, Grabowska E, Gronwald J, Huzarski T, Byrski T, Górski B, D Ecedil Bniak T, Narod SA, Lubiński J. Germline CHEK2 mutations and colorectal cancer risk: different effects of a missense and truncating mutations? *Eur J Hum Genet* 2007; **15**: 237-241 [PMID: 17106448 DOI: 10.1038/sj.ejhg.5201734]

21 **Lee JM**, Hays JL, Annunziata CM, Noonan AM, Minasian L, Zujewski JA, Yu M, Gordon N, Ji J, Sissung TM, Figg WD, Azad N, Wood BJ, Doroshow J, Kohn EC. Phase I/Ib study of olaparib and carboplatin in BRCA1 or BRCA2 mutation-associated breast or ovarian cancer with biomarker analyses. *J Natl Cancer Inst* 2014;

106: dju089 [PMID: 24842883 DOI: 10.1093/jnci/dju089]
22 **Oza AM**, Cibula D, Benzaquen AO, Poole C, Mathijssen RH, Sonke GS, Colombo N, Špaček J, Vuylsteke P, Hirte H, Mahner S, Plante M, Schmalfeldt B, Mackay H, Rowbottom J, Lowe ES, Dougherty B, Barrett JC, Friedlander M. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial. *Lancet Oncol* 2015; **16**: 87-97 [PMID: 25481791 DOI: 10.1016/S1470-2045(14)71135-0]
23 **Kaufman B**, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmaña J, Mitchell G, Fried G, Stemmer SM, Hubert A, Rosengarten O, Steiner M, Loman N, Bowen K, Fielding A, Domchek SM. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. *J Clin Oncol* 2015; **33**: 244-250 [PMID: 25366685 DOI: 10.1200/JCO.2014.56.2728]
24 **Edwards SL**, Brough R, Lord CJ, Natrajan R, Vatcheva R, Levine DA, Boyd J, Reis-Filho JS, Ashworth A. Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* 2008; **451**: 1111-1115 [PMID: 18264088 DOI: 10.1038/nature06548]
25 **Sakai W**, Swisher EM, Karlan BY, Agarwal MK, Higgins J, Friedman C, Villegas E, Jacquemont C, Farrugia DJ, Couch FJ, Urban N, Taniguchi T. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature* 2008; **451**: 1116-1120 [PMID: 18264087 DOI: 10.1038/nature06633]
26 **Swisher EM**, Sakai W, Karlan BY, Wurz K, Urban N, Taniguchi T. Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. *Cancer Res* 2008; **68**: 2581-2586 [PMID: 18413725 DOI: 10.1158/0008-5472.CAN-08-0088]
27 **Rottenberg S**, Jaspers JE, Kersbergen A, van der Burg E, Nygren AO, Zander SA, Derksen PW, de Bruin M, Zevenhoven J, Lau A, Boulter R, Cranston A, O'Connor MJ, Martin NM, Borst P, Jonkers J. High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. *Proc Natl Acad Sci USA* 2008; **105**: 17079-17084 [PMID: 18971340 DOI: 10.1073/pnas.0806092105]

P- Reviewer: Lakatos PL, Matsuda A, Meshikhes AW
S- Editor: Gong ZM **L- Editor**: Filipodia **E- Editor**: Liu WX

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