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

World Journal of *Gastroenterology*

World J Gastroenterol 2015 October 14; 21(38): 10709-10930

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 1377 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 68 countries, including Algeria (2), Argentina (7), Australia (31), Austria (9), Belgium (11), Brazil (20), Brunei Darussalam (1), Bulgaria (2), Cambodia (1), Canada (26), Chile (4), China (164), Croatia (2), Cuba (1), Czech (6), Denmark (2), Egypt (9), Estonia (2), Finland (6), France (20), Germany (58), Greece (31), Guatemala (1), Hungary (15), Iceland (1), India (33), Indonesia (2), Iran (10), Ireland (9), Israel (18), Italy (194), Japan (150), 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*
Mirko Diksic, *Montreal*
Jane A Foster, *Hamilton*
Hugh J Freeman, *Vancouver*
Shahrokh M Ghobadloo, *Ottawa*
Yuewen Gong, *Winnipeg*
Philip H Gordon, *Quebec*
Rakesh Kumar, *Edmonton*
Wolfgang A Kunze, *Hamilton*
Patrick Labonte, *Laval*
Zhikang Peng, *Winnipeg*
Jayadev Raju, *Ottawa*
Maitreyi Raman, *Calgary*
Giada Sebastiani, *Montreal*
Maida J Sewitch, *Montreal*
Eldon A Shaffer, *Alberta*
Christopher W Teshima, *Edmonton*
Jean Sévigny, *Québec*
Pingchang Yang, *Hamilton*
Pingchang Yang, *Hamilton*
Eric M Yoshida, *Vancouver*
Bin Zheng, *Edmonton*

Chile

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

China

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

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

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

Cuba
Damian Casadesus, *Havana*

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

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

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

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

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

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

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

Greece
Alexandra Alexopoulou, *Athens*
Nikolaos Antonakopoulos, *Athens*
Stelios F Assimakopoulos, *Patras*
Grigoris Chatzimavroudis, *Thessaloniki*
Evangelos Cholongitas, *Thessaloniki*
Gregory Christodoulidis, *Larisa*
George N Dalekos, *Larissa*
Urania Georgopoulou, *Athens*
Eleni Gigi, *Thessaloniki*

Stavros Gourgiotis, *Athens*
Leontios J Hadjileontiadis, *Thessaloniki*
Thomas Hyphantis, *Ioannina*
Ioannis Kanellos, *Thessaloniki*
Stylianos Karatapanis, *Rhodes*
Michael Koutsilieris, *Athens*
Spiros D Ladas, *Athens*
Theodoros K Liakakos, *Athens*
Emanuel K Manesis, *Athens*
Spilios Manolakopoulos, *Athens*
Gerassimos John Mantzaris, *Athens*
Athanasios D Marinis, *Piraeus*
Nikolaos Ioannis Nikiteas, *Athens*
Konstantinos X Papamichael, *Athens*
George Sgourakis, *Athens*
Konstantinos C Thomopoulos, *Patras*
Konstantinos Triantafyllou, *Athens*
Christos Triantos, *Patras*
Georgios Zacharakis, *Athens*
Petros Zezos, *Alexandroupolis*
Demosthenes E Ziogas, *Ioannina*

Guatemala

Carlos Maria Parellada, *Guatemala*

Hungary

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

Iceland

Tryggvi Bjorn Stefánsson, *Reykjavík*

India

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

Indonesia

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

Iran

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

Ireland

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

Israel

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

Italy

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

Massimo Falconi, *Ancona*
Ezio Falletto, *Turin*
Silvia Fargion, *Milan*
Matteo Fassan, *Verona*
Gianfranco Delle Fave, *Roma*
Alessandro Federico, *Naples*
Francesco Feo, *Sassari*
Davide Festi, *Bologna*
Natale Figura, *Siena*
Vincenzo Formica, *Rome*
Mirella Fraquelli, *Milan*
Marzio Frazzoni, *Modena*
Walter Fries, *Messina*
Gennaro Galizia, *Naples*
Andrea Galli, *Florence*
Matteo Garcovich, *Rome*
Eugenio Gaudio, *Rome*
Paola Ghiorzo, *Genoa*
Edoardo G Giannini, *Genova*
Luca Gianotti, *Monza*
Maria Cecilia Giron, *Padova*
Alberto Grassi, *Rimini*
Gabriele Grassi, *Trieste*
Francesco Greco, *Bergamo*
Luigi Greco, *Naples*
Antonio Grieco, *Rome*
Fabio Grizzi, *Rozzano*
Laurino Grossi, *Pescara*
Simone Guglielmetti, *Milan*
Tiberiu Hershcovici, *Jerusalem*
Calogero Iacono, *Verona*
Enzo Ierardi, *Bari*
Amedeo Indriolo, *Bergamo*
Raffaele Iorio, *Naples*
Paola Iovino, *Salerno*
Angelo A Izzo, *Naples*
Loreta Kondili, *Rome*
Filippo La Torre, *Rome*
Giuseppe La Torre, *Rome*
Giovanni Latella, *L'Aquila*
Salvatore Leonardi, *Catania*
Massimo Libra, *Catania*
Anna Licata, *Palermo*
C armela Loguercio, *Naples*
Amedeo Lonardo, *Modena*
Carmelo Luigiano, *Catania*
Francesco Luzza, *Catanzaro*
Giovanni Maconi, *Milano*
Antonio Macrì, *Messina*
Mariano Malaguarnera, *Catania*
Francesco Manguso, *Napoli*
Tommaso Maria Manzia, *Rome*
Daniele Marrelli, *Siena*
Gabriele Masselli, *Rome*
Sara Massironi, *Milan*
Giuseppe Mazzarella, *Avellino*
Michele Milella, *Rome*
Giovanni Milito, *Rome*
Antonella d'Arminio Monforte, *Milan*
Fabrizio Montecucco, *Genoa*
Giovanni Monteleone, *Rome*
Mario Morino, *Torino*
Vincenzo La Mura, *Milan*
Gerardo Nardone, *Naples*
Riccardo Nascimbeni, *Brescia*
Gabriella Nesi, *Florence*
Giuseppe Nigri, *Rome*
Erica Novo, *Turin*
Veronica Ojetti, *Rome*
Michele Orditura, *Naples*
Fabio Pace, *Seriate*
Lucia Pacifico, *Rome*
Omero Alessandro Paoluzi, *Rome*
Valerio Pazienza, *San Giovanni Rotondo*
Rinaldo Pellicano, *Turin*
Adriano M Pellicelli, *Rome*
Nadia Peparini, *Ciampino*
Mario Pescatori, *Rome*
Antonio Picardi, *Rome*
Alberto Pilotto, *Padova*
Alberto Piperno, *Monza*
Anna Chiara Piscaglia, *Rome*
Maurizio Pompili, *Rome*
Francesca Romana Ponziani, *Rome*
Cosimo Prantera, *Rome*
Girolamo Ranieri, *Bari*
Carlo Ratto, *Tome*
Barbara Renga, *Perugia*
Alessandro Repici, *Rozzano*
Maria Elena Riccioni, *Rome*
Lucia Ricci-Vitiani, *Rome*
Luciana Rigoli, *Messina*
Mario Rizzetto, *Torino*
Ballarin Roberto, *Modena*
Roberto G Romanelli, *Florence*
Claudio Romano, *Messina*
Luca Roncucci, *Modena*
Cesare Ruffolo, *Treviso*
L ucia Sacchetti, *Napoli*
Rodolfo Sacco, *Pisa*
Lapo Sali, *Florence*
Romina Salpini, *Rome*
Giulio Aniello, *Santoro Treviso*
Armando Santoro, *Rozzano*
Edoardo Savarino, *Padua*
Marco Senzolo, *Padua*
Annalucia Serafino, *Rome*
Giuseppe S Sica, *Rome*
Pierpaolo Sileri, *Rome*
Cosimo Sperti, *Padua*
Vincenzo Stanghellini, *Bologna*
Cristina Stasi, *Florence*
Gabriele Stocco, *Trieste*
Roberto Tarquini, *Florence*
Mario Testini, *Bari*
Guido Torzilli, *Milan*
Guido Alberto Massimo, *Tiberio Brescia*
Giuseppe Toffoli, *Aviano*
Alberto Tommasini, *Trieste*
Francesco Tonelli, *Florence*
Cesare Tosetti Porretta, *Terme*
Lucio Trevisani, *Cona*
Guglielmo M Trovato, *Catania*
Mariapia Vairetti, *Pavia*
Luca Vittorio Valenti, *Milano*
Mariateresa T Ventura, *Bari*
Giuseppe Verlato, *Verona*
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*
H Shimada, *Tokyo*
Tomohiko Shimatani, *Kure*
Yukihiro Shimizu, *Nanto*
Tadashi Shimoyama, *Hirosaki*
Masayuki Sho, *Nara*
Ikuo Shoji, *Kobe*
Atsushi Sofuni, *Tokyo*
Takeshi Suda, *Niigata*
M Sugimoto, *Hamamatsu*
Ken Sugimoto, *Hamamatsu*
Haruhiko Sugimura, *Hamamatsu*
Shoichiro Sumi, *Kyoto*
Hidekazu Suzuki, *Tokyo*
Masahiro Tajika, *Nagoya*
Hitoshi Takagi, *Takasaki*
Toru Takahashi, *Niigata*
Yoshihisa Takahashi, *Tokyo*
Shinsuke Takeno, *Fukuoka*
Akihiro Tamori, *Osaka*
Kyosuke Tanaka, *Tsu*
Shinji Tanaka, *Hiroshima*
Atsushi Tanaka, *Tokyo*
Yasuhito Tanaka, *Nagoya*
Shinji Tanaka, *Tokyo*
Minoru Tomizawa, *Yotsukaido City*
Kyoko Tsukiyama-Kohara, *Kagoshima*
Takuya Watanabe, *Niigata*
Kazuhiro Watanabe, *Sendai*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Yokkaichi*
Hiroshi Yamamoto, *Otsu*
Kosho Yamanouchi, *Nagasaki*
Ichiro Yasuda, *Gifu*
Yutaka Yata, *Maebashi-city*
Shin-ichi Yokota, *Sapporo*
Norimasa Yoshida, *Kyoto*
Hiroshi Yoshida, *Tama-City*
Hitoshi Yoshiji, *Kashihara*
Kazuhiko Yoshimatsu, *Tokyo*
Kentaro Yoshioka, *Toyoake*
Nobuhiro Zaima, *Nara*

Jordan
Khaled Ali Jadallah, *Irbid*

Kuwait
Islam Khan, *Kuwait*

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

Lithuania
Antanas Mickevicius, *Kaunas*

Malaysia
Huck Joo Tan, *Petaling Jaya*

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

Morocco
Samir Ahboucha, *Khouribga*

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

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

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

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

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

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

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

Filipa F Vale, *Lisbon*

Puerto Rico

Caroline B Appleyard, *Ponce*

Qatar

Abdulbari Bener, *Doha*

Romania

Mihai Ciocirlan, *Bucharest*
Dan LucianDumitrascu, *Cluj-Napoca*
Carmen Fierbinteanu-Braticevici, *Bucharest*
Romeo G Mihaila, *Sibiu*
Lucian Negreanu, *Bucharest*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*
Ioan Sporea, *Timisoara*
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*

Xiaonan Han, *Cincinnati*
Mohamed Hassan, *Jackson*
Martin Hauer-Jensen, *Little Rock*
Koichi Hayano, *Boston*
Yingli Hee, *Atlanta*
Samuel B Ho, *San Diego*
Jason Ken Hou, *Houston*
Lifang Hou, *Chicago*
K-Qin Hu, *Orange*
Jamal A Ibdah, *Columbia*
Robert Thomas Jensen, *Bethesda*
Huanguang "Charlie" Jia, *Gainesville*
Rome Jutabha, *Los Angeles*
Andreas M Kaiser, *Los Angeles*
Avinash Kambadakone, *Boston*
David Edward Kaplan, *Philadelphia*
Randeep Kashyap, *Rochester*
Rashmi Kaul, *Tulsa*
Ali Keshavarzian, *Chicago*
Amir Maqbul Khan, *Marshall*
Nabeel Hasan Khan, *New Orleans*
Sahil Khanna, *Rochester*
Kusum K Kharbanda, *Omaha*
Hyun Sik Kim, *Pittsburgh*
Joseph Kim, *Duarte*
Jae S Kim, *Gainesville*
Miran Kim, *Providence*
Timothy R Koch, *Washington*
Burton I Korelitz, *New York*
Betsy Kren, *Minneapolis*
Shiu-Ming Kuo, *Buffalo*
Michelle Lai, *Boston*
Andreas Larentzakis, *Boston*
Edward Wolfgang Lee, *Los Angeles*
Daniel A Leffler, *Boston*
Michael Leitman, *New York*
Suthat Liangpunsakul, *Indianapolis*
Joseph K Lim, *New Haven*
Elaine Y Lin, *Bronx*
Henry C Lin, *Albuquerque*
Rohit Loomba, *La Jolla*
James David Luketich, *Pittsburgh*
Li Ma, *Stanford*
Mohammad F Madhoun, *Oklahoma City*
Thomas C Mahl, *Buffalo*
Ashish Malhotra, *Bettendorf*
Pranoti Mandrekar, *Worcester*
John Marks, *Wynnewood*
Wendy M Mars, *Pittsburgh*
Julien Vahe Matricon, *San Antonio*
Craig J McClain, *Louisville*
Tamir Miloh, *Phoenix*
Ayse Leyla Mindikoglu, *Baltimore*
Huanbiao Mo, *Denton*
Klaus Monkemuller, *Birmingham*
John Morton, *Stanford*
Adnan Muhammad, *Tampa*
Michael J Nowicki, *Jackson*
Patrick I Okolo, *Baltimore*
Giusepp Orlando, *Winston Salem*
Natalia A Osna, *Omaha*
Virendra N Pandey, *Newark*
Mansour A Parsi, *Cleveland*
Michael F Picco, *Jacksonville*
Daniel S Pratt, *Boston*
Xiaofa Qin, *Newark*
Janardan K Reddy, *Chicago*
Victor E Reyes, *Galveston*
Jon Marc Rhoads, *Houston*
Giulia Roda, *New York*
Jean-Francois Armand Rossignol, *Tampa*
Paul A Rufo, *Boston*
Madhusudana Girija Sanal, *New York*
Miguel Saps, *Chicago*
Sushil Sarna, *Galveston*
Ann O Scheimann, *Baltimore*
Bernd Schnabl, *La Jolla*
Matthew J Schuchert, *Pittsburgh*
Ekihiro Seki, *La Jolla*
Chanjuan Shi, *Nashville*
David Quan Shih, *Los Angeles*
Shadab A Siddiqi, *Orlando*
William B Silverman, *Iowa City*
Shashideep Singhal, *New York*
Bronislaw L Slomiany, *Newark*
Steven F Solga, *Bethlehem*
Byoung-Joon Song, *Bethesda*
Dario Sorrentino, *Roanoke*
Scott R Steele, *Fort Lewis*
Branko Stefanovic, *Tallahassee*
Arun Swaminath, *New York*
Kazuaki Takabe, *Richmond*
Naoki Tanaka, *Bethesda*
Hans Ludger Tillmann, *Durham*
George Triadafilopoulos, *Stanford*
John Richardson Thompson, *Nashville*
Andrew Ukleja, *Weston*
Miranda AL van Tilburg, *Chapel Hill*
Gilberto Vaughan, *Atlanta*
Vijayakumar Velu, *Atlanta*
Gebhard Wagener, *New York*
Kasper Saonun Wang, *Los Angeles*
Xiangbing Wang, *New Brunswick*
Daoyan Wei, *Houston*
Theodore H Welling, *Ann Arbor*
C Mel Wilcox, *Birmingham*
Jacqueline Lee Wolf, *Boston*
Laura Ann Woollett, *Cincinnati*
Harry Hua-Xiang Xia, *East Hanover*
Wen Xie, *Pittsburgh*
Guang Yu Yang, *Chicago*
Michele T Yip-Schneider, *Indianapolis*
Sam Zakhari, *Bethesda*
Kezhong Zhang, *Detroit*
Huiping Zhou, *Richmond*
Xiao-Jian Zhou, *Cambridge*
Richard Zubarik, *Burlington*

Venezuela
Miguel Angel Chiurillo, *Barquisimeto*

Vietnam
Van Bang Nguyen, *Hanoi*

World Journal of Gastroenterology

Contents

Weekly Volume 21 Number 38 October 14, 2015

EDITORIAL

TOPIC HIGHLIGHT

SYSTEMATIC REVIEWS

CASE REPORT

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Shuai Ma*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Ze-Mao Gong*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Gastroenterology

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

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

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

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

PUBLICATION DATE
October 14, 2015

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

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

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10709

World J Gastroenterol 2015 October 14; 21(38): 10709-10713
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Advanced pancreatic cancer - how to choose an adequate treatment option

Eija A Korkeila

Eija A Korkeila, Department of Oncology, Turku University Hospital and University of Turku, 20521 Turku, Finland

Author contributions: Korkeila EA wrote this manuscript.

Conflict-of-interest statement: The author Eija A Korkeila has received a fee for serving as a speaker and an advisory board member for Abbott and Roche.

Correspondence to: Eija A Korkeila, MD, PhD, Specialist in Oncology and Radiotherapy, Department of Oncology, Turku University Hospital and University of Turku, Hämeentie 11 PB 52, 20521 Turku, Finland. eija.korkeila@tyks.fi
Telephone: +358-2-3130000
Fax: +358-2-3132850

Received: January 31, 2015
Peer-review started: February 1, 2015
First decision: April 13, 2015
Revised: May 26, 2015
Accepted: August 25, 2015
Article in press: August 25, 2015
Published online: October 14, 2015

Abstract

The prognosis of pancreatic adenocarcinoma is poor, making it one of the leading causes of cancer-related death. The 5-year overall survival rate remains below 5% and little progress is made during the past decade. Only about 10%-20% of patients are eligible for curative-intent surgery and the majority end up having recurring disease even after radical surgery and postoperative adjuvant chemotherapy. Chemotherapy in metastatic disease is palliative at best, aiming at disease and symptom control and prolongation of life. Treatment always causes side effects, the degree of which varies from patient to patient, depending on the patient's general condition, concomitant morbidities as well as on the chosen treatment modality. Why is pancreatic cancer so resistant to treatment? How to best help the patient to reach the set treatment goals?

Key words: Pancreatic cancer; Chemotherapy; Palliative treatment; Prognosis; Side effects

Core tip: The prognosis of metastatic pancreatic adenocarcinoma is poor. Chemotherapy is palliative at best. Some patients benefit from treatment, while some have rapidly progressing treatment-resistant disease. There are several options for single-agent and combined treatment. Some patients may even gain benefit from treatment in second and even further lines and live substantially longer than average. Why is pancreatic cancer so resistant to treatment?

Korkeila EA. Advanced pancreatic cancer - how to choose an adequate treatment option. *World J Gastroenterol* 2015; 21(38): 10709-10713 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10709.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10709

INTRODUCTION

Why is pancreatic cancer resistant to treatment?

Symptoms of pancreatic adenocarcinoma, including vague upper abdominal or back pain, nausea, fatigue

and weight loss, are associated with more advanced disease. Tumours of the pancreatic head cause icterus, which tends to lead to somewhat earlier diagnosis[1]. There are no effective and sensitive, non-invasive cost-effective methods to screen asymptomatic pancreatic cancer, with the exception of patients who have high-risk precursor lesions, including intraductal papillary mucinous neoplasms, and pancreatic intraepithelial neoplasia[2]. However, among a substantial proportion of patients the diagnosis is inevitably late, making cure unreachable[3-11].

Pancreatic cancer is associated with desmoplastic reaction, *i.e.*, the tumour mass consisting of not only cancer cells but also of an exceptionally high percentage of stromal cells, namely fibroblasts and inflammatory cells, as well as a substantial amount of rigid extracellular matrix[1,12,13]. These factors result in inadequate blood and lymphatic vessels as well as poor vascularisation and hypoxia, leading to poor delivery of chemotherapeutic agents, as focused by Chu *et al*[12] and Feig *et al*[13]. These micro-environmental factors, together with several genetic mutations, among them *KRAS*, and *SMAD4*, *AKT*, *MYC* and *P13K* as well as tumour suppressor genes *TP53* and *PTEN*, support tumour growth and survival, making pancreatic cancer one of the most lethal human malignancies[12-14].

FIRST-LINE CHEMOTHERAPEUTIC OPTIONS

Gemcitabine

Gemcitabine is a nucleoside analogue that blocks DNA replication[1]. Gemcitabine was compared to 5-fluorouracil (5-FU) in a randomized phase Ⅲ trial of 126 patients diagnosed with advanced pancreatic cancer. Treatment efficacy was analyzed using clinical benefit response, consisting of pain evaluation, Karnofsky performance status and weight. Clinical benefit rate and median survival were superior among patients treated with gemcitabine as compared with 5-FU (23.8% *vs* 4.8%, $P = 0.0022$; 5.65 mo *vs* 4.41 mo, respectively)[15]. Thereafter, gemcitabine has been the mainstay of treatment in pancreatic cancer. The general side effects of treatment, including fever, infection and elevation of liver enzymes are usually transient and easily manageable. Hemolytic-uremic syndrome is a rare, serious side effect, which can be fatal[16].

Gemcitabine combinations

Gemcitabine combined with either 5-FU, cisplatin, oxaliplatin, or capecitabine has been studied in several trials, but no statistically significant survival advantage has been shown in pre-nab-paclitaxel-era[17-21]. A randomized phase Ⅲ study reported by Cunningham and colleagues, showed higher response rate and progression-free survival for the combination treatment as well as a trend for superior overall survival. However, in a meta-analysis a survival benefit could be reached[22].

Combination chemotherapy without gemcitabine

The PRODIGE group trial randomized 342 patients with good performance status (Zubrovsky 0/1) diagnosed with metastatic pancreatic cancer to receive either a combination of oxaliplatin, irinotecan, leucovorin, 5-FU bolus and 5-FU continuous infusion (FOLFIRINOX) or single gemcitabine. FOLFIRINOX treatment was associated with a statistically superior overall survival as compared to gemcitabine (11.1 mo *vs* 6.8 mo, HR = 0.47, $P < 0.001$). Combined treatment was, however, associated with a higher incidence of grade 3-4 side effects, including neutropenia, febrile neutropenia, thrombocytopenia, diarrhoea and sensory neuropathy[23]. Hence, treatment-related toxicity has limited the use of FOLFIRINOX in everyday clinical practice in full doses.

Nab-paclitaxel-gemcitabine

Nab-paclitaxel is a nanoparticle albumin-bound chemotherapeutic agent, which has synergistic effects with gemcitabine[24]. MPACT-study randomized 342 patients with metastatic pancreatic cancer to receive nab-paclitaxel plus gemcitabine or gemcitabine alone. This study showed the combination treatment to improve median overall survival (8.5 mo *vs* 6.7 mo, $P = 0.000015$)[25], although the survival difference was more modest than expected on the basis of the previous phase Ⅱ trial (12.2 mo)[24]. The side effects of treatment included fatigue, febrile neutropenia and reversible sensory neuropathy. However, treatment effect in the majority of pre-specified subgroups favoured the combination treatment arm. Moreover, even patients with less favourable disease features, including performance status 2, benefited from treatment[25].

Targeted therapy

The addition of bevacizumab or cetuximab to gem-citabine has not shown improvement in survival among patients with pancreatic cancer[26-30].

Erlotinib is an oral tyrosine kinase inhibitor that blocks the activity of human epidermal growth factor receptor type 1 (HER1/EGFR)[30]. The combination of erlotinib and gemcitabine was compared to gemcitabine alone among 569 patients with advanced pancreatic cancer in a phase Ⅲ trial[31]. Overall survival was significantly longer in the combined treatment arm than gemcitabine alone arm (6.24 mo *vs* 5.91 mo, $P = 0.038$). Patients in the combination arm had higher incidence of skin rash, infection, diarrhoea, stomatitis and interstitial pneumonitis. Patients with grade 2 skin rash benefited from the combined treatment, as compared with those who developed no rash[31]. Erlotinib is the only targeted therapy shown to improve

Table 1 Phase Ⅲ trials of combined treatment showing statistically significant survival benefit in metastatic pancreatic cancer

Ref.	Primary endpoint	Treatment arms	No. of patients	OS (mo)
Moore *et al*[31]	OS	Gemcitabine + erlotinib *vs* gemcitabine	569	6.24 *vs* 5.91 HR = 0.82 CI: 0.69-0.99 *P* = 0.038
Cunningham *et al*[22]	OS	Gemcitabine + capecitabine *vs* gemcitabine	533	7.1 *vs* 6.2 HR = 0.86 CI: 0.72-1.02 *P* = 0.08
			Meta-analysis 935	OS NA HR = 0.86 CI: 0.75-0.98 *P* = 0.02
Conroy *et al*[23]	OS	FOLFIRINOX *vs* gemcitabine	342	11.1 *vs* 6.8 HR = 0.57 CI: 0.45-0.73 *P* < 0.0001
Von Hoff *et al*[25]	OS	Nab-paclitaxel + gemcitabine *vs* gemcitabine + placebo	861	8.5 *vs* 6.7 HR = 0.77 CI: 0.62-0.83 *P* < 0.0001

OS: Overall survival; NA: Not available.

survival so far, albeit the prolongation of life was only 2 wk.

HOW TO CHOOSE? WHAT ABOUT SECOND LINE?

What is comforting enough, we now have choices for treatment. Phase Ⅲ trials showing survival benefit of combined treatment are displayed in Table 1. Gemcitabine-nab-paclitaxel combination and FOLFIRINOX have provided the longest survival benefit in pancreatic cancer. These two treatment modalities have not yet been compared in head-to-head-studies. Both options are valid. Nab-paclitaxel combined to gemcitabine is relatively well tolerated, even though it is associated with increased risk of, *e.g.*, infection and sensory neuropathy. The latter is transient and subsides rapidly after cessation of treatment. However, the original FOLFIRINOX treatment carries an increased risk of side effects and thereby is only suitable for patients with a very good performance status. Hence, when used, it has generally been delivered with reduced doses. Gemcitabine alone or in combination with erlotinib are still options for some patients. All patients are not eligible to combined treatments; some patients have a widely advanced disease and are not candidates for any form of chemotherapy. Whichever treatment is chosen in first-line, its efficacy lasts 4-5 mo at most. Some patients may benefit from second-line treatment and even in subsequent lines. Most often, an oxaliplatin-based regimen is chosen, if not used in first-line, although no data from randomized phase Ⅲ trials are available[32]. All patients should receive treatment for their symptoms and psychological support as needed.

CONCLUSION

The basis for taking care of a patient with a highly malignant incurable disease rests on a good patient-physician interaction. The patient needs to know where he stands, in order to form an opinion how he wants to proceed. Hope is at least as crucial as honesty. It is important for the patient to know what can be done to help him, rather than what cannot. The symptoms can usually be controlled at least to some extent; bile obstruction managed with a stent, and importantly, pain alleviated with the help of medication or special techniques. Even though some patients are not fit for active chemotherapeutic treatment, some do gain benefit from therapy and a few live considerably longer than average. In my opinion, every person has a right to know the basic facts of his disease, have his questions answered (if there is an answer) and have a chance to participate in deciding, how he is going to spend probably the last weeks or months of his life. Especially, the patient needs time to think and discuss with family and friends, before returning to possible treatment options and details or referral to symptomatic care.

REFERENCES

1 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]
2 **Greer JB**, Brand RE. Screening for pancreatic cancer: current evidence and future directions. *Gastroenterol Hepatol* (N Y) 2007; **3**: 929-938 [PMID: 21960811]
3 **Ryan DP**, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med* 2014; **371**: 1039-1049 [PMID: 25207767 DOI: 10.1056/NEJMra1404198]
4 **Hariharan D**, Saied A, Kocher HM. Analysis of mortality rates for pancreatic cancer across the world. *HPB* (Oxford) 2008; **10**: 58-62 [PMID: 18695761 DOI: 10.1080/13651820701883148]
5 Finnish Cancer Registry. 26.1.2015 Available from: URL: http://www.cancer.fi/syoparekisteri/en/statistics/newest-survival-ratios/
6 **Chao YJ**, Sy ED, Hsu HP, Shan YS. Predictors for resectability and survival in locally advanced pancreatic cancer after gemcitabine-based neoadjuvant therapy. *BMC Surg* 2014; **14**: 72 [PMID: 25258022 DOI: 10.1186/1471-2482-14-72]
7 **Shaib Y**, Davila J, Naumann C, El-Serag H. The impact of curative intent surgery on the survival of pancreatic cancer patients: a U.S. Population-based study. *Am J Gastroenterol* 2007; **102**: 1377-1382 [PMID: 17403071]
8 **Zuckerman DS**, Ryan DP. Adjuvant therapy for pancreatic cancer: a review. *Cancer* 2008; **112**: 243-249 [PMID: 18050292 DOI: 10.1002/cncr.23174]
9 **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]

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

11 **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]

12 **Chu GC**, Kimmelman AC, Hezel AF, DePinho RA. Stromal biology of pancreatic cancer. *J Cell Biochem* 2007; **101**: 887-907 [PMID: 17266048 DOI: 10.1002/jcb.21209]

13 **Feig C**, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. *Clin Cancer Res* 2012; **18**: 4266-4276 [PMID: 22896693 DOI: 10.1158/1078-0432.CCR-11-3114]

14 **Le A**, Rajeshkumar NV, Maitra A, Dang CV. Conceptual framework for cutting the pancreatic cancer fuel supply. *Clin Cancer Res* 2012; **18**: 4285-4290 [PMID: 22896695]

15 **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]

16 **Fung MC**, Storniolo AM, Nguyen B, Arning M, Brookfield W, Vigil J. A review of hemolytic uremic syndrome in patients treated with gemcitabine therapy. *Cancer* 1999; **85**: 2023-2032 [PMID: 10223245 DOI: 10.1002/(SICI)1097-0142]

17 **Berlin JD**, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson AB. Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* 2002; **20**: 3270-3275 [PMID: 12149301 DOI: 10.1200/JCO.2002.11.149]

18 **Heinemann V**, Quietzsch D, Gieseler F, Gonnermann M, Schönekäs H, Rost A, Neuhaus H, Haag C, Clemens M, Heinrich B, Vehling-Kaiser U, Fuchs M, Fleckenstein D, Gesierich W, Uthgenannt D, Einsele H, Holstege A, Hinke A, Schalhorn A, Wilkowski R. Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. *J Clin Oncol* 2006; **24**: 3946-3952 [PMID: 16921047 DOI: 10.1200/JCO.2009.25.4433]

19 **Louvet C**, Labianca R, Hammel P, Lledo G, Zampino MG, André T, Zaniboni A, Ducreux M, Aitini E, Taïeb J, Faroux R, Lepere C, de Gramont A. Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial. *J Clin Oncol* 2005; **23**: 3509-3516 [PMID: 15908661 DOI: 10.1200/JCO.2005.06.023]

20 **Scheithauer W**, Schüll B, Ulrich-Pur H, Schmid K, Raderer M, Haider K, Kwasny W, Depisch D, Schneeweiss B, Lang F, Kornek GV. Biweekly high-dose gemcitabine alone or in combination with capecitabine in patients with metastatic pancreatic adenocarcinoma: a randomized phase II trial. *Ann Oncol* 2003; **14**: 97-104 [PMID: 12488300]

21 **Herrmann R**, Bodoky G, Ruhstaller T, Glimelius B, Bajetta E, Schüller J, Saletti P, Bauer J, Figer A, Pestalozzi B, Köhne CH, Mingrone W, Stemmer SM, Tàmas K, Kornek GV, Koeberle D, Cina S, Bernhard J, Dietrich D, Scheithauer W. Gemcitabine plus capecitabine compared with gemcitabine alone in advanced pancreatic cancer: a randomized, multicenter, phase III trial of the Swiss Group for Clinical Cancer Research and the Central European Cooperative Oncology Group. *J Clin Oncol* 2007; **25**: 2212-2217 [PMID: 17538165 DOI: 10.1200/JCO.2006.09.0886]

22 **Cunningham D**, Chau I, Stocken DD, Valle JW, Smith D, Steward W, Harper PG, Dunn J, Tudur-Smith C, West J, Falk S, Crellin A, Adab F, Thompson J, Leonard P, Ostrowski J, Eatock M, Scheithauer W, Herrmann R, Neoptolemos JP. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2009; **27**: 5513-5518 [PMID: 19858379 DOI: 10.1200/JCO.2009.24.2446]

23 **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]

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

25 **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]

26 **Kindler HL**, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, Picus J, Bhargava P, Mayer RJ, Schilsky RL, Goldberg RM. Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J Clin Oncol* 2010; **28**: 3617-3622 [PMID: 20606091 DOI: 10.1200/JCO.2010.28.1386]

27 **Van Cutsem E**, Vervenne WL, Bennouna J, Humblet Y, Gill S, Van Laethem JL, Verslype C, Scheithauer W, Shang A, Cosaert J, Moore MJ. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**: 2231-2237 [PMID: 19307500 DOI: 10.1200/JCO.2008.20.0238]

28 **Philip PA**, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, Khorana AA, Goldman B, Fenoglio-Preiser CM, Abbruzzese JL, Blanke CD. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. *J Clin Oncol* 2010; **28**: 3605-3610 [PMID: 20606093 DOI: 10.1200/JCO.2009.25.7550]

29 **Cascinu S**, Berardi R, Labianca R, Siena S, Falcone A, Aitini E, Barni S, Di Costanzo F, Dapretto E, Tonini G, Pierantoni C, Artale S, Rota S, Floriani I, Scartozzi M, Zaniboni A. Cetuximab plus gemcitabine and cisplatin compared with gemcitabine and cisplatin alone in patients with advanced pancreatic cancer: a randomised, multicentre, phase II trial. *Lancet Oncol* 2008; **9**: 39-44 [PMID: 18077217 DOI: 10.1016/S1470-2045(07)70383-2]

30 **Xiong HQ**, Abbruzzese JL. Epidermal growth factor receptor-targeted therapy for pancreatic cancer. *Semin Oncol* 2002; **29**: 31-37 [PMID: 12422311 DOI: 10.1200/JCO.2004.12.040]

31 **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]

32 **Seufferlein T**, Bachet JB, Van Cutsem E, Rougier P. Pancreatic adenocarcinoma: ESMO-ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23** Suppl 7: vii33-vii40 [PMID: 22997452 DOI: 10.1093/annonc/mds224]

P- Reviewer: Delitala AP, Mizuno N, Plaza MA, Yip-Schneider MT
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10714

World J Gastroenterol 2015 October 14; 21(38): 10714-10720
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis B virus

Hepatitis B virus infection in Indonesia

Yoshihiko Yano, Takako Utsumi, Maria Inge Lusida, Yoshitake Hayashi

Yoshihiko Yano, Takako Utsumi, Yoshitake Hayashi, Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan

Takako Utsumi, Maria Inge Lusida, Indonesia-Japan Collaborative Research Center for Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Airlangga University, Surabaya 60115, Indonesia

Author contributions: All the authors made a substantial contribution to the conception and design of the study, data acquisition, and to drafting and critically revising the manuscript; the authors approved the final version.

Supported by Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) program from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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

Correspondence to: Takako Utsumi, PhD, Center for Infectious Diseases, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. tutsumi@people.kobe-u.ac.jp
Telephone: +81-78-3825700
Fax: +81-78-3825719

Received: April 28, 2015
Peer-review started: May 6, 2015
First decision: June 23, 2015
Revised: July 14, 2015
Accepted: September 15, 2015
Article in press: September 15, 2015
Published online: October 14, 2015

Abstract

Approximately 240 million people are chronically infected with hepatitis B virus (HBV), 75% of whom reside in Asia. Approximately 600000 of infected patients die each year due to HBV-related diseases or hepatocellular carcinoma (HCC). The endemicity of hepatitis surface antigen in Indonesia is intermediate to high with a geographical difference. The risk of HBV infection is high in hemodialysis (HD) patients, men having sex with men, and health care workers. Occult HBV infection has been detected in various groups such as blood donors, HD patients, and HIV-infected individuals and children. The most common HBV subgenotype in Indonesia is B3 followed by C1. Various novel subgenotypes of HBV have been identified throughout Indonesia, with the novel HBV subgenotypes C6-C16 and D6 being successfully isolated. Although a number of HBV subgenotypes have been discovered in Indonesia, genotype-related pathogenicity has not yet been elucidated in detail. Therefore, genotype-related differences in the prognosis of liver disease and their effects on treatments need to be determined. A previous study conducted in Indonesia revealed that hepatic steatosis was associated with disease progression. Pre-S2 mutations and mutations at C1638T and T1753V in HBV/B3 have been associated with advanced liver diseases including HCC. However, drug resistance to lamivudine, which is prominent in Indonesia, remains obscure. Although the number of studies on HBV in Indonesia has been increasing, adequate databases on HBV infection are limited. We herein provided an overview of the epidemiology and clinical characteristics of HBV infection in Indonesia.

Key words: Hepatitis B virus; Epidemiology; Prevention; Clinical characteristics; Indonesia

Core tip: Hepatitis B virus (HBV) infection is an important public concern and its prevalence varies greatly in different parts of the world. The high prevalence of HBV in Indonesia highlights the need to improve prevention and control measures because few evidence-based prevention strategies are currently available. Although studies on HBV in Indonesia are increasing, it is still not fully understood. We herein reviewed epidemiologically important aspects of HBV infection in Indonesia.

Yano Y, Utsumi T, Lusida MI, Hayashi Y. Hepatitis B virus infection in Indonesia. *World J Gastroenterol* 2015; 21(38): 10714-10720 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10714.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10714

INTRODUCTION

Hepatitis B virus (HBV) infection is associated with a diverse range of liver damage including asymptomatic carriers, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Approximately 240 million people are chronically infected with HBV[1], 75% of whom reside in Asia[2]. Approximately 600000 of infected patients die each year of HBV-related diseases or HCC[3]. The prevalence of HBV infection varies according to the geographic region, and is categorized as high ($\geqslant$ 8%), intermediate (2%-7%), or low (< 2%) endemicity. The endemicity of HBV in Indonesia is moderate to high[4,5], ranging from 2.5% to 10% for hepatitis B surface antigen (HBsAg)[2,5,6]. HBV has been classified into at least 9 genotypes (A through H and J) and has been shown to have a distinct geographical distribution[7,8]. The most common HBV subgenotype is HBV B3 (HBV/B3), followed by HBV/C1[9]; however, various novel genotypes have been detected in Indonesia.

Indonesia is the largest archipelago in the world, consisting of five major islands and approximately 30 smaller groups. The archipelago is located between two oceans, the Pacific and Indian oceans, and bridges two continents, Asia and Australia. This strategic position has influenced the serological and virological aspects of HBV infection (Figure 1). Although the number of studies conducted on HBV in Indonesia has increased, adequate databases on HBV infection are still limited[10]. The epidemiology of HBV remains obscure in Indonesia[11]. Therefore, we herein provided an overview of HBV infection among the Indonesian population.

PREVALENCE OF HBV INFECTION AND RISK FACTORS IN INDONESIA

The prevalence of chronic HBV infection varies greatly in different areas of the world and the prevalence of chronic HBV infection can be categorized as high, intermediate, or low endemicity. Table 1 shows the prevalence of the HBsAg in not only the general population, but also risk groups such as commercial sex workers (CSW) and men having sex with men (MSM). The prevalence of HBV in the general population in Indonesia is higher than that of HCV (2%)[12], with the highest rates being reported in Makassar (7.1%)[13] in Sulawesi Island and the lowest rates being reported in Jakarta (4.0%)[14] in Java Island; however, another study reported that the prevalence of HBV in Jakarta was 5.8% in the general population[15]. Hasan previously reported that the prevalence of HBV infection in the general population was the highest in Pontianak (9.1%) in the Kalimantan Island[5]. Furthermore, the prevalence of HBsAg was markedly higher in habitants in the highland of Papua (12.8%) and North Sulawesi (33.0%)[16]. The prevalence of HBsAg in pregnant women was found to be the same as that in the general population in Indonesia[17,18]. These findings demonstrated that the endemicity of HBsAg among the general population in Indonesia is intermediate to high, as reported previously[2,19].

HBV infection was not detected in children in Tahuna, North Sulawesi, and Surabaya, East Java, suggesting the efficacy of Hepatitis B (HB) vaccinations in pre-school children[11,20].

The highest risk group of HBV infection was previously reported to be hemodialysis (HD) patients (11.2%) in Yogyakarta[12], followed by MSM (9.8%)[21] in Solo in the Java Island (Table 1). The prevalence of HBV/HIV co-infection was found to be higher than that of HBV infection alone in Indonesia[22-24] as well as in neighboring countries such as Vietnam and India. The incidence of HIV and HBV burden are currently increasing in Indonesia[22,23,25]; however, no HBV/HIV co-infection cases have been identified in CSW[26]. The prevalence of HBsAg has been classified as high endemicity (8.8%) in health care workers[27] and intermediate endemicity in staff in HDU (5.7%) throughout Indonesia (Table 1). A previous study also revealed that the prevalence of HBsAg was high among medical employees in Padang (11.2%), Mataram (13.3%), and Irian Jaya (13.3%)[5].

Many unique animals exist in Indonesia because of its specific ecosystem. Gibbons in Kalimantan were previously reported to be infected with HBV having their own genotype[28].

OCCULT HBV INFECTION

Occult HBV infection (OBI) is defined as the presence of HBV DNA in the serum and/or liver tissue of individuals with HBV core antibodies (anti-HBc) without HBsAg[29]. Several studies have been conducted on OBI in Indonesia. OBI was detected in 8.1% of blood donors with amino acid mutations (T123A, M133L, and T143M) in the a determinant of HBsAg, which

Figure 1 Map of Indonesia. Indonesia is the largest archipelago in the world, consisting of five major islands and approximately 30 smaller groups.

Table 1 Prevalence of hepatitis B surface antigen and risk factors in Indonesian populations

Region	Prevalence (%)	Subject	Main genotype	Ref.
Java				
Bandung	4.7	Pregnant women		Reniers *et al*[17], 1987
Jakarta	4.0	General population		Akbar *et al*[14], 1997
Jakarta	5.8	General population		Budihusodo *et al*[15], 1991
Jakarta	2.2	Parturient women		Gunardi *et al*[6], 2014
Surabaya	4.0	CSW		Kotaki *et al*[26], 2013
Solo	9.8	MSM		Prasetyo *et al*[21], 2014
Four prisons in Central Java[1]	3.2	Drug abuser inmates in prisons	B3, C1	Prasetyo *et al*[10], 2013
Yogyakarta	11.2	HD patients	B3	Rinonce *et al*[12], 2013
	5.7	Staff in HDU	B3	
Sumatra				
Padang		HBV carriers	C1, B3	Siburian *et al*[9], 2013
Kalimantan				
Banjarmasin	4.6	General population	B, C	Darmawan *et al*[32], 2015
Sulawesi				
Tahuna	4.9	General population	C5	Achwan *et al*[11], 2007
Makassar	7.1	General population		Amirudin *et al*[13], 1991
Bali[1]	1.9	Pregnant women		Surya *et al*[18], 2005
Papua				
Jayapura	4.6	General population	C6, D6, B3	Lusida *et al*[36], 2008

[1]Exact place is not mentioned. MSM: Men having sex with men; CSW: Commercial sex workers; HD: Hemodialysis; HDU: Hemodialysis unit.

resulted in changes in predicted antigenicity[30]. Several OBI cases were detected among school children with the variant T126I, which may be one of the viral mechanisms helping the virus to escape from current HB vaccines in Indonesia[31]. In Banjarmasin, Kalimantan, OBI was identified in healthy young adults with 13 amino acid substitutions[32]. Awareness of the reactivation of OBI has increased in Indonesia, especially in HBV endemic areas[33]. A total of 27.1% and 14.7% of HIV-infected individuals and HD patients, respectively, were considered to have OBI[12,22], suggesting that the prevalence of HBV infection regardless of HBsAg was high in immunosuppressive patients.

HBV GENOTYPES/SUBGENOTYPES IN INDONESIA

HBV is currently grouped into at least 9 genotypes (A through H and J, with I still being controversial)[34]. The HBV sequence is characterized by more than 8% nucleotide (nt) differences for genotypes and 4%-8% for subgenotypes. The most common HBV subgenotype in Indonesia is HBV/B3, followed by HBV/C1[35,36] (Table 1), with various novel subgenotypes of HBV being identified throughout Indonesia. Ten

novel HBV subgenotypes (HBV/C7-C16) were isolated in Indonesia between 2008 and 2012[37-41]. HBV/C6, HBV/C11, and HBV/D6 were identified in a Papuan population[36,40,42]. Genotype J (HBV/J) was detected in a Japanese patient with HCC who was thought to have been infected in Kalimantan, Indonesia, during World War Ⅱ[28,43]. HBV isolates from subjects from Sulawesi clustered within the HBV/C5, together with known isolates from the Philippines and Vietnam[11]. The distribution of genotypes/subgenotypes varies even in different regions of a country, which may partly be related to the ethnic origin of the infected patients.

PREVENTION

The most significant achievement in the prevention of HB is the implementation of a universal infant vaccination for HB. The HB universal vaccination was introduced in Indonesia in 1997, with the Indonesian government attempting to ensure that every newborn was vaccinated against HBV infection during the first 7 d of life. The immunization project in Lombok decreased the prevalence of HBsAg from 6.2% to 1.4% among children less than 5 years old[44]. In Surabaya and North Sulawesi, the prevalence of HBsAg in preschool children was reported to be 0%[11,20], a result that was attributed to the universal HB vaccination. Although the prevalence of HBsAg among children varies by region, for example, 3.1% in Lamongan in East Java[31] and 4.2% in Papua, the HB vaccination history is obscure. Since the HB vaccination is one of the Expanded Program on Immunization projects being run by the government, communication with the local government is of great importance for better practices.

CHARACTERISTICS OF CHRONIC HBV INFECTION IN INDONESIA

Previous studies revealed that the clinical characteristics of chronic HB (CHB) differed among genotypes, and the prognosis of genotype B was better than that of genotype C[45-47]. However, most studies in Asia were conducted in Taiwan, China, Hong Kong, and Japan. The main subgenotype in Indonesia is HBV/B3, which is different from the subgenotypes HBV/B1 and HBV/B2 mainly analyzed in other Asian countries[48]. Furthermore, HBV/C in Indonesia is mainly HBV/C1 in Java and HBV/C6 in Papua, and is different from HBV/C, which is spreading in East Asian countries such as China and Japan[36,42]. Since most clinical studies conducted in Indonesia involved patients with HBV/B, the clinical course of HBV/C in Indonesia currently remains unclear. Although HCC is prevalent in Indonesia, further studies are needed to determine clinical characteristics in relation to the genotypes in Indonesia.

Recent studies revealed that hepatic steatosis was associated with disease progression[49]. Steatosis was identified as an independent risk factor for HCC and the progression of hepatitis was found to be more rapid in HCV patients with steatosis[50,51]. However, a meta-analysis revealed that hepatic steatosis was not related to the clinical course of HBV patients[52]. A previous study reported that hepatic steatosis was more strongly associated with genotype C (37.9%) than with genotype B (24.0%)[53]. Lesmana *et al*[54] examined 179 CHB patients in Jakarta and found the prevalence of hepatic steatosis to be approximately 30%. Obesity is a serious social issue in Indonesia, as in other countries[55]. However, studies on steatosis in Indonesia are still limited, and, as such, further investigations are warranted.

Although the prevalence of HBV and HCV infections in Southeast Asia including Indonesia is high, clinical studies remain limited. Lamivudine, adefovir, and telbivudine, therapeutic drugs for CHB, are currently covered by health insurance in Indonesia. Although drug resistance to lamivudine has not yet been examined, it is common for the cheapest drug, lamivudine, to be prescribed or antiviral therapy to be discontinued due to economic reasons. Therefore, the prevalence of lamivudine-resistant HBV may increase. Telbivudine, which was recently approved in Indonesia, was found to be effective for Indonesian HBV carriers. Sulaiman *et al*[56] reported that HBeAg loss and the seroconversion rate for HBeAg-positive patients were 28.8% and 14.1% at week 52 of telbivudine therapy, respectively. Furthermore, undetectable HBV DNA (PCR negativity) was 51.8% at week 24 and 62.7% at week 52 of this therapy. However, a large-scale study has not yet been conducted on interferon therapy for CHB and, thus, its effectiveness currently remains unknown.

Previous studies revealed a hepatitis virus co-infection among HIV patients. Anggorowati *et al*[23] examined 126 HIV patients in Yogyakarta city and found that 8% had the HBsAg and were considered to be co-infected with HBV. Utsumi *et al*[22] examined 118 HIV patients in Surabaya City, and reported that 15.3% were HBsAg-positive while 27.1% were positive for HBV-DNA regardless of being HBsAg-negative and were considered to have OBI. These findings suggested that HBV co-infection including OBI was frequent among HIV patients and serological examinations were sometimes insufficient for detecting co-infections because of a compromised immune system. Fibriani *et al*[24] recently examined 616 HIV patients in Bandung city in West Java, found HBV co-infection in approximately 7% of these patients, and identified the clinical characteristics of co-infection as being male and having a history of injection drug use.

HBV infection was examined in HD patients. Rinonce *et al*[12] examined 161 HD patients in Yogyakarta, and revealed that the prevalence of HBsAg positivity was 11.8% and also that the viral

genomes of several strains were identical, suggesting nosocomial infection.

LIVER CANCER IN RELATION TO HBV IN INDONESIA

Cancer-related death is a major public health problem in Indonesia and accounts for the seventh largest cause of death. According to the Jakarta Cancer Registry, the ratio of liver cancer between 2005 and 2007 was 1.4 per 100000 (eleventh place) among women and 4.0 per 100000 (third place) among men[57]. On the other hand, HBV is the most common cause of HCC. Sulaiman[58] and Marwoto *et al*[59] firstly reported in 1985 that the frequency of HBV among HCC was 67.0%, while Sulaiman showed that it was 47.6%. Wang *et al*[60] examined the epidemiology of HCC in Japan, India, China, and Indonesia, and found that the positive prevalences of the HBsAg and HCV antibody were 21.0% and 40.0% in Indonesian patients with liver cancer, respectively. HBV is a well-known oncogenic virus, and previous studies revealed that the Pre-S mutation, X mutation, and BCP-PC mutation were associated with hepatocarcinogenesis[61,62]. The Pre-S1 deletion, Pre-S2 deletion, T53C mutation were found to be related to HCC at the S domain. Utama *et al*[63] examined the prevalence of the Pre-S2 mutation among 268 HBV carriers in Bantan, and showed it was 2.7%, 18.2%, 40.9%, and 28.6% in asymptomatic carriers, chronic hepatitis, liver cirrhosis, and HCC, respectively, indicating that the Pre-S2 mutation was an independent factor of progressive liver disease. A meta-analysis revealed that the X domain including an A1762T/G1764A double mutant, T1753V, C1653T, G1896A, and G1899A were related to HCC[64]. Heriyanto *et al*[35] compared 40 cirrhosis and liver cancer patients with 109 chronic hepatitis patients in Yogyakarta city, and a multivariate analysis identified being older than 45 years old (OR = 2.61, P = 0.034), having a C1638T variation (OR = 1074.57, P = 0.005), and having a T1753V variation (OR = 6.39, P = 0.047) as independent factors participating in disease progression.

HBV INFECTION AND HOST FACTORS

Recent technological advances revealed that various kinds of genetic factors are associated with cancers. The genome-wide association study showed that a large number of single nucleotide polymorphisms (SNPs) were related to various kinds of cancers. In case-control and retrospective studies on liver cancers, numerous candidate genes for SNPs were found to be associated with HCC. In 2009, Kamatani *et al*[65] examined 188 Japanese CHB patients and 934 controls and was the first to show that SNPs in the human leukocyte antigen (HLA)-DP region were associated with chronic HBV carriers. The HLA gene is located in 6p21.3 and plays an important role in antigen presentation. Polymorphisms in this region were also identified not only in Japanese patients, but also in Chinese patients[66,67]. In Indonesian populations, several SNPs including rs3135363 in HLA-DR, rs9277535 in HLA-DP, and rs9267665 in a gene-rich HLA class Ⅲ interval were associated with HB vaccine responses[68]. Host factors are also important for HBV infection and disease progression. Further analyses are needed to confirm these findings.

CONCLUSION

The endemicity of HBsAg in Indonesia is intermediate to high with a geographical difference. HD patients, MSM, and health care workers are at high risk of HBV infection. OBI has also been detected in various groups such as blood donors, HD patients, and HIV-infected individuals and children. Appropriate national immunization programs are required in HBV endemic countries such as Indonesia in order to reduce HBV infection. Although a number of HBV subgenotypes have been discovered in Indonesia, genotype-related pathogenicity has not yet been elucidated in detail. Therefore, genotype-related differences in the prognosis of liver disease and their effects on treatments are eagerly awaited.

REFERENCES

1 HIV/AIDS Programme. Guidance on prevention of viral hepatitis B and C among people WHO inject drugs. WHO, 2012. Available from: URL: http://apps.who.int/iris/bitstream/10665/75357/1/9789241504041_eng.pdf

2 **Merican I**, Guan R, Amarapuka D, Alexander MJ, Chutaputti A, Chien RN, Hasnian SS, Leung N, Lesmana L, Phiet PH, Sjalfoellah Noer HM, Sollano J, Sun HS, Xu DZ. Chronic hepatitis B virus infection in Asian countries. *J Gastroenterol Hepatol* 2000; **15**: 1356-1361 [PMID: 11197043]

3 **Abbas Z**, Siddiqui AR. Management of hepatitis B in developing countries. *World J Hepatol* 2011; **3**: 292-299 [PMID: 22216369 DOI: 10.4254/wjh.v3.i12.292]

4 **Fitria L**, Gunardi H, Akib AAP. Influence of hepatitis B immunization to prevent vertical transmission of Hep-B virus in infants born from Hep-B positive mother. *Paediatr Indones* 2010; **50**: 321-325

5 **Hasan I**. Epidemiology of hepatitis B. *Acta Med Indones* 2005; **37**: 231-234 [PMID: 16317222]

6 **Gunardi H**, Zaimi LF, Soedjatmiko AR, Muljono DH. Current prevalence of hepatitis B infection among parturient women in Jakarta, Indonesia. *Acta Med Indones* 2014; **46**: 3-9 [PMID: 24760802]

7 **Norder H**, Couroucé AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; **47**: 289-309 [PMID: 15564741]

8 **Locarnini S**, Littlejohn M, Aziz MN, Yuen L. Possible origins and evolution of the hepatitis B virus (HBV). *Semin Cancer Biol* 2013; **23**: 561-575 [PMID: 24013024 DOI: 10.1016/j.semcancer.2013.08.006]

9 **Siburian MD**, Utama A, Dhenni R, Arnelis N, Fanany I, Intan MD, Kurniasih TS, Andriani F, Afadlal S, Julianto EB, Rasman WS, Zubir N, Mathew G. High prevalence of hepatitis B virus genotype

C/C1 in the Minangkabau ethnic group in Indonesia. *Virol J* 2013; **10**: 27 [PMID: 23336976 DOI: 10.1186/1743-422X-10-27]

10 **Prasetyo AA**, Dirgahayu P, Sari Y, Hudiyono H, Kageyama S. Molecular epidemiology of HIV, HBV, HCV, and HTLV-1/2 in drug abuser inmates in central Javan prisons, Indonesia. *J Infect Dev Ctries* 2013; **7**: 453-467 [PMID: 23771289 DOI: 10.3855/jidc.2965]

11 **Achwan WA**, Muttaqin Z, Zakaria E, Depamede SA, Mulyanto S, Tsuda F, Takahashi K, Abe N, Mishiro S. Epidemiology of hepatitis B, C, and E viruses and human immunodeficiency virus infections in Tahuna, Sangihe-Talaud Archipelago, Indonesia. *Intervirology* 2007; **50**: 408-411 [PMID: 18185013 DOI: 10.1159/000112915]

12 **Rinonce HT**, Yano Y, Utsumi T, Heriyanto DS, Anggorowati N, Widasari DI, Lusida MI, Soetjipto H, Hotta H, Hayashi Y. Hepatitis B and C virus infection among hemodialysis patients in Yogyakarta, Indonesia: Prevalence and molecular evidence for nosocomial transmission. *J Med Virol* 2013; **85**: 1348-1361 [PMID: 23919229]

13 **Amirudin R**, Akil H, Akahane Y, Suzuki H. Hepatitis B and C virus infection in Ujung Pandang, Indonesia. *Gastroenterol Jpn* 1991; **26** Suppl 3: 184-188 [PMID: 1909264]

14 **Akbar N**, Basuki B, Mulyanto DH, Sulaiman A, Noer HM. Ethnicity, socioeconomic status, transfusions and risk of hepatitis B and hepatitis C infection. *J Gastroenterol Hepatol* 1997; **12**: 752-757 [PMID: 9430042]

15 **Budihusodo U**, Sulaiman HA, Akbar HN, Lesmana LA, Waspodo AS, Noer HM, Akahane Y, Suzuki H. Seroepidemiology of HBV and HCV infection in Jakarta, Indonesia. *Gastroenterol Jpn* 1991; **26**: 196-201 [PMID: 1909267]

16 **Wolff AP**, Ruys AH, Dolmans WM, Van Loon AM, Pangalila PF. Hepatitis B virus infection in patients with chronic liver disease and healthy controls in north-Sulawesi, Indonesia. *Trop Geogr Med* 1990; **42**: 221-225 [PMID: 2293430]

17 **Reniers J**, Vranckx R, Ngantung W, Sugita E, Meheus A. Prevalence and determinants of hepatitis B virus markers in pregnant women in West Java, Indonesia. *J Trop Med Hyg* 1987; **90**: 249-253 [PMID: 3669127]

18 **Surya IG**, Kornia K, Suwardewa TG, Mulyanto F, Mishiro S. Serological markers of hepatitis B, C, and E viruses and human immunodeficiency virus type-1 infections in pregnant women in Bali, Indonesia. *J Med Virol* 2005; **75**: 499-503 [PMID: 15714491]

19 **Creati M**, Saleh A, Ruff TA, Stewart T, Otto B, Sutanto A, Clements CJ. Implementing the birth dose of hepatitis B vaccine in rural Indonesia. *Vaccine* 2007; **25**: 5985-5993 [PMID: 17604881]

20 **Utsumi T**, Lusida MI, Yano Y, Purwono PB, Amin M, Soetjipto, Hotta H, Hayashi Y. Progress in the Control of Hepatitis B Virus Infection among Children in Indonesia. *J Vaccines Vaccin* 2014; **5**: 247

21 **Prasetyo AA**, Ariapramuda R, Kindi EA, Dirgahayu P, Sari Y, Dharmawan R, Kageyama S. Men having sex with men in Surakarta, Indonesia: demographics, behavioral characteristics and prevalence of blood borne pathogens. *Southeast Asian J Trop Med Public Health* 2014; **45**: 1032-1047 [PMID: 25507232]

22 **Utsumi T**, Yano Y, Lusida MI, Nasronudin M, Juniastuti H, Hayashi Y. Detection of highly prevalent hepatitis B virus co-infection with HIV in Indonesia. *Hepatol Res* 2013; **43**: 1032-1039 [PMID: 23336705 DOI: 10.1111/hepr.12053]

23 **Anggorowati N**, Yano Y, Heriyanto DS, Rinonce HT, Utsumi T, Mulya DP, Subronto YW, Hayashi Y. Clinical and virological characteristics of hepatitis B or C virus co-infection with HIV in Indonesian patients. *J Med Virol* 2012; **84**: 857-865 [PMID: 22499006 DOI: 10.1002/jmv.23293]

24 **Fibriani A**, Wisaksana R, Alisjahbana B, Indrati A, Schutten M, van Crevel R, van der Ven A, Boucher CA. Hepatitis B virus prevalence, risk factors and genotype distribution in HIV infected patients from West Java, Indonesia. *J Clin Virol* 2014; **59**: 235-241 [PMID: 24529845 DOI: 10.1016/j.jcv.2014.01.012]

25 **Zaw SK**, Tun ST, Thida A, Aung TK, Maung W, Shwe M, Aye MM, Clevenbergh P. Prevalence of hepatitis C and B virus among patients infected with HIV: a cross-sectional analysis of a large HIV care programme in Myanmar. *Trop Doct* 2013; **43**: 113-115 [PMID: 23800421 DOI: 10.1177/0049475513493416]

26 **Kotaki T**, Khairunisa SQ, Sukartiningrum SD, Arfijanto MV, Utsumi T, Normalina I, Handajani R, Widiyanti P, Rusli M, Rahayu RP, Lusida MI, Hayashi Y, Nasronudin M. High prevalence of HIV-1 CRF01_AE viruses among female commercial sex workers residing in Surabaya, Indonesia. *PLoS One* 2013; **8**: e82645 [PMID: 24367533 DOI: 10.1371/journal.pone.0082645]

27 **Agustian D**, Yusnita S, Susanto H, Sukandar H, de Schryver A, Meheus A. An estimation of the occupational risk of HBV, HCV and HIV infection among Indonesian health-care workers. *Acta Med Indones* 2009; **41** Suppl 1: 33-37 [PMID: 19920296]

28 **Utsumi T**, Wahyuni RM, Lusida MI, Yano Y, Priambada NP, Amin M, Purwono PB, Istimagfiroh A, Soetjipto A, Hotta H, Hayashi Y. Full genome characterization and phylogenetic analysis of hepatitis B virus in gibbons and a caretaker in Central Kalimantan, Indonesia. *Arch Virol* 2015; **160**: 685-692 [PMID: 25559671 DOI: 10.1007/s00705-014-2323-9]

29 **Hu KQ**. Occult hepatitis B virus infection and its clinical implications. *J Viral Hepat* 2002; **9**: 243-257 [PMID: 12081601]

30 **Thedja MD**, Roni M, Harahap AR, Siregar NC, Ie SI, Muljono DH. Occult hepatitis B in blood donors in Indonesia: altered antigenicity of the hepatitis B virus surface protein. *Hepatol Int* 2010; **4**: 608-614 [PMID: 21063484 DOI: 10.1007/s12072-010-9203-5]

31 **Utsumi T**, Yano Y, Lusida MI, Amin M, Soetjipto H, Hayashi Y. Serologic and molecular characteristics of hepatitis B virus among school children in East Java, Indonesia. *Am J Trop Med Hyg* 2010; **83**: 189-193 [PMID: 20595500 DOI: 10.4269/ajtmh.2010.09-0589]

32 **Darmawan E**, Turyadi KE, Nursanty NK, Thedja MD, Muljono DH. Seroepidemiology and occult hepatitis B virus infection in young adults in Banjarmasin, Indonesia. *J Med Virol* 2015; **87**: 199-207 [PMID: 25521058 DOI: 10.1002/jmv.24045]

33 **Wijaya I**, Hasan I. Reactivation of hepatitis B virus associated with chemotherapy and immunosuppressive agent. *Acta Med Indones* 2013; **45**: 61-66 [PMID: 23585411]

34 **Utsumi T**, Yano Y, Hotta H. Molecular epidemiology of hepatitis B virus in Asia. *World J Med Genet* 2014; **4**: 19-26 [DOI: 10.5496/wjmg.v4.i2.19]

35 **Heriyanto DS**, Yano Y, Utsumi T, Anggorowati N, Rinonce HT, Lusida MI, Soetjipto C, Ratnasari N, Maduseno S, Purnama PB, Nurdjanah S, Hayashi Y. Mutations within enhancer II and BCP regions of hepatitis B virus in relation to advanced liver diseases in patients infected with subgenotype B3 in Indonesia. *J Med Virol* 2012; **84**: 44-51 [PMID: 22095534 DOI: 10.1002/jmv.22266]

36 **Lusida MI**, Nugrahaputra VE, Soetjipto R, Nagano-Fujii M, Sasayama M, Utsumi T, Hotta H. Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. *J Clin Microbiol* 2008; **46**: 2160-2166 [PMID: 18463220 DOI: 10.1128/JCM.01681-07]

37 **Mulyanto SN**, Surayah K, Tsuda F, Ichiyama K, Takahashi M, Okamoto H. A nationwide molecular epidemiological study on hepatitis B virus in Indonesia: identification of two novel subgenotypes, B8 and C7. *Arch Virol* 2009; **154**: 1047-1059 [PMID: 19499283 DOI: 10.1007/s00705-009-0406-9]

38 **Mulyanto SN**, Surayah K, Tjahyono AA, Jirintai S, Takahashi M, Okamoto H. Identification and characterization of novel hepatitis B virus subgenotype C10 in Nusa Tenggara, Indonesia. *Arch Virol* 2010; **155**: 705-715 [PMID: 20306210 DOI: 10.1007/s00705-010-0628-x]

39 **Mulyanto SN**, Wahyono A, Jirintai S, Takahashi M, Okamoto H. Analysis of the full-length genomes of novel hepatitis B virus subgenotypes C11 and C12 in Papua, Indonesia. *J Med Virol* 2011; **83**: 54-64 [PMID: 21108339 DOI: 10.1002/jmv.21931]

40 **Utsumi T**, Nugrahaputra VE, Amin M, Hayashi Y, Hotta H, Lusida MI. Another novel subgenotype of hepatitis B virus genotype C from papuans of Highland origin. *J Med Virol* 2011; **83**: 225-234 [PMID: 21181916 DOI: 10.1002/jmv.21963]

41 **Mulyanto P**, Depamede SN, Wahyono A, Jirintai S, Nagashima S, Takahashi M, Nishizawa T, Okamoto H. Identification of

four novel subgenotypes (C13-C16) and two inter-genotypic recombinants (C12/G and C13/B3) of hepatitis B virus in Papua province, Indonesia. *Virus Res* 2012; **163**: 129-140 [PMID: 21925554 DOI: 10.1016/j.virusres.2011.09.002]

42 **Utsumi T**, Lusida MI, Yano Y, Nugrahaputra VE, Amin M, Juniastuti Y, Hotta H. Complete genome sequence and phylogenetic relatedness of hepatitis B virus isolates in Papua, Indonesia. *J Clin Microbiol* 2009; **47**: 1842-1847 [PMID: 19386834 DOI: 10.1128/JCM.02328-08]

43 **Tatematsu K**, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *J Virol* 2009; **83**: 10538-10547 [PMID: 19640977 DOI: 10.1128/JVI.00462-09]

44 **Ruff TA**, Gertig DM, Otto BF, Gust ID, Sutanto A, Soewarso TI, Kandun N, Marschner IC, Maynard JE. Lombok Hepatitis B Model Immunization Project: toward universal infant hepatitis B immunization in Indonesia. *J Infect Dis* 1995; **171**: 290-296 [PMID: 7844364]

45 **Lee CM**, Chen CH, Lu SN, Tung HD, Chou WJ, Wang JH, Chen TM, Hung CH, Huang CC, Chen WJ. Prevalence and clinical implications of hepatitis B virus genotypes in southern Taiwan. *Scand J Gastroenterol* 2003; **38**: 95-101 [PMID: 12608471]

46 **Orito E**, Mizokami M, Sakugawa H, Michitaka K, Ishikawa K, Ichida T, Okanoue T, Yotsuyanagi H, Iino S. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. *Hepatology* 2001; **33**: 218-223 [PMID: 11124839]

47 **Zhang AM**, Wang HF, Wang HB, Hu JH, He WP, Su HB, Chen J, Du N, Duan XZ. [Association between HBV genotype and chronic/severe liver disease with HBV infection in Chinese patients]. *Zhonghua Shiyan He Linchuang Bingduxue Zazhi* 2010; **24**: 178-180 [PMID: 21186519]

48 **Utama A**, Purwantomo S, Siburian MD, Dhenni R, Gani RA, Hasan I, Sanityoso A, Miskad UA, Akil F, Yusuf I, Achwan WA, Soemohardjo S, Lelosutan SA, Martamala R, Lukito B, Budihusodo U, Lesmana LA, Sulaiman A, Tai S. Hepatitis B virus subgenotypes and basal core promoter mutations in Indonesia. *World J Gastroenterol* 2009; **15**: 4028-4036 [PMID: 19705499]

49 **Petta S**, Cammà C, Di Marco V, Macaluso FS, Maida M, Pizzolanti G, Belmonte B, Cabibi D, Di Stefano R, Ferraro D, Guarnotta C, Venezia G, Craxì A. Hepatic steatosis and insulin resistance are associated with severe fibrosis in patients with chronic hepatitis caused by HBV or HCV infection. *Liver Int* 2011; **31**: 507-515 [PMID: 21382161 DOI: 10.1111/j.1478-3231.2011.02453.x]

50 **Koike K**. Steatosis, liver injury, and hepatocarcinogenesis in hepatitis C viral infection. *J Gastroenterol* 2009; **44** Suppl 19: 82-88 [PMID: 19148799 DOI: 10.1007/s00535-008-2276-4]

51 **Takuma Y**, Nouso K, Makino Y, Saito S, Takayama H, Takahara M, Takahashi H, Murakami I, Takeuchi H. Hepatic steatosis correlates with the postoperative recurrence of hepatitis C virus-associated hepatocellular carcinoma. *Liver Int* 2007; **27**: 620-626 [PMID: 17498246]

52 **Machado MV**, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. *J Gastroenterol Hepatol* 2011; **26**: 1361-1367 [PMID: 21649726 DOI: 10.1111/j.1440-1746.2011.06801.x]

53 **Lin CL**, Kao JH. The clinical implications of hepatitis B virus genotype: Recent advances. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 123-130 [PMID: 21199523]

54 **Lesmana LA**, Lesmana CR, Pakasi LS, Krisnuhoni E. Prevalence of hepatic steatosis in chronic hepatitis B patients and its association with disease severity. *Acta Med Indones* 2012; **44**: 35-39 [PMID: 22451183]

55 **Roemling C**, Qaim M. Obesity trends and determinants in Indonesia. *Appetite* 2012; **58**: 1005-1013 [PMID: 22402303 DOI: 10.1016/j.appet.2012.02.053]

56 **Sulaiman A**, Lesmana LA. An observational study to evaluate the safety and efficacy of telbivudine in adults with chronic hepatitis B. *Acta Med Indones* 2014; **46**: 38-43 [PMID: 24760807]

57 **Wahidin M**, Noviani R, Hermawan S, Andriani V, Ardian A, Djarir H. Population-based cancer registration in Indonesia. *Asian Pac J Cancer Prev* 2012; **13**: 1709-1710 [PMID: 22799393]

58 **Sulaiman HA**. Hepatitis B virus infection in liver cirrhosis and hepatocellular carcinoma in Jakarta Indonesia. *Gastroenterol Jpn* 1989; **24**: 434-441 [PMID: 2550307]

59 **Marwoto W**, Diana S, Roostini ES. Epidemiology of liver cancer in Indonesia. *Southeast Asian J Trop Med Public Health* 1985; **16**: 607-608 [PMID: 3012789]

60 **Wang BE**, Ma WM, Sulaiman A, Noer S, Sumoharjo S, Sumarsidi D, Tandon BN, Nakao K, Mishiro S, Miyakawa Y, Akahane Y, Suzuki H. Demographic, clinical, and virological characteristics of hepatocellular carcinoma in Asia: survey of 414 patients from four countries. *J Med Virol* 2002; **67**: 394-400 [PMID: 12116033]

61 **Qu LS**, Liu JX, Liu TT, Shen XZ, Chen TY, Ni ZP, Lu CH. Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in Qidong, China. *PLoS One* 2014; **9**: e98257 [PMID: 24849936 DOI: 10.1371/journal.pone.0098257]

62 **Liao Y**, Hu X, Chen J, Cai B, Tang J, Ying B, Wang H, Wang L. Precore mutation of hepatitis B virus may contribute to hepatocellular carcinoma risk: evidence from an updated meta-analysis. *PLoS One* 2012; **7**: e38394 [PMID: 22675557 DOI: 10.1371/journal.pone.0038394]

63 **Utama A**, Siburian MD, Fanany I, Intan MD, Dhenni R, Kurniasih TS, Lelosutan SA, Achwan WA, Zubir N, Arnelis B, Yusuf I, Lesmana LA, Sulaiman A. Hepatitis B virus pre-S2 start codon mutations in Indonesian liver disease patients. *World J Gastroenterol* 2012; **18**: 5418-5426 [PMID: 23082059 DOI: 10.3748/wjg.v18.i38.5418]

64 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082 [PMID: 19574418 DOI: 10.1093/jnci/djp180]

65 **Kamatani Y**, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, Hosono N, Kubo M, Tsunoda T, Kamatani N, Kumada H, Puseenam A, Sura T, Daigo Y, Chayama K, Chantratita W, Nakamura Y, Matsuda K. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. *Nat Genet* 2009; **41**: 591-595 [PMID: 19349983 DOI: 10.1038/ng.348]

66 **Guo X**, Zhang Y, Li J, Ma J, Wei Z, Tan W, O'Brien SJ. Strong influence of human leukocyte antigen (HLA)-DP gene variants on development of persistent chronic hepatitis B virus carriers in the Han Chinese population. *Hepatology* 2011; **53**: 422-428 [PMID: 21274863 DOI: 10.1002/hep.24048]

67 **Hu L**, Zhai X, Liu J, Chu M, Pan S, Jiang J, Zhang Y, Wang H, Chen J, Shen H, Hu Z. Genetic variants in human leukocyte antigen/DP-DQ influence both hepatitis B virus clearance and hepatocellular carcinoma development. *Hepatology* 2012; **55**: 1426-1431 [PMID: 22105689 DOI: 10.1002/hep.24799]

68 **Png E**, Thalamuthu A, Ong RT, Snippe H, Boland GJ, Seielstad M. A genome-wide association study of hepatitis B vaccine response in an Indonesian population reveals multiple independent risk variants in the HLA region. *Hum Mol Genet* 2011; **20**: 3893-3898 [PMID: 21764829 DOI: 10.1093/hmg/ddr302]

P- Reviewer: Blum HE, Chen RF **S- Editor**: Yu J **L- Editor**: A
E- Editor: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10721

World J Gastroenterol 2015 October 14; 21(38): 10721-10731
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis B virus

Restoring homeostasis of CD4$^+$ T cells in hepatitis-B-virus-related liver fibrosis

Li-Sha Cheng, Yun Liu, Wei Jiang

Li-Sha Cheng, Yun Liu, Wei Jiang, Department of Gastroenterology, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Author contributions: Cheng LS and Liu Y acquired data; Cheng LS drafted the manuscript; Jiang W critically revised the manuscript for important intellectual content.

Supported by The National Natural Science Foundation of China, No. 81070341 and No. 81270517.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest in this study.

Correspondence to: Wei Jiang, Professor, Department of Gastroenterology, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai 200032, China. jiang.wei@zs-hospital.sh.cn
Telephone: +86-21-64041990-2424
Fax: +86-21-64432583

Received: April 26, 2015
Peer-review started: April 28, 2015
First decision: June 2, 2015
Revised: June 19, 2015
Accepted: September 2, 2015
Article in press: September 2, 2015
Published online: October 14, 2015

Abstract

Immune-mediated liver injury is widely seen during hepatitis B virus (HBV) infection. Unsuccessful immune clearance of HBV results in chronic hepatitis and increases the risk of liver cirrhosis and hepatocellular carcinoma. HBV-related liver fibrosis (HBVLF), occurring as a result of HBV-induced chronic hepatitis, is a reversible, intermediate stage of chronic hepatitis B (CHB) and liver cirrhosis. Therefore, defining the pathogenesis of HBVLF is of practical significance for achieving better clinical outcomes. Recently, the homeostasis of CD4$^+$ T cells was considered to be pivotal in the process of HBVLF. To better uncover the underlying mechanisms, in this review, we systematically retrospect the impacts of different CD4$^+$ T-cell subsets on CHB and HBVLF. We emphasize CD4$^+$ T-cell homeostasis and the important balance between regulatory T (Treg) and T helper 17 (Th17) cells. We discuss some cytokines associated with Treg and Th17 cells such as interleukin (IL)-17, IL-22, IL-21, IL-23, IL-10, IL-35 and IL-33, as well as surface molecules such as programmed cell death protein 1, cytotoxic T lymphocyte-associated antigen 4, T cell immunoglobulin domain and mucin domain-containing molecule 3 and cannabinoid receptor 2 that have potential therapeutic implications for the homeostasis of CD4$^+$ T cells in CHB and HBVLF.

Key words: Homeostasis; Regulatory T cells; T helper 17 cells; CD4$^+$ T cells; Liver fibrosis; Chronic hepatitis B; Pathogenesis; Therapy

Core tip: Hepatitis B virus (HBV)-related liver fibrosis (HBVLF) is a reversible, intermediate stage of chronic hepatitis B and liver cirrhosis. The homeostasis of CD4$^+$ T cells, especially the balance between regulatory T (Treg) cells and T helper 17 (Th17) cells is pivotal in HBVLF. Therefore, uncovering the underlying mechanisms of CD4$^+$ T cell homeostasis regulating

HBVLF may help achieve better clinical outcomes. We discuss Treg and Th17 cell-related cytokines and surface molecules that may be targeted therapeutically to alter CD4+ T-cell homeostasis in chronic HBV infection.

Cheng LS, Liu Y, Jiang W. Restoring homeostasis of CD4+ T cells in hepatitis-B-virus-related liver fibrosis. *World J Gastroenterol* 2015; 21(38): 10721-10731 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10721.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10721

INTRODUCTION

Over 350 million people worldwide are chronically infected with hepatitis B virus (HBV). According to the World Health Organization, HBV puts people at high risk of death from liver cirrhosis (LC) and hepatocellular carcinoma (HCC), thus causing a heavy global health burden. HBV is hepatotropic but not cytopathic, and interactions between HBV, hepatocytes and the host immune system determine the natural history of infected individuals[1]. CD4+ T cells play key roles in HBV infection. On one hand, CD4+ T cells substantially impact the clearance of HBV by aiding cytotoxic CD8+ T cells, B cells and natural killer T cells[2]. On the other hand, CD4+ T cells contribute to the pathogenesis of inflammation progression *via* production of an array of pro-inflammatory and pro-fibrotic cytokines[2,3].

Liver fibrosis is recognized as a wound-healing response driven primarily by inflammation in response to various parenchymal injuries[4]. HBV-related liver fibrosis (HBVLF) is a reversible, intermediate stage of chronic hepatitis B (CHB) and LC[5]. As conventional subsets of CD4+ T cells, T helper 1 (Th1) and Th2 cells are well-known. Th1 cells produce high levels of interferon γ (IFN-γ), which helps to develop an efficient, specific antiviral immune response and attenuate tissue fibrosis[6,7]. Th2 cells produce interleukin (IL)-4, IL-5 and IL-13, which suppress Th1 cells, resulting in persistent HBV replication and chronic liver immunopathology, and are directly involved in fibrogenesis[6-8]. However, detailed study of the immunity of liver fibrosis has shown that the Th1/Th2 dichotomy is not appropriate. Nowadays, the crucial roles of newly-identified CD4+ T-cell subsets are widely recognized and extensively researched in the progression of CHB.

CD4+ T-CELL SUBSETS AND THEIR IMPACT ON HBV-RELATED CHRONIC HEPATITIS AND LIVER FIBROSIS

On the basis of characteristic transcription factors, unique cytokine profiles and discrete functional properties, CD4+ T cells can be subdivided into new subsets. These include Th17, Th9, Th22, T follicular helper (Tfh) and regulatory T (Treg) cells, in addition to the conventional Th1 and Th2 cells.

Th17 cells

IL-17 and its potential role in immunity were discovered two decades ago[9], then Th17 cells were defined as an independent lineage of T-helper cells in 2005[10,11]. Since then, IL-17 and Th17 cells have been extensively studied to define their properties and roles. At present, the pathogenic role of Th17 cells in promoting liver injury and fibrosis is widely recognized[12-15]. Circulating and intrahepatic Th17 cell numbers are increased in HBV-infected patients with CHB or HBV-related acute-on-chronic liver failure (ACLF), and IL-17 expressions positively related to the severity of liver injury and inflammation progression[12,13]. Th17 cell numbers also increase with the severity of liver fibrosis in humans and mice[14,15].

Until now, the role of Th17 cells in the pathogenesis of liver fibrosis has not yet been fully elucidated. Several studies have found that IL-17 affects hepatic stellate cells (HSCs), by recruiting neutrophils and monocytes[14-17]. However, the whole is greater than the sum of its parts. When naïve CD4+ T cells are exposed to transforming growth factor (TGF)-β and IL-6 during antigen activation, the cells upregulate the Th17 cell-specific transcriptional factor retinoid orphan nuclear receptor γt (RORγt) and differentiate into Th17 cells[10,11]. In addition, IL-21 may allow amplification of Th17 cells with or without IL-6 and TGF-β, and IL-23 is indispensable for the proliferation and function of Th17 cells[18-22]. After activation, Th17 cells secrete a mixture of cytokines including IL-17, IL-21, IL-22, IL-6, IL-9 and tumor necrosis factor α (TNF-α). Although most Th17 cell-mediated pathogenic effects are attributed to IL-17, the impact of Th17 cells is more complex than IL-17-mediated effects.

IL-22 is produced primarily by Th17 cells, and exerts hepatoprotective or pathological effects under different settings of liver diseases, such as acute liver damage induced by carbon tetrachloride (CCl_4), concanavalin A or Fas ligand, alcoholic liver diseases, and chronic hepatitis caused by HBV or hepatitis C virus (HCV) infection[23-26]. Zhao *et al*[26] found that IL-22 was positively related to hepatitis and fibrosis in HBV-infected patients with LC, and using an HBV transgenic mouse model, the authors suggested that IL-22 exacerbated chronic hepatitis and fibrosis by promoting Th17 cell recruitment[26]. Other researchers have noted that the predominance of IL-22's pathological functions over its protective functions in patients with HBV was due to the cytokine's ability to upregulate chemokine expression to recruit inflammatory cells into the liver[23]. However, there are also some researchers have observed that the levels of IL-22 were significantly reduced in severe liver injuries during CHB[27].

Another important Th17 cell-related cytokine is IL-21. Recent studies have indicated that both

circulating IL-21$^+$CD3$^+$CD8$^-$ T cell numbers and intrahepatic IL-21 levels are correlated with the severity of liver damage in patients with active CHB, HBV-related LC and HBV-related ACLF[28-30]. In addition, IL-21 causes HSC activation *in vitro*, thus facilitating the fibrogenesis of LC[29].

The effects of Th17 cells in HBV-related liver injury and fibrosis are comprehensive, and need to be further elucidated. Moreover, there are still mysteries surrounding Th17 cells. Apart from the integrated effects of Th17-related cytokines, other sources of these cytokines [such as IL-17 produced by neutrophils, natural killer T (NKT) cells, macrophages, and γδT cells] may make it difficult to define the exact roles of Th17 cells in liver fibrosis[31]. In recent years, the plasticity of Th17 cells during inflammation has been widely reported[32,33], revealing the importance of crosstalk between different CD4$^+$ T-cell subsets.

Treg cells

CD4$^+$CD25$^+$ Treg cells are a lineage of CD4$^+$ T cells characterized by production of TGF-β and expression of the transcription factor Forkhead box P3 (FoxP3)[34]. Treg cells exhibit immunosuppressive and self-tolerant functions by direct cell contact and by secreting inhibitory cytokines such as IL-10, TGF-β and IL-35.

IL-10 can inhibit Th1 and Th2 cell responses through antigen-presenting cells[35]. IL-10 can also prevent the induction of Th17 cell responses, but cannot suppress an established Th17 cell-mediated chronic inflammation[36]. During HBV-related disease progression, IL-10 may serve as a negative feedback mechanism to regulate pro-inflammatory Th17 cell responses[37]. Moreover, activated HSCs produce IL-10, which constrains the cells' ability to produce collagen, thereby blocking the progression of liver fibrosis[38].

IL-35 is a novel inhibitory cytokine produced by Treg cells, and has been receiving increasing attention[39,40]. However, Bardel *et al*[41] argued that Treg cells do not express sufficient levels of IL-35 in humans. Recent studies have shown that IL-35 can be detected in circulating CD4$^+$ T cells from CHB patients and can inhibit the pathogenesis of HBVLF and LC[42,43].

Many studies have shown that Treg cells are significantly correlated with HBV infection and the degree of liver fibrosis[44-48]. Treg cell numbers increase with the number of HBV antigens. The virus also uses the cells for shelter, avoiding immune attack due to the cells' immunosuppressive activities[44], while the same immunosuppressive function works on other cell types to alleviate liver injury[45]. Simultaneously, Treg cells inhibit HSC activation and proliferation, thus limiting liver fibrosis[46,47]. However, the specific actions of Treg cells in HBVLF remain to be elucidated; in particular, the increasing evidence that Treg cells can convert to effector T cells adds complexity to the situation[49,50].

Th9, Th22 and Tfh cells

In the presence of high levels of TGF-β and IL-4, naïve CD4$^+$ T cells differentiate to Th9 cells, which produce IL-9[51]. As a newly-identified subset of CD4$^+$ T cells, Th9 cells have been studied only in allergic inflammation, autoimmune disease and tumor immunity[52]; the role of Th9 cells in liver injury is unknown.

Th22 cells predominantly produce IL-22, and develop from naïve CD4$^+$ T cells in the presence of IL-6 and TNF-α[53]. Th22 cells and intrahepatic IL-22 have been reported to have hepatoprotective effects in drug-induced hepatocellular injury[54]. However, the role of Th22 cells and IL-22 in HBVLF is unknown. Defining this role may be challenging, since IL-22 is also produced by other cells, especially Th17 cells.

Tfh cells express high levels of chemokine receptor 5, inducible co-stimulator, programmed cell death protein1 (PD-1), and CD40L[55]. Expression of these surface molecules, along with cytokines IL-4 and IL-21, allows Tfh cells to regulate T cells and B cells. Recently, HCC patients were found to have significantly fewer circulating Tfh cells with impaired IL-21 production and B cell regulatory properties, compared with HBV-infected LC patients and healthy controls[56]. This suggests that Tfh cells may negatively involve in the progression of HBV-associated HCC, but the role of Tfh cells in liver fibrosis is unknown.

HOMEOSTASIS OF TREG AND TH17 CELLS IN HBVLF

Given the specific roles of Th1, Th2, Th17 and Treg cells in HBV-related chronic hepatitis and fibrosis, and the way these subsets affect each other, current studies mainly focus on the subsets and their homeostasis. Treg and Th17 cells are the most intensively studied subsets for HBVLF.

Significance of the balance between Treg and Th17 cells during HBVLF

As discussed above, Treg and Th17 cells are important CD4$^+$ T cell subsets that are developmentally correlated and functionally reciprocal during inflammation. Recent reports have proved their close interactions and transitions. Thus, there is a balance between Treg and Th17 cells.

In our previous work, we highlighted the significance of the balance between Treg and Th17 cells in the progression of HBVLF[3]. We found that the ratio Treg/Th17 was negatively related to the severity of liver fibrosis[3]. Other researchers found this correlation in patients with HBV-related LC and mouse models of liver fibrosis[46,47]. A dominance of Th17 cells is closely correlated with liver fibrosis[3,46,47]. In addition, an imbalance in the ratio was reported in HBV-related ACLF, and liver injury was alleviated when the balance was restored[57-59]. Several groups found that improved liver function after transplantation of autologous bone marrow mesenchymal stem cells might be mediated by changes in the Treg/Th17 ratio[60]. Therefore, the

balance between Treg and Th17 cells is not only of great significance in indicating the severity of liver injury, but also has potential therapeutic value.

Mechanisms of the balance between Treg and Th17 cells regulating liver fibrosis

Recently, a growing number of studies have investigated the underlying mechanisms where by the Treg/Th17 balance regulates the process of liver fibrosis. When $CD4^+CD25^-$ cells were co-cultured with HSCs, an anti-IL-17 antibody down-regulated - and recombinant IL-17 upregulated - HSC proliferation and pro-fibrotic cytokine production[3]. Using a transwell co-culture system, we found that $CD4^+CD25^+$ Treg cells directly down-regulated the pro-fibrotic features of HSCs by cell contact rather than through the release of TGF-β or IL-10[3]. Other researchers found that a Th17 cell dominance over Treg cells could activate HSCs in CCl_4-treated mice with liver fibrosis[46]. Although these studies have demonstrated the crucial effects of the Treg/Th17 balance on liver fibrosis through an impact on HSCs, whether there are other mechanisms remains to be elucidated.

Regulation of the balance between Treg and Th17 cells

Because the balance between Treg and Th17 cells is important in the pathogenesis of HBVLF, many studies have investigated factors that regulate the Treg/Th17 balance in order to achieve better clinical outcomes.

From the perspective of developmental pathways, TGF-β might be the first candidate for consideration. High concentrations of TGF-β induce FoxP3 expression in naïve $CD4^+$ T cells, driving their differentiation into Treg cells[34]. In contrast, TGF-β plus IL-6 or IL-21 induce the expression of RORγt and signal transducer and activator of transcription 3 (STAT3), promoting Th17 cell differentiation[21]. Although several groups have indicated that TGF-β is dispensable for the differentiation of Th17 cells[19,20], the modulatory effect of TGF-β cannot be ignored.

IL-21 suppresses FoxP3 expression and promotes Th17 cell differentiation by regulating TGF-β signaling[21,61]. The vitamin A metabolite retinoic acid is a key regulator of TGF-β-mediated Treg cell differentiation and inhibits Th17 cell differentiation by directly counteracting the activity of IL-6[62]. IL-2, together with TGF-β, can drive Treg cell differentiation, and IL-2 inhibits Th17 cell differentiation through a STAT5-dependent pathway[63].

Interactions between Treg and Th17 cells directly affect their balance. During HBV infection, Treg cells inhibit Th17 cells, either through Treg cell cytotoxicity or through inhibitory cytokines such as IL-10, TGF-β or IL-35[42,64,65]. Depletion of Treg cells enhances Th17 cell responses, leading to more severe liver damage[3,64,65]. Treg cells expressing CD39 have been reported to effectively limit Th17 cell-responses[66].

However, there is accumulating evidence that Treg cells also upregulate the production of Th17 cell-associated pro-inflammatory cytokines, mainly IL-17 and IL-22[67,68]. The TNF-TNFR2 pathway might play a part in this phenomenon[69]. Zhou *et al*[69] found that Treg cells deficient in TNFR2 support lower production of IL-17A (also called IL-17) and TNF by co-cultured Th17 cells. Furthermore, the authors found that exogenously-generated Th17 cells supported the expansion and phenotypic stability of Treg cells *in vivo via* the same TNF-TNFR2 pathway[69]. Although the effects of Th17 cells on Treg cells are unclear in liver injury, the bidirectional interactions between Treg and Th17 cells likely affect their homeostasis.

The plasticity of Treg and Th17 cells also affects their balance. The stability of Treg cells is openly discussed[70]. However, multiple groups have shown that Treg cells secrete IL-17 when activated under certain conditions, for example, by Toll-like receptor 2 (TLR2), TLR4 or TLR9 and Th17-biasing cytokine conditions such as IL-6, IL-21, IL-23 or IL-1β[71-75]. These IL-17-producing Treg cells retain expression of FoxP3 but lose their suppressive functionality[71]. However, the lost suppressive function can recover *in vitro*[71,72]. Whether the ability to secrete IL-17 by Treg cells can be regarded as plasticity or an adaptive response remains to be elucidated, but the discovery of IL-17-producing $FoxP3^+$ cells supports an additional mechanism maintaining the balance between Treg and Th17 cells.

Unlike Treg cells, the plasticity of Th17 cells is widely reported. During chronic inflammation, Th17 cells can convert to Th1 or Th2 cells[32,33]. Notably, in the presence of IL-12 and TNF-α, Th17 cells rapidly shift towards an IFN-γ-producing Th1 cell phenotype, and lose the capacity for IL-17-production[76,77]. Intriguingly, Ye *et al*[78] reported that human tumor-infiltrating Th17 cells from melanoma, ovarian, breast and colon cancers can express FoxP3 in response to T-cell receptor stimulation and subsequent epigenetic modification and gene reprogramming. In these studies, the $FoxP3^+$ cells derived from tumor-infiltrating Th17 cells had potent suppressive activity and did not convert back to Th17 cells under Th17 cell differentiation conditions[78]. These results provide another example of Th17 cell plasticity, although whether this Th17-to-Treg cell event occurs *in vivo* remains to be determined.

IMPLICATIONS FOR HBVLF TREATMENT STRATEGIES

Current treatment strategies for chronic HBV infection primarily target the virus directly or attempt to restore an effective antiviral immune response. As for the process of HBVLF, the inappropriate immune response induced by $CD4^+$ T cells is responsible for causing the disease. Treatment strategies aimed at mitigating or even eliminating the progression of inflammation and fibrosis can focus on the homeostasis of $CD4^+$ T cells,

Table 1 Role of Treg and Th17 cell-related interleukins in chronic hepatitis

Treg and Th17-related interleukins	Cellular sources	Roles in CD4+ T cell differentiation and function	Roles in liver inflammation and fibrosis	Ref.
IL-17	Th17, neutrophils, NKT cells, macrophages, γδT cells	Characteristic cytokine of Th17 cells: pro-inflammatory	Pro-inflammatory Pro-fibrotic	[6-8,10,31]
IL-21	Th17, Tfh, NKT cells	Promotes differentiation of Th17 cells; Inhibits differentiation of Treg cells	Promotes HBV-related liver injury and fibrogenesis	[18,21,28-30]
IL-23	DCs, macrophages	Promotes Th17 cell proliferation and stabilizes effector Th17 cells	Promotes HBV-related liver injury	[22,80]
IL-22	Th17, Th22, activated NK and NKT cells	Characteristic cytokine of Th22 cells: pro-inflammatory	Hepatoprotective? Pro-inflammatory? Pro-fibrotic? Anti-fibrotic?	[23,24,26,27]
IL-10	Treg, hepatocytes, Kupffer cells, LSECs, HSCs, Breg	Inhibits Th1, Th2, Th17 cell differentiation and cytokine production	Anti-inflammatory Anti-fibrotic	[35,38,81]
IL-35	Treg, Breg	Immunosuppressive	Anti-inflammatory Anti-fibrotic?	[30,31,33,34]
IL-33	LSECs, activated HSCs	Promotes Th2 differentiation and cytokine production; Increases Treg cells? Activates Tfh cells?	Pro-fibrotic Anti-inflammatory?	[86-88,90,91]

Treg: Regulatory T cells; Tfh: T follicular helper cells; HBV: Hepatitis B virus; NK: Natural killer cells; NKT: Natural killer T cells; DCs: Dendritic cells; Breg: Regulatory B cells; LSECs: Liver sinusoidal endothelial cells; HSCs: Hepatic stellate cells.

Table 2 Role of surface molecules in chronic hepatitis

Surface molecules	Expression on T cells and CD4+ T cell subsets	General effects	Role in liver inflammation and fibrosis	CD4+ T cell response to blocking	Ref.
PD-1	CD8+ T cells CD4+ T cells	Inhibits T cell activation; Maintains tolerance	Causes exhaustion of HBV-specific CD8+ and CD4+ T cells	Partially-revived proliferation and function	[96-98,104]
TIM-3	CD8+ T cells CD4+ T cells Treg cells	Inhibits T cell activation; Maintains tolerance	Promotes progression of HBV infection	Revived more (blocked with PD-1)	[99,100,102-104]
CTLA-4	Activated CD4+ T cells Treg cells	Inhibits CD4+ T cell over-activation; Maintains tolerance	Promotes persistence of HBV and progression of HBV infection	Unknown	[105,107,109,110]
CB2	CD4+ T cells CD8+ T cells Th17 cells	Immunoregulatory: pro-inflammatory or anti-inflammatory; Anti-fibrotic	Anti-inflammatory; Anti-fibrotic; Hepatoprotective?	Decreased frequency and function of Th17 cells	[112-114]

PD-1: Programmed cell-death protein 1; CTLA-4: Cytotoxic T lymphocyte-associated antigen 4; TIM-3: T-cell immunoglobulin domain and mucin domain-containing molecule 3; CB2: Cannabinoid receptor 2; HBV: Hepatitis B virus.

in particular the balance between Treg and Th17 cells. Any treatment that achieves an anti-fibrotic effect by targeting Treg and/or Th17 cells has potential therapeutic value for chronic HBV-induced liver injury. As described above, the treatment can work in different ways: cell development, cell function, or cell conversion. In this section, we mainly concentrate on Treg/Th17 cell function, discussing the cytokines (Table 1) and surface molecules (Table 2) that regulate the homeostasis of CD4+ T cells.

Interleukins associated with Treg and Th17 cells

IL-17A: IL-17A is the dominant member of Th17 cell-associated cytokines. As discussed above, IL-17A levels correlate positively with hepatitis and the severity of liver fibrosis. Targeting IL-17A has yielded substantial results in animal models. In our previous work with concanavalin A-treated mice, blockade of IL-17A using an anti-IL-17 monoclonal antibody markedly down-regulated the expression of α-smooth muscle actin and decreased the level of serum alanine aminotransferase (ALT), thus alleviating liver injury and fibrosis[3]. In addition, Tan *et al*[17] found that IL-17A receptor-deficient mice exhibited decreased pro-inflammatory cytokine levels, reduced neutrophil recruitment, and less hepatocellular necrosis in the CCl_4 model than did wild-type mice. Similarly, Meng *et al*[16] reported that liver fibrosis induced by either bile duct ligation or CCl_4 was reduced in IL-17AR-deficient mice. Zheng *et al*[79] found that most patients with HBV-related decompensated cirrhosis who underwent bone marrow-derived stem cell transplantation displayed significantly improved liver function, due in part to decreased levels of IL-17.

IL-21 and IL-23: IL-21 is important in initiating

and amplifying the differentiation of Th17 cells[18,21]. Korn *et al*[21] observed that Th17 cell-frequencies were reduced by 50% when IL-21 receptor-deficient T cells were cultured with IL-6 and TGF-β. The authors also indicated that IL-21 was one of the most efficient alternative cytokines to IL-6 in inhibiting TGF-β-driven FoxP3+ Treg cell differentiation in IL-6-deficient mice[21]. Another indispensable factor for Th17 cell differentiation, IL-23 promotes Th17 cell proliferation and stabilizes Th17 cell function. IL-23- or IL-23 receptor-deficient cells failed to stimulate enough functional IL-17-producing cells[22]. Wang *et al*[80] observed high levels of IL-23 and IL-23 receptor in HBV-infected CHB and ACLF patients, and an IL-23 neutralizing antibody significantly decreased the production of IL-17 *in vitro*. Accordingly, the authors emphasized the importance of IL-23 and Th17 cells in HBV-related liver damage[80].

IL-22: The context-dependent pro- and anti-inflammatory nature of IL-22 has been described under different conditions of liver diseases[23-26]. Although several studies showed that administration of IL-22 ameliorates liver fibrosis in mouse models, Zhao *et al*[26] pointed out that these models had differences with HBV-induced immune-mediated liver fibrosis. Using HBV-transgenic mice, the authors observed that blockade of IL-22 reduced Th17 cell recruitment and ameliorated liver inflammation and fibrosis *in vivo*[26]. In another HBV-transgenic mouse model, Zhang *et al*[23] found that the severity of liver injury was reduced by neutralization of IL-22 when splenocytes were transferred from HBV-immunized mice. The authors indicated that this effect was not dependent on HBV inhibition, but instead due to decreased recruitment of all leukocyte subsets into the liver and reduced intrahepatic chemokine expression[23]. The contrasting effects exhibited by IL-22 in different etiology-induced immunity need to be clarified in future studies and maybe of significance for the development of new therapeutic approaches.

IL-10: IL-10 is an important multi-sourced, anti-inflammatory cytokine[35]. In the liver, IL-10 can be produced by hepatocytes, Kupffer cells, HSCs, regulatory B (Breg) cells, and Treg cells[81]. As mentioned above, IL-10 can inhibit Th1, Th2 and Th17 responses as well as restrain activation of HSCs[38]. In CCl_4-treated mice, deletion of the IL-10 gene resulted in significantly more severe fibrosis[82]. In addition, in thioacetamide-treated IL-10 knockout mice, exogenous administration of IL-10 gene reversed an established hepatic fibrosis[83]. Taken together, IL-10 might have potential for future treatment of HBV infection and liver fibrosis. However, since IL-10 exhibits broad biological effects, future studies should also focus on decreasing the side effects.

IL-35: IL-35 is a novel immunosuppressive cytokine produced by Treg and Breg cells, and is being studied for its therapeutic utilities[39,40,84]. Recent studies showed that IL-35 was negatively involved in the pathogenesis of HBVLF and cirrhosis[42]. However, studies of the association between IL-35 and chronic HBV infection are still limited. Nowadays, IL-35 is mainly investigated in autoimmune diseases. One study on primary biliary cirrhosis showed that in dominant-negative TGF-β receptor type Ⅱ mice, deletion of the gene encoding the IL-12p35 subunit, which deletes IL-12 and IL-35, induced a Th17 cell response, inhibited a Th1 cell response, and caused liver fibrosis[85]. This results suggests that IL-35 might be closely associated with liver fibrosis.

IL-33: IL-33 belongs to the IL-1 family, and is associated with liver injury and fibrosis in chronic hepatitis[86,87]. In the liver, IL-33 is made by sinusoidal endothelial cells and activated HSCs[87]. Through soluble receptor ST2, IL-33 promotes Th2 cell responses and increases production of Th2 cytokines (IL-4, IL-5 and IL-13) *in vitro* and *in vivo*[86-88]. However, some researchers have provided evidence that the IL-33/ST2 axis can ameliorate liver inflammation[89,90]. In mice with concanavalin A-induced liver injury, administration of IL-33 attenuated hepatitis, whereas deletion of ST2 caused significantly more severe hepatitis[89,90]. Zhao *et al*[91] found that IL-33 might activate Tfh cells, which facilitate humoral immunity against HBV. The specific roles of IL-33 need to be further investigated, in particular the influence of IL-33 on different CD4+ T subsets.

Surface molecules on CD4+ T cells

CD4+ T cells express surface co-inhibitory molecules, including PD-1, cytotoxic T lymphocyte-associated antigen4 (CTLA-4), T-cell immunoglobulin domain and mucin domain-containing molecule 3 (TIM-3), lymphocyte activation gene 3, and CD244. Upregulation of these molecules can result in HBV-specific T cell exhaustion, which is a crucial mechanism in the deviation of homeostasis of adaptive immunity and the consequent persistence and progression of HBV infection[92,93]. These co-inhibitory molecules interact with their ligands expressed on antigen-presenting cells, then deliver signals which decrease cell proliferation and cytokine production[93]. Studies on these co-inhibitory molecules in HBV infection have focused mainly on exhausted CD8+ T cells, but recently more attention has been paid to CD4+ T cells in consideration of their pivotal roles in cell immunity[94]. Furthermore, the expression of these co-inhibitory molecules on CD4+ T cells was shown to be important in other chronic viral diseases such as hepatitis C[95].

PD-1: PD-1 is a member of the CD28 superfamily, and exerts a wide range of immunoregulatory roles in T-cell activation and tolerance through binding to its ligands PD-L1 and PD-L2[96]. Several studies found that high

levels of PD-1 on CD4+ T cells are strongly linked to exhaustion of HBV-specific CD4+ T cells[93,97,98]. Using a DRB1*01-restricted major histocompatibility complex (MHC) class Ⅱ tetramer, Raziorrouh *et al*[98] found that CD4+ T cells had elevated PD-1 expression; moreover, PD-L1/PD-L2 neutralization reactivated cell proliferation and partially increased production of IFN-γ, IL-2 and TNF-α[98]. Notably, the four patients who responded to the PD-L1/PD-L2 blockade achieved long-term HBV suppression, while the other nine patients who failed to revive T-cell proliferation continued to have high viral loads[98]. Blockade of PD-L1/PD-L2 increased the frequencies of HCV-specific CD4+ T cells and induced cell expansion and production of IFN-γ and TNF-α *in vitro*, whereas influenza- and Epstein-Barr virus-specific CD4+ T cells did not respond significantly to the blockade[95]. The responses of CD4+ T cells to the blockade of PD-L1/PD-L2 may differ from different chronic virus infection. There is limited data on the relationship between PD-1 expression and progression of chronic HBV infection. Xu *et al*[97] reported that although PD-1 expression was upregulated in LC and HCC, the magnitude was small and there was no correlation between PD-1 levels and the severity of liver injury. The role of PD-1 in chronic HBV infection and related liver fibrosis needs further investigation.

TIM-3: TIM-3, which is expressed on CD4+ and CD8+ T cells, negatively regulates T-cell responses and induces tolerance through binding to its ligand galectin-9[99,100]. The Tim-3/galectin-9 axis is also essential for the homeostasis of Treg cells[100,101]. Recent studies have indicated that expression of *Tim-3* on CD4+ T cells is upregulated in patients with CHB compared to healthy controls, and TIM-3 levels are positively associated with the extent of HBV infection[93,102]. In addition, the levels of *Tim-3* are decreased after antiviral treatment[102]. However, Raziorrouh *et al*[98] observed a persistent low level of TIM-3 in CHB patients, and TIM-3 blockade had little influence on CD4+ T-cell function. The difference between these studies might be due to the relative paucity of CD4+ T cells and the DRB1*01-restricted MHC class Ⅱ tetramer, as DRB1*01+CD4+ T cells are specific only to HBV core epitope 61-80. In a recent study of mice with chronic lymphocytic choriomeningitis virus infection, treatment with vinegar-processed floss of *Daphne genkwa*, a traditional folk medicine extract, restored function of exhausted virus-specific CD4+ and CD8+ T cells[103]. This restoration might have occurred *via* down-regulation of PD-1 and Tim-3. Moreover, it has been reported that targeting both PD-1 and Tim-3 is an effective strategy to restore exhausted CD8+ T cells during chronic viral infection[104]. Future studies should focus on the blockade of both TIM-3 and PD-1 on HBV-specific CD4+ T cells.

CTLA-4: CTLA-4 is expressed on activated and regulatory CD4+ T cells to prevent over-activation and maintain tolerance[105]. It is widely reported that CTLA-4 has a close correlation with HBV infection in promoting Th2 cell responses[106-108]. Several groups found that CTLA-4 gene polymorphisms might be associated with HBV progression and viral persistence[107,109]. However, expression of CTLA-4 on virus-specific CD4+ T cells in chronic HBV infection is still controversial. Recent years have witnessed prominent effects of CTLA-4 blocking. Blockade of CTLA-4 by the monoclonal antibody tremelimumab has been tested in patients with HCC and HCV-induced LC, and shown substantial antitumor and antiviral effects[110]. In *Propionibacterium acnes* and lipopolysaccharide-induced mouse models of fulminant hepatitis, all mice injected with adenovirus encoding a CTLA-4 immunoglobulin construct survived, whereas most of the control mice died, suggesting that the construct could be useful for treatment of severe liver injury[111]. Together, these studies have shown the importance of CTLA-4 in chronic viral infection and associated disease progression. Future studies should investigate the correlation of CTLA-4 with the homeostasis of HBV-specific CD4+ T cells.

Cannabinoid receptor 2: In addition to co-inhibitory molecules on the surface of CD4+ T cells, surface receptors such as cannabinoid receptor 2 (CB2) have received attention because of their anti-inflammatory and anti-fibrotic properties in mouse liver[112]. CB2 is abundantly expressed on almost all immune cells[113]. In CB2-deficient mice with bile duct ligation, intrahepatic Th17 cells and IL-17 levels were increased compared with wild-type mice, whereas the CB2 agonist JWH-133 reduced the differentiation and function of Th17 cells *in vitro*[112]. In CB2-deficient mice treated with CCl_4, increased ALT levels and hepatocyte apoptosis, and delayed liver regeneration were shown, while JWH-133 displayed hepatoprotective property in CCl_4 treated wild types[114]. Thus, activating CB2 on Th17 cells may be effective for treatment of liver fibrosis. It will be interesting to investigate the relation of CB2 with HBV infection and subsequent diseases.

CONCLUSION

HBVLF is an intricate disease process that cannot be regulated by a single cytokine or immune cell. The disequilibrium of CD4+ T cells contributes to the disease, and restoring homeostasis may help greatly to reestablish effective immunity against HBV-related pathological processes. However, there are still many problems to be resolved. First, the specific regulatory mechanisms of CD4+ T-cell homeostasis, especially the balance between Treg and Th17 cells, are still not fully elucidated in the process of HBV-induced liver injury. Second, the practical situations are not always as good as in theory, and naturally developing HBV-specific immunity in CHB patients may not be the same as that in mouse models. Moreover, how to apply current findings from mice to humans with good therapeutic effects and few side effects is always worthy of further

research.

There is still a long way to go in the restoration of homeostasis of CD4$^+$ T cells in HBVLF, but future studies will be meaningful in elucidating the pathogenesis and resistance of chronic HBV infection. It is noteworthy that the homeostasis of CD4$^+$ T cells is only part of the immunoregulatory network. We should be concerned with the local regulation of CD4$^+$ T cells, as well as their interactions with other cells, especially their links with innate immunity. For instance, we previously found that the fibrotic factor high-mobility group box1 (HMGB1), a damage-associated molecular pattern molecule, could transmit signals from necrotic cells to innate immune cells and then to CD4$^+$ T cells in CHB patients through the axis of HMGB1-TLR4-IL-6-Treg/Th17 balance[115]. As a result, HMGB1 promotes Th17 cell responses and inhibits Treg cell responses, thus exerting pro-inflammatory and pro-fibrotic effects[115]. Therefore, future studies on the homeostasis of CD4$^+$ T cells should include their links with innate immunity.

REFERENCES

1 **Trépo C**, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014; **384**: 2053-2063 [PMID: 24954675 DOI: 10.1016/S0140-6736(14)60220-8]

2 **Wang FS**, Zhang Z. Host immunity influences disease progression and antiviral efficacy in humans infected with hepatitis B virus. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 499-512 [PMID: 19817672 DOI: 10.1586/egh.09.50]

3 **Li J**, Qiu SJ, She WM, Wang FP, Gao H, Li L, Tu CT, Wang JY, Shen XZ, Jiang W. Significance of the balance between regulatory T (Treg) and T helper 17 (Th17) cells during hepatitis B virus related liver fibrosis. *PLoS One* 2012; **7**: e39307 [PMID: 22745730 DOI: 10.1371/journal.pone.0039307]

4 **Xu R**, Zhang Z, Wang FS. Liver fibrosis: mechanisms of immune-mediated liver injury. *Cell Mol Immunol* 2012; **9**: 296-301 [PMID: 22157623 DOI: 10.1038/cmi.2011.53]

5 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]

6 **Marra F**, Aleffi S, Galastri S, Provenzano A. Mononuclear cells in liver fibrosis. *Semin Immunopathol* 2009; **31**: 345-358 [PMID: 19533130 DOI: 10.1007/s00281-009-0169-0]

7 **Wynn TA**. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; **214**: 199-210 [PMID: 18161745 DOI: 10.1002/path.2277]

8 **Navarro-Partida J**, Martinez-Rizo AB, Gonzalez-Cuevas J, Arrevillaga-Boni G, Ortiz-Navarrete V, Armendariz-Borunda J. Pirfenidone restricts Th2 differentiation in vitro and limits Th2 response in experimental liver fibrosis. *Eur J Pharmacol* 2012; **678**: 71-77 [PMID: 22222821 DOI: 10.1016/j.ejphar.2011.12.025]

9 **Yao Z**, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, Cohen JI, Spriggs MK. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* 1995; **3**: 811-821 [PMID: 8777726]

10 **Harrington LE**, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; **6**: 1123-1132 [PMID: 16200070 DOI: 10.1038/ni1254]

11 **Park H**, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; **6**: 1133-1141 [PMID: 16200068 DOI: 10.1038/ni1261]

12 **Zhang JY**, Zhang Z, Lin F, Zou ZS, Xu RN, Jin L, Fu JL, Shi F, Shi M, Wang HF, Wang FS. Interleukin-17-producing CD4(+) T cells increase with severity of liver damage in patients with chronic hepatitis B. *Hepatology* 2010; **51**: 81-91 [PMID: 19842207 DOI: 10.1002/hep.23273]

13 **Yang B**, Wang Y, Zhao C, Yan W, Che H, Shen C, Zhao M. Increased Th17 cells and interleukin-17 contribute to immune activation and disease aggravation in patients with chronic hepatitis B virus infection. *Immunol Lett* 2013; **149**: 41-49 [PMID: 23237940 DOI: 10.1016/j.imlet.2012.12.001]

14 **Sun HQ**, Zhang JY, Zhang H, Zou ZS, Wang FS, Jia JH. Increased Th17 cells contribute to disease progression in patients with HBV-associated liver cirrhosis. *J Viral Hepat* 2012; **19**: 396-403 [PMID: 22571901 DOI: 10.1111/j.1365-2893.2011.01561.x]

15 **Du WJ**, Zhen JH, Zeng ZQ, Zheng ZM, Xu Y, Qin LY, Chen SJ. Expression of interleukin-17 associated with disease progression and liver fibrosis with hepatitis B virus infection: IL-17 in HBV infection. *Diagn Pathol* 2013; **8**: 40 [PMID: 23448394 DOI: 10.1186/1746-1596-8-40]

16 **Meng F**, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, Cong M, Iwaisako K, Liu X, Zhang M, Osterreicher CH, Stickel F, Ley K, Brenner DA, Kisseleva T. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology* 2012; **143**: 765-76.e1-3 [PMID: 22687286 DOI: 10.1053/j.gastro.2012.05.049]

17 **Tan Z**, Qian X, Jiang R, Liu Q, Wang Y, Chen C, Wang X, Ryffel B, Sun B. IL-17A plays a critical role in the pathogenesis of liver fibrosis through hepatic stellate cell activation. *J Immunol* 2013; **191**: 1835-1844 [PMID: 23842754 DOI: 10.4049/jimmunol.1203013]

18 **Fina D**, Sarra M, Fantini MC, Rizzo A, Caruso R, Caprioli F, Stolfi C, Cardolini I, Dottori M, Boirivant M, Pallone F, Macdonald TT, Monteleone G. Regulation of gut inflammation and th17 cell response by interleukin-21. *Gastroenterology* 2008; **134**: 1038-1048 [PMID: 18395085 DOI: 10.1053/j.gastro.2008.01.041]

19 **Santarlasci V**, Maggi L, Capone M, Frosali F, Querci V, De Palma R, Liotta F, Cosmi L, Maggi E, Romagnani S, Annunziato F. TGF-beta indirectly favors the development of human Th17 cells by inhibiting Th1 cells. *Eur J Immunol* 2009; **39**: 207-215 [PMID: 19130583 DOI: 10.1002/eji.200838748]

20 **Ghoreschi K**, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE, Ramos HL, Wei L, Davidson TS, Bouladoux N, Grainger JR, Chen Q, Kanno Y, Watford WT, Sun HW, Eberl G, Shevach EM, Belkaid Y, Cua DJ, Chen W, O'Shea JJ. Generation of pathogenic T(H)17 cells in the absence of TGF-β signalling. *Nature* 2010; **467**: 967-971 [PMID: 20962846 DOI: 10.1038/nature09447]

21 **Korn T**, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, Oukka M, Kuchroo VK. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* 2007; **448**: 484-487 [PMID: 17581588 DOI: 10.1038/nature05970]

22 **McGeachy MJ**, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, McClanahan TK, O'Shea JJ, Cua DJ. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat Immunol* 2009; **10**: 314-324 [PMID: 19182808 DOI: 10.1038/ni.1698]

23 **Zhang Y**, Cobleigh MA, Lian JQ, Huang CX, Booth CJ, Bai XF, Robek MD. A proinflammatory role for interleukin-22 in the immune response to hepatitis B virus. *Gastroenterology* 2011; **141**: 1897-1906 [PMID: 21708106 DOI: 10.1053/j.gastro.2011.06.051]

24 **Kong X**, Feng D, Wang H, Hong F, Bertola A, Wang FS, Gao B. Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. *Hepatology* 2012; **56**: 1150-1159 [PMID: 22473749 DOI: 10.1002/hep.25744]

25 **Kong X**, Feng D, Mathews S, Gao B. Hepatoprotective and anti-fibrotic functions of interleukin-22: therapeutic potential for the treatment of alcoholic liver disease. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 56-60 [PMID: 23855297 DOI: 10.1111/jgh.12032]

26 **Zhao J**, Zhang Z, Luan Y, Zou Z, Sun Y, Li Y, Jin L, Zhou C, Fu J, Gao B, Fu Y, Wang FS. Pathological functions of interleukin-22 in chronic liver inflammation and fibrosis with hepatitis B virus infection by promoting T helper 17 cell recruitment. *Hepatology* 2014; **59**: 1331-1342 [PMID: 24677193 DOI: 10.1002/hep.26916]

27 **Xiang X**, Gui H, King NJ, Cole L, Wang H, Xie Q, Bao S. IL-22 and non-ELR-CXC chemokine expression in chronic hepatitis B virus-infected liver. *Immunol Cell Biol* 2012; **90**: 611-619 [PMID: 21946664 DOI: 10.1038/icb.2011.79]

28 **Pan Q**, Yu Y, Tang Z, Xi M, Jiang H, Xun Y, Liu X, Liu H, Hu J, Zang G. Increased levels of IL-21 responses are associated with the severity of liver injury in patients with chronic active hepatitis B. *J Viral Hepat* 2014; **21**: e78-e88 [PMID: 24611989 DOI: 10.1111/jvh.12242]

29 **Feng G**, Zhang JY, Zeng QL, Yu X, Zhang Z, Lv S, Xu X, Wang FS. Interleukin-21 mediates hepatitis B virus-associated liver cirrhosis by activating hepatic stellate cells. *Hepatol Res* 2014; **44**: E198-E205 [PMID: 23905760 DOI: 10.1111/hepr.12215]

30 **Hu X**, Ma S, Huang X, Jiang X, Zhu X, Gao H, Xu M, Sun J, Abbott WG, Hou J. Interleukin-21 is upregulated in hepatitis B-related acute-on-chronic liver failure and associated with severity of liver disease. *J Viral Hepat* 2011; **18**: 458-467 [PMID: 21692955 DOI: 10.1111/j.1365-2893.2011.01475.x]

31 **Busman-Sahay KO**, Walrath T, Huber S, O'Connor W. Cytokine crowdsourcing: multicellular production of TH17-associated cytokines. *J Leukoc Biol* 2015; **97**: 499-510 [PMID: 25548251 DOI: 10.1189/jlb.3RU0814-386R]

32 **Peck A**, Mellins ED. Plasticity of T-cell phenotype and function: the T helper type 17 example. *Immunology* 2010; **129**: 147-153 [PMID: 19922424 DOI: 10.1111/j.1365-2567.2009.03189.x]

33 **Geginat J**, Paroni M, Maglie S, Alfen JS, Kastirr I, Gruarin P, De Simone M, Pagani M, Abrignani S. Plasticity of human CD4 T cell subsets. *Front Immunol* 2014; **5**: 630 [PMID: 25566245 DOI: 10.3389/fimmu.2014.00630]

34 **Walker MR**, Kasprowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH, Ziegler SF. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *J Clin Invest* 2003; **112**: 1437-1443 [PMID: 14597769 DOI: 10.1172/JCI19441]

35 **Sabat R**, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J. Biology of interleukin-10. *Cytokine Growth Factor Rev* 2010; **21**: 331-344 [PMID: 21115385 DOI: 10.1016/j.cytogfr.2010.09.002]

36 **Naundorf S**, Schröder M, Höflich C, Suman N, Volk HD, Grütz G. IL-10 interferes directly with TCR-induced IFN-gamma but not IL-17 production in memory T cells. *Eur J Immunol* 2009; **39**: 1066-1077 [PMID: 19266486 DOI: 10.1002/eji.200838773]

37 **Wu W**, Li J, Chen F, Zhu H, Peng G, Chen Z. Circulating Th17 cells frequency is associated with the disease progression in HBV infected patients. *J Gastroenterol Hepatol* 2010; **25**: 750-757 [PMID: 20492330 DOI: 10.1111/j.1440-1746.2009.06154.x]

38 **Wang SC**, Ohata M, Schrum L, Rippe RA, Tsukamoto H. Expression of interleukin-10 by in vitro and in vivo activated hepatic stellate cells. *J Biol Chem* 1998; **273**: 302-308 [PMID: 9417080]

39 **Collison LW**, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 2007; **450**: 566-569 [PMID: 18033300 DOI: 10.1038/nature06306]

40 **Collison LW**, Chaturvedi V, Henderson AL, Giacomin PR, Guy C, Bankoti J, Finkelstein D, Forbes K, Workman CJ, Brown SA, Rehg JE, Jones ML, Ni HT, Artis D, Turk MJ, Vignali DA. IL-35-mediated induction of a potent regulatory T cell population. *Nat Immunol* 2010; **11**: 1093-1101 [PMID: 20953201 DOI: 10.1038/ni.1952]

41 **Bardel E**, Larousserie F, Charlot-Rabiega P, Coulomb-L'Herminé A, Devergne O. Human CD4+ CD25+ Foxp3+ regulatory T cells do not constitutively express IL-35. *J Immunol* 2008; **181**: 6898-6905 [PMID: 18981109]

42 **Shi M**, Wei J, Dong J, Meng W, Ma J, Wang T, Wang N, Wang Y. Function of interleukin-17 and -35 in the blood of patients with hepatitis B-related liver cirrhosis. *Mol Med Rep* 2015; **11**: 121-126 [PMID: 25323532 DOI: 10.3892/mmr.2014.2681]

43 **Liu F**, Tong F, He Y, Liu H. Detectable expression of IL-35 in CD4+ T cells from peripheral blood of chronic hepatitis B patients. *Clin Immunol* 2011; **139**: 1-5 [PMID: 21285006 DOI: 10.1016/j.clim.2010.12.012]

44 **Xu D**, Fu J, Jin L, Zhang H, Zhou C, Zou Z, Zhao JM, Zhang B, Shi M, Ding X, Tang Z, Fu YX, Wang FS. Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006; **177**: 739-747 [PMID: 16785573]

45 **Stross L**, Günther J, Gasteiger G, Asen T, Graf S, Aichler M, Esposito I, Busch DH, Knolle P, Sparwasser T, Protzer U. Foxp3+ regulatory T cells protect the liver from immune damage and compromise virus control during acute experimental hepatitis B virus infection in mice. *Hepatology* 2012; **56**: 873-883 [PMID: 22487943 DOI: 10.1002/hep.25765]

46 **Sun XF**, Gu L, Deng WS, Xu Q. Impaired balance of T helper 17/T regulatory cells in carbon tetrachloride-induced liver fibrosis in mice. *World J Gastroenterol* 2014; **20**: 2062-2070 [PMID: 24616573 DOI: 10.3748/wjg.v20.i8.2062]

47 **Yu X**, Guo R, Ming D, Su M, Lin C, Deng Y, Lin Z, Su Z. Ratios of regulatory T cells/T-helper 17 cells and transforming growth factor-β1/interleukin-17 to be associated with the development of hepatitis B virus-associated liver cirrhosis. *J Gastroenterol Hepatol* 2014; **29**: 1065-1072 [PMID: 24236690 DOI: 10.1111/jgh.12459]

48 **Niu Y**, Liu H, Yin D, Yi R, Chen T, Xue H, Zhang S, Lin S, Zhao Y. The balance between intrahepatic IL-17(+) T cells and Foxp3(+) regulatory T cells plays an important role in HBV-related end-stage liver disease. *BMC Immunol* 2011; **12**: 47 [PMID: 21851644 DOI: 10.1186/1471-2172-12-47]

49 **Beyer M**, Schultze JL. Plasticity of T(reg) cells: is reprogramming of T(reg) cells possible in the presence of FOXP3? *Int Immunopharmacol* 2011; **11**: 555-560 [PMID: 21115121 DOI: 10.1016/j.intimp.2010.11.024]

50 **da Silva Martins M**, Piccirillo CA. Functional stability of Foxp3+ regulatory T cells. *Trends Mol Med* 2012; **18**: 454-462 [PMID: 22771168 DOI: 10.1016/j.molmed.2012.06.001]

51 **Kaplan MH**. Th9 cells: differentiation and disease. *Immunol Rev* 2013; **252**: 104-115 [PMID: 23405898 DOI: 10.1111/imr.12028]

52 **Schmitt E**, Klein M, Bopp T. Th9 cells, new players in adaptive immunity. *Trends Immunol* 2014; **35**: 61-68 [PMID: 24215739 DOI: 10.1016/j.it.2013.10.004]

53 **Raphael I**, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. *Cytokine* 2015; **74**: 5-17 [PMID: 25458968 DOI: 10.1016/j.cyto.2014.09.011]

54 **Lai R**, Xiang X, Mo R, Bao R, Wang P, Guo S, Zhao G, Gui H, Wang H, Bao S, Xie Q. Protective effect of Th22 cells and intrahepatic IL-22 in drug induced hepatocellular injury. *J Hepatol* 2015; **63**: 148-155 [PMID: 25681556 DOI: 10.1016/j.jhep.2015.02.004]

55 **Ma CS**, Deenick EK, Batten M, Tangye SG. The origins, function, and regulation of T follicular helper cells. *J Exp Med* 2012; **209**: 1241-1253 [PMID: 22753927 DOI: 10.1084/jem.20120994]

56 **Jia Y**, Zeng Z, Li Y, Li Z, Jin L, Zhang Z, Wang L, Wang FS. Impaired function of CD4+ T follicular helper (Tfh) cells associated with hepatocellular carcinoma progression. *PLoS One* 2015; **10**: e0117458 [PMID: 25689070 DOI: 10.1371/journal.pone.0117458]

57 **Niu YH**, Yin DL, Liu HL, Yi RT, Yang YC, Xue HA, Chen TY, Zhang SL, Lin SM, Zhao YR. Restoring the Treg cell to Th17 cell ratio may alleviate HBV-related acute-on-chronic liver failure. *World J Gastroenterol* 2013; **19**: 4146-4154 [PMID: 23864777 DOI: 10.3748/wjg.v19.i26.4146]

58 **Zhang GL**, Xie DY, Lin BL, Xie C, Ye YN, Peng L, Zhang SQ, Zhang YF, Lai Q, Zhu JY, Zhang Y, Huang YS, Hu ZX, Gao ZL. Imbalance of interleukin-17-producing CD4 T cells/regulatory T cells axis occurs in remission stage of patients with hepatitis B virus-related acute-on-chronic liver failure. *J Gastroenterol Hepatol* 2013; **28**: 513-521 [PMID: 23215950 DOI: 10.1111/jgh.12082]

59 **Liang XS**, Li CZ, Zhou Y, Yin W, Liu YY, Fan WH. Changes in circulating Foxp3(+) regulatory T cells and interleukin-17-producing T helper cells during HBV-related acute-on-chronic liver failure. *World J Gastroenterol* 2014; **20**: 8558-8571 [PMID: 25024610 DOI: 10.3748/wjg.v20.i26.8558]

60 **Xu L**, Gong Y, Wang B, Shi K, Hou Y, Wang L, Lin Z, Han Y, Lu

L, Chen D, Lin X, Zeng Q, Feng W, Chen Y. Randomized trial of autologous bone marrow mesenchymal stem cells transplantation for hepatitis B virus cirrhosis: regulation of Treg/Th17 cells. *J Gastroenterol Hepatol* 2014; **29**: 1620-1628 [PMID: 24942592 DOI: 10.1111/jgh.12653]

61 **Fantini MC**, Rizzo A, Fina D, Caruso R, Becker C, Neurath MF, Macdonald TT, Pallone F, Monteleone G. IL-21 regulates experimental colitis by modulating the balance between Treg and Th17 cells. *Eur J Immunol* 2007; **37**: 3155-3163 [PMID: 17918200 DOI: 10.1002/eji.200737766]

62 **Mucida D**, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, Cheroutre H. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007; **317**: 256-260 [PMID: 17569825 DOI: 10.1126/science.1145697]

63 **Laurence A**, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, Blank RB, Meylan F, Siegel R, Hennighausen L, Shevach EM, O' shea JJ. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity* 2007; **26**: 371-381 [PMID: 17363300 DOI: 10.1016/j.immuni.2007.02.009]

64 **Roh YS**, Park S, Lim CW, Kim B. Depletion of Foxp3+ Regulatory T Cells Promotes Profibrogenic Milieu of Cholestasis-Induced Liver Injury. *Dig Dis Sci* 2015; **60**: 2009-2018 [PMID: 25416630 DOI: 10.1007/s10620-014-3438-2]

65 **Li J**, Shi J, Ren W, Wu W, Chen Z. Regulatory role of CD4(+)CD25(+)Foxp3 (+) regulatory T cells on IL-17-secreting T cells in chronic hepatitis B patients. *Dig Dis Sci* 2014; **59**: 1475-1483 [PMID: 24442238 DOI: 10.1007/s10620-013-3022-1]

66 **Tang Y**, Jiang L, Zheng Y, Ni B, Wu Y. Expression of CD39 on FoxP3+ T regulatory cells correlates with progression of HBV infection. *BMC Immunol* 2012; **13**: 17 [PMID: 22489829 DOI: 10.1186/1471-2172-13-17]

67 **Pandiyan P**, Conti HR, Zheng L, Peterson AC, Mathern DR, Hernández-Santos N, Edgerton M, Gaffen SL, Lenardo MJ. CD4(+)CD25(+)Foxp3(+) regulatory T cells promote Th17 cells in vitro and enhance host resistance in mouse Candida albicans Th17 cell infection model. *Immunity* 2011; **34**: 422-434 [PMID: 21435589 DOI: 10.1016/j.immuni.2011.03.002]

68 **Lin S**, Yang X, Liang D, Zheng SG. Treg cells: a potential regulator for IL-22 expression? *Int J Clin Exp Pathol* 2014; **7**: 474-480 [PMID: 24551268]

69 **Zhou Q**, Hu Y, Howard OM, Oppenheim JJ, Chen X. In vitro generated Th17 cells support the expansion and phenotypic stability of CD4(+)Foxp3(+) regulatory T cells in vivo. *Cytokine* 2014; **65**: 56-64 [PMID: 24080164 DOI: 10.1016/j.cyto.2013.09.008]

70 **Sakaguchi S**, Vignali DA, Rudensky AY, Niec RE, Waldmann H. The plasticity and stability of regulatory T cells. *Nat Rev Immunol* 2013; **13**: 461-467 [PMID: 23681097 DOI: 10.1038/nri3464]

71 **Beriou G**, Costantino CM, Ashley CW, Yang L, Kuchroo VK, Baecher-Allan C, Hafler DA. IL-17-producing human peripheral regulatory T cells retain suppressive function. *Blood* 2009; **113**: 4240-4249 [PMID: 19171879 DOI: 10.1182/blood-2008-10-183251]

72 **Voo KS**, Wang YH, Santori FR, Boggiano C, Wang YH, Arima K, Bover L, Hanabuchi S, Khalili J, Marinova E, Zheng B, Littman DR, Liu YJ. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. *Proc Natl Acad Sci USA* 2009; **106**: 4793-4798 [PMID: 19273860 DOI: 10.1073/pnas.0900408106]

73 **Nyirenda MH**, Sanvito L, Darlington PJ, O'Brien K, Zhang GX, Constantinescu CS, Bar-Or A, Gran B. TLR2 stimulation drives human naive and effector regulatory T cells into a Th17-like phenotype with reduced suppressive function. *J Immunol* 2011; **187**: 2278-2290 [PMID: 21775683 DOI: 10.4049/jimmunol.1003715]

74 **Adjobimey T**, Satoguina J, Oldenburg J, Hoerauf A, Layland LE. Co-activation through TLR4 and TLR9 but not TLR2 skews Treg-mediated modulation of Igs and induces IL-17 secretion in Treg: B cell co-cultures. *Innate Immun* 2014; **20**: 12-23 [PMID: 23529856 DOI: 10.1177/1753425913479414]

75 **Xu L**, Kitani A, Fuss I, Strober W. Cutting edge: regulatory T cells induce CD4+CD25-Foxp3- T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-beta. *J Immunol* 2007; **178**: 6725-6729 [PMID: 17513718]

76 **Cosmi L**, Liotta F, Maggi E, Romagnani S, Annunziato F. Th17 and non-classic Th1 cells in chronic inflammatory disorders: two sides of the same coin. *Int Arch Allergy Immunol* 2014; **164**: 171-177 [PMID: 25033972 DOI: 10.1159/000363502]

77 **Annunziato F**, Santarlasci V, Maggi L, Cosmi L, Liotta F, Romagnani S. Reasons for rarity of Th17 cells in inflammatory sites of human disorders. *Semin Immunol* 2013; **25**: 299-304 [PMID: 24211040 DOI: 10.1016/j.smim.2013.10.011]

78 **Ye J**, Su X, Hsueh EC, Zhang Y, Koenig JM, Hoft DF, Peng G. Human tumor-infiltrating Th17 cells have the capacity to differentiate into IFN-γ+ and FOXP3+ T cells with potent suppressive function. *Eur J Immunol* 2011; **41**: 936-951 [PMID: 21381020 DOI: 10.1002/eji.201040682]

79 **Zheng L**, Chu J, Shi Y, Zhou X, Tan L, Li Q, Cui L, Han Z, Han Y, Fan D. Bone marrow-derived stem cells ameliorate hepatic fibrosis by down-regulating interleukin-17. *Cell Biosci* 2013; **3**: 46 [PMID: 24314294 DOI: 10.1186/2045-3701-3-46]

80 **Wang Q**, Zhou J, Zhang B, Tian Z, Tang J, Zheng Y, Huang Z, Tian Y, Jia Z, Tang Y, van Velkinburgh JC, Mao Q, Bian X, Ping Y, Ni B, Wu Y. Hepatitis B virus induces IL-23 production in antigen presenting cells and causes liver damage via the IL-23/IL-17 axis. *PLoS Pathog* 2013; **9**: e1003410 [PMID: 23825942 DOI: 10.1371/journal.ppat.1003410]

81 **Hammerich L**, Tacke F. Interleukins in chronic liver disease: lessons learned from experimental mouse models. *Clin Exp Gastroenterol* 2014; **7**: 297-306 [PMID: 25214799 DOI: 10.2147/CEG.S43737]

82 **Thompson K**, Maltby J, Fallowfield J, McAulay M, Millward-Sadler H, Sheron N. Interleukin-10 expression and function in experimental murine liver inflammation and fibrosis. *Hepatology* 1998; **28**: 1597-1606 [PMID: 9828224 DOI: 10.1002/hep.510280620]

83 **Hung KS**, Lee TH, Chou WY, Wu CL, Cho CL, Lu CN, Jawan B, Wang CH. Interleukin-10 gene therapy reverses thioacetamide-induced liver fibrosis in mice. *Biochem Biophys Res Commun* 2005; **336**: 324-331 [PMID: 16126171 DOI: 10.1016/j.bbrc.2005.08.085]

84 **Xiang XG**, Xie Q. IL-35: a potential therapeutic target for controlling hepatitis B virus infection. *J Dig Dis* 2015; **16**: 1-6 [PMID: 25476593 DOI: 10.1111/1751-2980.12218]

85 **Tsuda M**, Zhang W, Yang GX, Tsuneyama K, Ando Y, Kawata K, Park O, Leung PS, Coppel RL, Ansari AA, Ridgway WM, Gao B, Lian ZX, Flavell R, He XS, Gershwin ME. Deletion of interleukin (IL)-12p35 induces liver fibrosis in dominant-negative TGFβ receptor type II mice. *Hepatology* 2013; **57**: 806-816 [PMID: 22576253 DOI: 10.1002/hep.25829]

86 **Wang J**, Cai Y, Ji H, Feng J, Ayana DA, Niu J, Jiang Y. Serum IL-33 levels are associated with liver damage in patients with chronic hepatitis B. *J Interferon Cytokine Res* 2012; **32**: 248-253 [PMID: 22304300 DOI: 10.1089/jir.2011.0109]

87 **Marvie P**, Lisbonne M, L'helgoualc'h A, Rauch M, Turlin B, Preisser L, Bourd-Boittin K, Théret N, Gascan H, Piquet-Pellorce C, Samson M. Interleukin-33 overexpression is associated with liver fibrosis in mice and humans. *J Cell Mol Med* 2010; **14**: 1726-1739 [PMID: 19508382 DOI: 10.1111/j.1582-4934.2009.00801.x]

88 **Schmitz J**, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005; **23**: 479-490 [PMID: 16286016 DOI: 10.1016/j.immuni.2005.09.015]

89 **Volarevic V**, Mitrovic M, Milovanovic M, Zelen I, Nikolic I, Mitrovic S, Pejnovic N, Arsenijevic N, Lukic ML. Protective role of IL-33/ST2 axis in Con A-induced hepatitis. *J Hepatol* 2012; **56**: 26-33 [PMID: 21703183 DOI: 10.1016/j.jhep.2011.03.022]

90 **Erhardt A**, Tiegs G. IL-33--a cytokine which balances on a knife's edge? *J Hepatol* 2012; **56**: 7-10 [PMID: 21703171 DOI: 10.1016/j.jhep.2011.05.007]

91 **Zhao PW**, Shi X, Li C, Ayana DA, Niu JQ, Feng JY, Wang J, Jiang YF. IL-33 Enhances Humoral Immunity Against Chronic HBV Infection Through Activating CD4(+)CXCR5(+) TFH Cells. *J Interferon Cytokine Res* 2015; **35**: 454-463 [PMID: 25714983 DOI:

10.1089/jir.2013.0122]
92 **Barboza L**, Salmen S, Peterson DL, Montes H, Colmenares M, Hernández M, Berrueta-Carrillo LE, Berrueta L. Altered T cell costimulation during chronic hepatitis B infection. *Cell Immunol* 2009; **257**: 61-68 [PMID: 19345343 DOI: 10.1016/j.cellimm.2009.02.008]
93 **Wang L**, Zhao C, Peng Q, Shi J, Gu G. Expression levels of CD28, CTLA-4, PD-1 and Tim-3 as novel indicators of T-cell immune function in patients with chronic hepatitis B virus infection. *Biomed Rep* 2014; **2**: 270-274 [PMID: 24649109 DOI: 10.3892/br.2014.217]
94 **Ye B**, Liu X, Li X, Kong H, Tian L, Chen Y. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. *Cell Death Dis* 2015; **6**: e1694 [PMID: 25789969 DOI: 10.1038/cddis.2015.42]
95 **Raziorrouh B**, Ulsenheimer A, Schraut W, Heeg M, Kurktschiev P, Zachoval R, Jung MC, Thimme R, Neumann-Haefelin C, Horster S, Wächtler M, Spannagl M, Haas J, Diepolder HM, Grüner NH. Inhibitory molecules that regulate expansion and restoration of HCV-specific CD4+ T cells in patients with chronic infection. *Gastroenterology* 2011; **141**: 1422-1431, 1431.e1-6 [PMID: 21763239 DOI: 10.1053/j.gastro.2011.07.004]
96 **Keir ME**, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* 2008; **26**: 677-704 [PMID: 18173375 DOI: 10.1146/annurev.immunol.26.021607.090331]
97 **Xu P**, Chen YJ, Chen H, Zhu XY, Song HF, Cao LJ, Wang XF. The expression of programmed death-1 in circulating CD4+ and CD8+ T cells during hepatitis B virus infection progression and its correlation with clinical baseline characteristics. *Gut Liver* 2014; **8**: 186-195 [PMID: 24672661 DOI: 10.5009/gnl.2014.8.2.186]
98 **Raziorrouh B**, Heeg M, Kurktschiev P, Schraut W, Zachoval R, Wendtner C, Wächtler M, Spannagl M, Denk G, Ulsenheimer A, Bengsch B, Pircher H, Diepolder HM, Grüner NH, Jung MC. Inhibitory phenotype of HBV-specific CD4+ T-cells is characterized by high PD-1 expression but absent coregulation of multiple inhibitory molecules. *PLoS One* 2014; **9**: e105703 [PMID: 25144233 DOI: 10.1371/journal.pone.0105703]
99 **Hastings WD**, Anderson DE, Kassam N, Koguchi K, Greenfield EA, Kent SC, Zheng XX, Strom TB, Hafler DA, Kuchroo VK. TIM-3 is expressed on activated human CD4+ T cells and regulates Th1 and Th17 cytokines. *Eur J Immunol* 2009; **39**: 2492-2501 [PMID: 19676072 DOI: 10.1002/eji.200939274]
100 **Ju Y**, Shang X, Liu Z, Zhang J, Li Y, Shen Y, Liu Y, Liu C, Liu B, Xu L, Wang Y, Zhang B, Zou J. The Tim-3/galectin-9 pathway involves in the homeostasis of hepatic Tregs in a mouse model of concanavalin A-induced hepatitis. *Mol Immunol* 2014; **58**: 85-91 [PMID: 24333756 DOI: 10.1016/j.molimm.2013.11.001]
101 **Wang F**, Wan L, Zhang C, Zheng X, Li J, Chen ZK. Tim-3-Galectin-9 pathway involves the suppression induced by CD4+CD25+ regulatory T cells. *Immunobiology* 2009; **214**: 342-349 [PMID: 19362679 DOI: 10.1016/j.imbio.2008.10.007]
102 **Wu W**, Shi Y, Li J, Chen F, Chen Z, Zheng M. Tim-3 expression on peripheral T cell subsets correlates with disease progression in hepatitis B infection. *Virol J* 2011; **8**: 113 [PMID: 21392402 DOI: 10.1186/1743-422X-8-113]
103 **Uyangaa E**, Choi JY, Patil AM, Kim JH, Kim SB, Kim K, Ryu HW, Oh SR, Eo SK. Functional restoration of exhausted CD4(+) and CD8(+) T cells in chronic viral infection by vinegar-processed flos of Daphne genkwa. *Comp Immunol Microbiol Infect Dis* 2015; **39**: 25-37 [PMID: 25744061 DOI: 10.1016/j.cimid.2015.02.001]
104 **Jin HT**, Anderson AC, Tan WG, West EE, Ha SJ, Araki K, Freeman GJ, Kuchroo VK, Ahmed R. Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. *Proc Natl Acad Sci USA* 2010; **107**: 14733-14738 [PMID: 20679213 DOI: 10.1073/pnas.1009731107]
105 **Carreno BM**, Bennett F, Chau TA, Ling V, Luxenberg D, Jussif J, Baroja ML, Madrenas J. CTLA-4 (CD152) can inhibit T cell activation by two different mechanisms depending on its level of cell surface expression. *J Immunol* 2000; **165**: 1352-1356 [PMID: 10903737]
106 **Ubaldi V**, Gatta L, Pace L, Doria G, Pioli C. CTLA-4 engagement inhibits Th2 but not Th1 cell polarisation. *Clin Dev Immunol* 2003; **10**: 13-17 [PMID: 14575153]
107 **Chen M**, Chang Y, Tang F, Xie QH, Li J, Yang H, He XX, Lin JS. Influence of cytotoxic T lymphocyte-associated antigen 4 polymorphisms on the outcomes of hepatitis B virus infection. *Mol Med Rep* 2014; **9**: 645-652 [PMID: 24270470 DOI: 10.3892/mmr.2013.1825]
108 **Yu Y**, Wu H, Tang Z, Zang G. CTLA4 silencing with siRNA promotes deviation of Th1/Th2 in chronic hepatitis B patients. *Cell Mol Immunol* 2009; **6**: 123-127 [PMID: 19403062 DOI: 10.1038/cmi.2009.17]
109 **Mohammad Alizadeh AH**, Hajilooi M, Ranjbar M, Fallahian F, Mousavi SM. Cytotoxic T-lymphocyte antigen 4 gene polymorphisms and susceptibility to chronic hepatitis B. *World J Gastroenterol* 2006; **12**: 630-635 [PMID: 16489681]
110 **Sangro B**, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, Barrera P, Riezu-Boj JI, Larrea E, Alfaro C, Sarobe P, Lasarte JJ, Pérez-Gracia JL, Melero I, Prieto J. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013; **59**: 81-88 [PMID: 23466307 DOI: 10.1016/j.jhep.2013.02.022]
111 **Nakayama Y**, Shimizu Y, Hirano K, Ebata K, Minemura M, Watanabe A, Sugiyama T. CTLA-4Ig suppresses liver injury by inhibiting acquired immune responses in a mouse model of fulminant hepatitis. *Hepatology* 2005; **42**: 915-924 [PMID: 16175605 DOI: 10.1002/hep.20872]
112 **Guillot A**, Hamdaoui N, Bizy A, Zoltani K, Souktani R, Zafrani ES, Mallat A, Lotersztajn S, Lafdil F. Cannabinoid receptor 2 counteracts interleukin-17-induced immune and fibrogenic responses in mouse liver. *Hepatology* 2014; **59**: 296-306 [PMID: 23813495 DOI: 10.1002/hep.26598]
113 **Basu S**, Dittel BN. Unraveling the complexities of cannabinoid receptor 2 (CB2) immune regulation in health and disease. *Immunol Res* 2011; **51**: 26-38 [PMID: 21626285 DOI: 10.1007/s12026-011-8210-5]
114 **Teixeira-Clerc F**, Belot MP, Manin S, Deveaux V, Cadoudal T, Chobert MN, Louvet A, Zimmer A, Tordjmann T, Mallat A, Lotersztajn S. Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration. *Hepatology* 2010; **52**: 1046-1059 [PMID: 20597071 DOI: 10.1002/hep.23779]
115 **Li J**, Wang FP, She WM, Yang CQ, Li L, Tu CT, Wang JY, Jiang W. Enhanced high-mobility group box 1 (HMGB1) modulates regulatory T cells (Treg)/T helper 17 (Th17) balance via toll-like receptor (TLR)-4-interleukin (IL)-6 pathway in patients with chronic hepatitis B. *J Viral Hepat* 2014; **21**: 129-140 [PMID: 24383926 DOI: 10.1111/jvh.12152]

P- Reviewer: Shimizu Y **S- Editor**: Ma YJ **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.v21.i38.10732

World J Gastroenterol 2015 October 14; 21(38): 10732-10738
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis B virus

Molecular mechanism of hepatitis B virus X protein function in hepatocarcinogenesis

Ming Geng, Xuan Xin, Li-Quan Bi, Lu-Ting Zhou, Xiao-Hong Liu

Ming Geng, Xuan Xin, Li-Quan Bi, Lu-Ting Zhou, Xiao-Hong Liu, Department of Pathology, General Hospital of Jinan Military Command, Jinan 250031, Shandong Province, China

Author contributions: Geng M and Xin X contributed equally to this work; all authors approved the final manuscript.

Supported by National Science Foundation of China, No. 81172261.

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

Correspondence to: Xiao-Hong Liu, MD, PhD, Department of Pathology, General Hospital of Jinan Military Command, Shifang Road 25, Jinan 250031, Shandong Province, China. liuxh333@126.com
Telephone: +86-531-51666857
Fax: +86-531-51666284

Received: April 28, 2015
Peer-review started: May 6, 2015
First decision: June 2, 2015
Revised: June 24, 2015
Accepted: September 2, 2015
Article in press: September 2, 2015
Published online: October 14, 2015

Abstract

Many factors are considered to contribute to hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC), including products of HBV, HBV integration and mutation, and host susceptibility. HBV X protein (HBx) can interfere with several signaling pathways associated with cell proliferation and invasion, and HBx C-terminal truncation has been suggested to impact the development of HCC. This review focuses on the pathological functions of HBx in HBV-induced hepatocarcinogenesis. As a transactivator, HBx can affect regulatory non-coding RNAs (ncRNAs), including microRNAs and long ncRNAs. HBx is also involved in epigenetic modification and DNA repair. HBx interacts with various signal-transduction pathways, such as the p53, Wnt, and nuclear factor-κB pathways. We conclude that HBx hastens the development of hepatoma.

Key words: Hepatocellular carcinoma; Hepatitis B virus; Hepatitis B virus X protein; Hepatocarcinogenesis

Core tip: The mechanisms underlying hepatitis B virus (HBV)-induced malignant transformation remain ambiguous, but research has suggested that HBV X (HBx) protein has a crucial function in the pathogenesis of hepatocellular carcinoma. This review focuses on the pathological functions of HBx in HBV-induced hepatocarcinogenesis.

Geng M, Xin X, Bi LQ, Zhou LT, Liu XH. Molecular mechanism of hepatitis B virus X protein function in hepatocarcinogenesis. *World J Gastroenterol* 2015; 21(38): 10732-10738 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10732.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10732

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most

common cause of cancer mortality[1]. Chronic hepatitis B virus (HBV) infection has been demonstrated to be a risk factor for liver carcinogenesis, accounting for 55% of cases worldwide. Notably, 80% or more of such cases are found in the eastern Pacific region and sub-Saharan Africa, areas with the highest tumor incidence[2,3]. The mechanisms underlying HBV-induced malignant transformation remain ambiguous, but previous research has suggested that HBV X (HBx) protein has a crucial role in the pathogenesis of HCC[4]. Here, we review the molecular mechanisms of HBx in the pathogenesis of HCC.

HBx GENE AND HBx PROTEIN

HBV is considered to be the smallest DNA virus and contains a 3.2 kb circular double-stranded viral DNA genome, including a long minus-strand that is complementary to viral mRNA sequences and a short plus-strand. The open reading frame (ORF) of HBx is 465 bp long, from nucleotides 1376 to 1837, and is translated into a 154 amino acid (aa) protein. The *HBx* gene is located upstream of gene *C* and close to the sticky end of the viral genome, where it also overlaps with other genes, including viral polymerase, Pre C, ORF5, and ORF6. Although HBx cannot directly bind to the DNA helix, it can activate other protein factors to further bind to their or other promoters and enhancers. Thus, HBx can trans-regulate gene transcription[5].

The plus-strand HBx viral genome contains several transcriptional regulation element sequences, including gene expression basic core promoter, core upstream regulatory sequence, negative regulatory element, enhancer Ⅱ, direct repeat 1 (DR1), and DR2. Also, the 5′ end of the *HBx* gene overlaps with the ORF of DNA polymerase P[6,7]. Thus, the *X* gene of HBV contains the longest overlapping region between structural and functional sequences in the viral genome. More importantly, because of the overlap between the coding region and regulation elements in the *X* gene of HBV, any DNA mutation and/or deletion can affect functionally both gene and transcriptional regulation.

HBx AND DNA REPAIR

Current studies indicate that DNA repair is one of the driving mechanisms of carcinogenesis. Accumulation of DNA damage causes genomic instability and eventually leads to mutations. Recent studies showed that the expression level of HBx positively correlated with that of 8-hydroxy-2 deoxyguanosine (8-OHdG), a key oxidative stress indicator that causes DNA mis-pairing. Meanwhile, a high level of HBx inhibited human DNA glycosylase α activity, which caused suppression of DNA repair machinery, long-term DNA damage, and tumorigenesis[8].

Jung *et al*[9] reported that HBx with C terminal truncation does not induce reactive oxygen species (ROS) production and has no effect on level of 8-OHdG. This indicated an important role for the HBx C terminal region in oxidative stress-induced ROS production, consequential mitochondrial DNA damage, and HCC pathogenesis. Another study also reported that HBx can regulate p53 expression and further depress the DNA repair capability[10].

HBx AND METHYLATION

Epigenetic studies allow us to understand how DNA methyltransferases (DNMTs) involved in DNA methylation can control gene expression through chromatin structural modification, changes in regional DNA accessibility, changes in DNA stability, and shifts in DNA-protein interactions. HBx can affect the cell cycle, proliferation, invasion, apoptosis, *etc*. of HCC cells by regulating DNMTs involved in DNA methylation of specific genes. A recent publication demonstrated that HBx can upregulate DNMT1 and DNMT3A through transactivation[11]. Wei *et al*[12] demonstrated that downregulation of miR-101 by HBx can lead to abnormal DNA methylation by miR-101-targeting of DNMT3A and promotion of HCC malignancy. A similar study showed that HBx upregulated DNMT1 and DNMT3A at both the transcriptional and translational levels, leading to induction of p16 (INK4A) promoter methylation and subsequent inhibition of p16 expression[13].

HBx AND NON-CODING RNAs

Non-coding RNAs (ncRNAs) compose a large group of RNAs transcribed from non-coding regions of the human genome. ncRNAs account for about 90% of the genome and can be categorized in two types: 18-200 nucleotide small ncRNAs, including microRNAs (miRNAs), small interfering (siRNAs), Piwi-interacting RNAs, small nuclear RNAs, small nucleolar RNAs, *etc.*; 200 nucleotide to 100 kb long ncRNAs (lncRNAs), including mRNA-like ncRNAs, long no-poly A tail ncRNAs, *etc.*[14,15]. Most of these RNAs have been rarely studied, and although their functions remain entirely unclear, they have a variety of important biological functions.

MiRNAs play a critical role in the control of gene expression and signal transduction in HCC carcinogenesis. Several *in vitro* studies demonstrated that HBx can promote early stage HCC progression by inducing high levels of miR-21 expression, which inhibiteds programmed cell death 4 in cancer cells[16,17]. Upregulated miR-21 and miR-222 also can directly target tumor suppressor p27 and Kipl, a key regulator of the cell cycle, to contribute to cancer progression[18]. In a previous study, Bandopadhyay *et al*[19] found that miR-21 and miR-222 were downregulated when HepG2 cells were transfected with HBx and HBV plasmid DNA or HepG2.2.15 cells were infected with HBV. This result was confirmed in clinical plasma samples from HCC patients. Interestingly, similar downregulated effects

also were observed in transfected HepG2 cells and patients' plasma for miR-145, whereas miR-145 was upregulated in an infected HepG2.2.15 cell line. These results suggested that HBx can control multiple miRNAs in different manners to promote HCC progression[19]. Additionally, an animal model showed that HBx inhibited the tumor suppressor p53 to control the expression of miR-148a and to increase the expression of hematopoietic pre-B cell leukemia transcription factor-interacting protein. This resulted in activation of Akt, extracellular-related kinase, and mammalian target of rapamycin signaling pathways to enhance tumor cell growth, invasion, and metastasis[20]. A recent study also showed that HBx can downregulate miR-192, suggesting that HBx may be anti-apoptotic in HCC[21].

lncRNAs play crucial roles in human cancers. It has been reported that the lncRNA highly up-regulated in liver cancer (HULC) was dramatically upregulated in HCC[22]. Du *et al*[23] reported that HBx can increase expression of HULC *via* the cAMP-response element binding protein activated promoter of lncRNA HULC. Furthermore, downregulation of P18, a gene downstream of HULC, can promote liver cell proliferation. Another lncRNA (termed lncRNA-Dreh) can be downregulated by HBx, which enhanced HCC cell invasion and migration *in vitro*[24]. It is known that deregulation of lncRNA is one of the key factors in HCC tumor initiation and progression.

HBx MUTANTS AND TUMOR IMITATION

HBV infection-induced HCC usually occurs within 10-30 years after the initial HBV infection. During this period, mutations of the HBV genome accumulate. Two dominant types of HBx mutations can be detected in chronic hepatitis: type Ⅰ are single nucleotide mutations at multiple sites, and type Ⅱ are C-terminal truncations that cause relatively higher levels of protein accumulation in the tumor region. Liver cells with these two types of mutations may have proliferative advantage in colony formation.

Previous studies have shown that HBV genome integration is random, and there are no specific integration sites or rules. HBx and HBV core gene (HBc) mutations and deletions commonly occur in viral genome integration[25-28]. A polymerase chain reaction DNA amplification study of 45 tumor samples and sequencing results of 19 samples showed a high frequency of HBx mutation in HCC. Those mutations were mostly located close to the carboxyl terminus. It is believed that a strong correlation exists between HBx mutation and liver cell cancer transformation[29]. Similarly, we determined that the hot spot of HBx mutation is highly regional. Blood tests of HBx mutations from patients in Europe and Africa showed a higher incidence of mutation at 130 and 131 aa of HBx for mild hepatitis patients and accumulation of HBx C-terminal truncation in HCC peri-tumor tissues[30-34]. In contrast, a study of 153 HCC patients from Vietnam showed more 130 and 131 aa mutations in tumor tissue, with only four out of 48 samples having HBx C-terminal truncation accumulation[35]. A report from Hong Kong claimed that more than 54 mutations were detected in 95.2% of tissue samples and 95.3% of blood samples from 113 patients, where there was at least one mutation in most of the samples[36]. There were 12 mutation sites in tissue samples and nine mutation sites in blood samples, which suggested a mutation-driven pathogenesis for HCC. Another study demonstrated that mutations were complicated and changeable in both HCC and peri-carcinoma liver tissue (PCLT). C-terminal truncation is more frequently found in HCC than in benign liver tissues. However, there is no single site mutation of a nucleic acid or amino acid that results in a distribution discrepancy between HCC and PCLT[37]. The reports described above indicated a regional distribution of HBx mutants, which reflects the high degree of complexity of HBV caused HCC.

The results of a comparative study between HBx C-terminal truncation and full-length HBx transfection indicated that each mutation plays a different role in cancer cell biology[38,39]. Specifically, overexpression of HBx 20 aa and 40 aa C-terminal deletion mutants can enhance cell growth, colony formation, tumor volume, and G1 to S phase cell cycle transition. In contrast, an HBx 30 aa C-terminal deletion mutant can inhibit cell proliferation. These results suggested that 125-134 aa of HBx is important for cell proliferation. More recent studies showed that HBx spontaneous deletion mutations were typically located in the same region. Liu *et al*[40] and Wang *et al*[41] reported that the HBx 127 mutant contributed to tumor cell proliferation metastasis more than wild-type HBx by promoting cell growth through a positive feedback loop involving 5-lipoxygenase, fatty acid synthase, and miR-215. This finding is consistent with a report from Fu *et al*[42] that concluded that the HBx-d382 deletion mutant (128-145 aa) enhanced cell proliferation. The dual mutations K130M/V131I strengthened the capability of HBx, as they upregulated the expression and transcriptional activity of hypoxia-inducible factor 1-alpha (HIF-1α). The C-terminal truncation and deletion mutations, however, weakened the ability of HBx to upregulate HIF-1α. Furthermore, the C-terminus was found to be essential for HBx stability and transactivation. A positive correlation was found between the HBx mutants and HIF-1α expression in clinical HCC samples[43]. In brief, it is believed that C-terminal truncation and deletion promoted tumor malignancy. However, the detailed mechanism needs to be investigated further.

HBx AND THE P53 SIGNALING PATHWAY

Mutations in the tumor suppressor gene *p53* are the most common in all types of cancers. p53 disorder plays an important role in the tumorigenesis of HCC.

Many studies have indicated a complex transactivation between HBx and p53, where HBx directly inhibits p53 activity by binding to its C-terminus[44]. In addition, overexpression of the p53 target gene murine double minute 2 can induce degradation of HBx in HCC[45]. Kew *et al*[46] investigated the effect of wild-type and mutant HBx on p53 and found that HBx mutants, but not wild-type HBx, can inhibit p53 expression and its downstream signaling.

Recent studies suggested that overexpression of a HBx C-terminal mutant in HHT4 cells, a normal liver cell line, significantly increased the colony forming efficiency (CFE), whereas its corresponding wild-type allele CNT significantly decreased the CFE in HHT4 cells. Meanwhile, the p53-249Ser mutant interacted with HBx mutants to regulate cell proliferation and mitochondrial stability[47]. A report from another group showed that the HBx gene overlapped with the HBV core promoter region. Thus, core promoter mutations can also lead to HBx mutants that further upregulate S-phase kinase-associated protein 2 (SKP2). SKP2 can downregulate p53 though ubiquitination and consequentially promote tumorigenesis[48].

HBx AND THE NUCLEAR FACTOR-κB SIGNALING PATHWAY

Nuclear factor (NF)-κB is one of the driving transcriptional factors in cancer biology and participates in cross talk with multiple pathways to control tumor initiation, development, invasion, and metastasis. Previous studies showed that HBx interacts with NF-κB to increase the expression of metastasis-associated protein 1 (MTA1). MTA1 is a major chromatin modulator that plays important roles in inflammation and tumor initiation. NF-κB cross talk with Notch signaling has also been demonstrated, and Notch 1 signaling can be blocked by HBx transfection in the normal liver cell line L02[49]. Lim *et al*[50] demonstrated that endogenous P22-FLIP, a cleavage product of c-FLIP$_L$, can interact with HBx to activate NF-κB signaling. Further investigation showed that P22-FLIP, HBx, and NEMO, a regulatory subunit of IκB kinase (IKK), also known as IKKγ, can form a trimer complex to activate NF-κB signaling and promote tumor formation.

Lee *et al*[51] showed that NF-κB is highly associated with HBx131, HBx130, HBx5, HBx94, and HBx38 mutants as well as the HBx130-HBx131 double mutation and the HBx5-HBx130-HBx131 triple mutation. These double and triple mutations increased HCC incidence to 3.75 and 5.34 times the normal risk level, respectively. HBx5 mutants and double mutants showed much higher NF-κB activity than wild-type and triple-mutation HBx. Notably, triple-mutation HBx cannot enhance NF-κB activity.

Many studies have demonstrated that HBx can promote HCC cell invasion and metastasis through NF-κB signaling. Zhang *et al*[52] reported that HBx activated NF-κB binding to the calpain small subunit 1 (Capn4) promoter and, thus, upregulated expression of Capn4 in HCC cell. This HBx-induced Capn4 upregulation can be significantly blocked by specific siRNA knockdown of NF-κB or pyrrolidinedithiocarbamic acid (PDTC). Studies from other groups also showed that HBx increased the expression of NF-κB target genes, including vascular endothelial growth factor (VEGF), matrix metalloproteinase 2 (MMP2), MMP9, and MMP14. In addition, PDTC inhibited HBx stimulation of NF-κB signaling, which led to a decrease in the expression of VEGF, MMP9, and MMP14 but not MMP2. PDTC also showed an anti-angiogenic effect in HepG2 tumor xenograft nude mice. These results demonstrated that HBx promoted tumor cell invasion, angiogenesis, and metastasis by activating NF-κB signaling and upregulating downstream target genes VEGF and MMPs[53]. HBx also can associate with peroxidase to enhance the level of ROS. This led to greater activation of NF-κB and the formation of a positive feedback loop in cancer cells. Peroxidase-associated HBx upregulated MMPs and downregulated E-cadherin to enhance tumor cell invasion[54].

HBx AND THE Wnt SIGNALING PATHWAY

Highly preserved Wnt signaling has important functions in embryo development, and abnormal Wnt signaling can stimulate tumorigenesis. Wnt signaling molecules can be divided in two categories: (1) canonical Wnt/β-catenin signaling molecules, including Wnt-1, Wnt-3a, Wnt-8a Wnt-8b, *etc.*[55]; and (2) non-canonical Wnt signaling molecules, including Wnt-4, Wnt5a, Wnt-11[56], as well as Wnt/Ca^{2+}, Wnt/planar cell polarity, and others[57,58].

Many studies have shown that HBx competitively binds to adenomatous polyposis coli to disassociate β-catenin from its degradation complex, resulting in nuclear β-catenin accumulation and activation of Wnt signaling to induce tumor transformation[59]. In addition, overexpression of HBx with Wnt-1 can activate Wnt/β-catenin signaling in Huh7 cells by stabilizing cytoplasmic β-catenin. Furthermore, stabilization of β-catenin by HBx can be achieved by inhibiting glycogen synthase kinase 3 activity *via* the activation of Src kinase[60].

Liu *et al*[61], Geng *et al*[62] and Lin *et al*[63] found that the Wnt5a gene is regulated by HBx mutants through gene expression library screening. Further research showed that Wnt-5a may suppress tumor progression in HBV-induced HCC[61-63]. An immunohistochemical study of 114 HCC samples demonstrated that Wnt-5a as well as its receptor, receptor tyrosine kinase-like orphan receptor 2 (ROR2), were downregulated in 80.7% (92/114) of samples. The expression of Wnt-5a was negatively correlated with β-catenin expression and positively correlated with E-cadherin

expression. Thus, the expression of Wnt-5a and ROR2 is associated with patient prognosis. Huh7 HCC cells transfected with Wnt-5a have a decreased proliferation rate, and Wnt-5a siRNA knockdown can increase cell proliferation[64]. These findings suggested that HBx mutants can control tumor growth *via* signaling through the Wnt pathway.

CONCLUSION

HBx is the only expressed HBV viral protein in malignant HCC and has been shown to be a key molecule in HCC carcinogenesis. However, the molecular mechanism of HBx-induced HCC progression remains unclear. HBx is maintained as an important player in HCC tumorigenesis. HBx functions in HCC through its nuclear translocation, protein-protein interactions, regulation of transcription factors, induction of chromosome instability, and nuclear localized HBx-involved signal transduction, thereby controlling cancer cell proliferation, transformation, invasion, and metastasis. After studying HBx mutants and their associated molecular pathways, it is clear that these mutants have different biological functions and activities compared to wild-type HBx and that they may play important regulatory roles in the pathogenesis of HCC.

REFERENCES

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

2 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73 [PMID: 16391218]

3 **Kew MC**. Epidemiology of chronic hepatitis B virus infection, hepatocellular carcinoma, and hepatitis B virus-induced hepatocellular carcinoma. *Pathol Biol* (Paris) 2010; **58**: 273-277 [PMID: 20378277 DOI: 10.1016/j.patbio.2010.01.005]

4 **Zhang XD**, Wang Y, Ye LH. Hepatitis B virus X protein accelerates the development of hepatoma. *Cancer Biol Med* 2014; **11**: 182-190 [PMID: 25364579 DOI: 10.7497/j.issn.2095-3941.2014.03.004]

5 **Seeger C**, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; **64**: 51-68 [PMID: 10704474]

6 **Venard V**, Corsaro D, Kajzer C, Bronowicki JP, Le Faou A. Hepatitis B virus X gene variability in French-born patients with chronic hepatitis and hepatocellular carcinoma. *J Med Virol* 2000; **62**: 177-184 [PMID: 11002246]

7 **Kramvis A**, Kew MC. The core promoter of hepatitis B virus. *J Viral Hepat* 1999; **6**: 415-427 [PMID: 10607259]

8 **Cheng B**, Zheng Y, Guo X, Wang Y, Liu C. Hepatitis B viral X protein alters the biological features and expressions of DNA repair enzymes in LO2 cells. *Liver Int* 2010; **30**: 319-326 [PMID: 19968784 DOI: 10.1111/j.1478-3231.2009.02167.x]

9 **Jung SY**, Kim YJ. C-terminal region of HBx is crucial for mitochondrial DNA damage. *Cancer Lett* 2013; **331**: 76-83 [PMID: 23246371 DOI: 10.1016/j.canlet.2012.12.004]

10 **Capovilla A**, Carmona S, Arbuthnot P. Hepatitis B virus X-protein binds damaged DNA and sensitizes liver cells to ultraviolet irradiation. *Biochem Biophys Res Commun* 1997; **232**: 255-260 [PMID: 9125143]

11 **Tian Y**, Yang W, Song J, Wu Y, Ni B. Hepatitis B virus X protein-induced aberrant epigenetic modifications contributing to human hepatocellular carcinoma pathogenesis. *Mol Cell Biol* 2013; **33**: 2810-2816 [PMID: 23716588 DOI: 10.1128/MCB.00205-13]

12 **Wei X**, Xiang T, Ren G, Tan C, Liu R, Xu X, Wu Z. miR-101 is down-regulated by the hepatitis B virus x protein and induces aberrant DNA methylation by targeting DNA methyltransferase 3A. *Cell Signal* 2013; **25**: 439-446 [PMID: 23124077 DOI: 10.1016/j.cellsig.2012.10.013]

13 **Zhu YZ**, Zhu R, Shi LG, Mao Y, Zheng GJ, Chen Q, Zhu HG. Hepatitis B virus X protein promotes hypermethylation of p16(INK4A) promoter through upregulation of DNA methyltransferases in hepatocarcinogenesis. *Exp Mol Pathol* 2010; **89**: 268-275 [PMID: 20620135 DOI: 10.1016/j.yexmp.2010.06.013]

14 **Nana-Sinkam SP**, Croce CM. Non-coding RNAs in cancer initiation and progression and as novel biomarkers. *Mol Oncol* 2011; **5**: 483-491 [PMID: 22079056 DOI: 10.1016/j.molonc.2011.10.003]

15 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438]

16 **Li CH**, Xu F, Chow S, Feng L, Yin D, Ng TB, Chen Y. Hepatitis B virus X protein promotes hepatocellular carcinoma transformation through interleukin-6 activation of microRNA-21 expression. *Eur J Cancer* 2014; **50**: 2560-2569 [PMID: 25087183 DOI: 10.1016/j.ejca.2014.07.008]

17 **Qiu X**, Dong S, Qiao F, Lu S, Song Y, Lao Y, Li Y, Zeng T, Hu J, Zhang L, Zhang L, Fan H. HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene* 2013; **32**: 3296-3305 [PMID: 23604124 DOI: 10.1038/onc.2013.150]

18 **Galardi S**, Mercatelli N, Giorda E, Massalini S, Frajese GV, Ciafrè SA, Farace MG. miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. *J Biol Chem* 2007; **282**: 23716-23724 [PMID: 17569667]

19 **Bandopadhyay M**, Banerjee A, Sarkar N, Panigrahi R, Datta S, Pal A, Singh SP, Biswas A, Chakrabarti S, Chakravarty R. Tumor suppressor micro RNA miR-145 and onco micro RNAs miR-21 and miR-222 expressions are differentially modulated by hepatitis B virus X protein in malignant hepatocytes. *BMC Cancer* 2014; **14**: 721 [PMID: 25260533 DOI: 10.1186/1471-2407-14-721]

20 **Xu X**, Fan Z, Kang L, Han J, Jiang C, Zheng X, Zhu Z, Jiao H, Lin J, Jiang K, Ding L, Zhang H, Cheng L, Fu H, Song Y, Jiang Y, Liu J, Wang R, Du N, Ye Q. Hepatitis B virus X protein represses miRNA-148a to enhance tumorigenesis. *J Clin Invest* 2013; **123**: 630-645 [PMID: 23321675 DOI: 10.1172/JCI64265]

21 **Xie QH**, He XX, Chang Y, Jiang X, Lin JS. [HBx gene down-regulates miR-192 expression and inhibits apoptosis of human hepatoma cell line HepG2]. *Zhonghua Gan Zang Bing Zazhi* 2011; **19**: 857-860 [PMID: 22433310 DOI: 10.3760/cma.j.issn.1007-3418.2011.11.015]

22 **He Y**, Meng XM, Huang C, Wu BM, Zhang L, Lv XW, Li J. Long noncoding RNAs: Novel insights into hepatocelluar carcinoma. *Cancer Lett* 2014; **344**: 20-27 [PMID: 24183851 DOI: 10.1016/j.canlet.2013.10.021]

23 **Du Y**, Kong G, You X, Zhang S, Zhang T, Gao Y, Ye L, Zhang X. Elevation of highly up-regulated in liver cancer (HULC) by hepatitis B virus X protein promotes hepatoma cell proliferation via down-regulating p18. *J Biol Chem* 2012; **287**: 26302-26311 [PMID: 22685290 DOI: 10.1074/jbc.M112.342113]

24 **Huang JF**, Guo YJ, Zhao CX, Yuan SX, Wang Y, Tang GN, Zhou WP, Sun SH. Hepatitis B virus X protein (HBx)-related long noncoding RNA (lncRNA) down-regulated expression by HBx (Dreh) inhibits hepatocellular carcinoma metastasis by targeting the intermediate filament protein vimentin. *Hepatology* 2013; **57**: 1882-1892 [PMID: 23239537 DOI: 10.1002/hep.26195]

25 **Murakami S**. Hepatitis B virus X protein: a multifunctional viral regulator. *J Gastroenterol* 2001; **36**: 651-660 [PMID: 11686474]

26 **Tu H**, Bonura C, Giannini C, Mouly H, Soussan P, Kew M, Paterlini-Bréchot P, Bréchot C, Kremsdorf D. Biological impact of natural COOH-terminal deletions of hepatitis B virus X protein in hepatocellular carcinoma tissues. *Cancer Res* 2001; **61**: 7803-7810

[PMID: 11691796]
27 **Wang Q**, Zhang T, Ye L, Wang W, Zhang X. Analysis of hepatitis B virus X gene (HBx) mutants in tissues of patients suffered from hepatocellular carcinoma in China. *Cancer Epidemiol* 2012; **36**: 369-374 [PMID: 22178505 DOI: 10.1016/j.canep]
28 **Xie Y**, Liu S, Zhao Y, Guo Z, Xu J. X protein mutations in hepatitis B virus DNA predict postoperative survival in hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 10325-10331 [PMID: 25034530 DOI: 10.1007/s13277-014-2331-0]
29 **Liu XH**, Lin J, Zhang SH, Zhang SM, Feitelson MA, Gao HJ, Zhu MH. COOH-terminal deletion of HBx gene is a frequent event in HBV-associated hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1346-1352 [PMID: 18322946 DOI: 10.3748/wjg.14.1346]
30 **Hsia CC**, Yuwen H, Tabor E. Hot-spot mutations in hepatitis B virus X gene in hepatocellular carcinoma. *Lancet* 1996; **348**: 625-626 [PMID: 8774611]
31 **Takahashi K**, Akahane Y, Hino K, Ohta Y, Mishiro S. Hepatitis B virus genomic sequence in the circulation of hepatocellular carcinoma patients: comparative analysis of 40 full-length isolates. *Arch Virol* 1998; **143**: 2313-2326 [PMID: 9930189]
32 **Kim DC**, Chung WJ, Lee JH, Jang BK, Hwang JS, Kang KJ, Kwon SY. Clinicopathological characteristics of PIK3CA and HBx mutations in Korean patients with hepatocellular carcinomas. *APMIS* 2014; **122**: 1001-1006 [PMID: 24673525 DOI: 10.1111/apm.12245]
33 **Baptista M**, Kramvis A, Kew MC. High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. *Hepatology* 1999; **29**: 946-953 [PMID: 10051502]
34 **Iavarone M**, Trabut JB, Delpuech O, Carnot F, Colombo M, Kremsdorf D, Bréchot C, Thiers V. Characterisation of hepatitis B virus X protein mutants in tumour and non-tumour liver cells using laser capture microdissection. *J Hepatol* 2003; **39**: 253-261 [PMID: 12873823]
35 **Song LH**, Duy DN, Binh VQ, Luty AJ, Kremsner PG, Bock CT. Low frequency of mutations in the X gene, core promoter and precore region of hepatitis B virus infected Vietnamese. *J Viral Hepat* 2005; **12**: 160-167 [PMID: 15720531]
36 **Chen GG**, Li MY, Ho RL, Chak EC, Lau WY, Lai PB. Identification of hepatitis B virus X gene mutation in Hong Kong patients with hepatocellular carcinoma. *J Clin Virol* 2005; **34**: 7-12 [PMID: 16087118]
37 **Wang D**, Cai H, Yu WB, Yu L. Identification of hepatitis B virus X gene variants between hepatocellular carcinoma tissues and pericarcinoma liver tissues in Eastern China. *Int J Clin Exp Pathol* 2014; **7**: 5988-5996 [PMID: 25337243]
38 **Liu X**, Wang L, Zhang S, Lin J, Zhang S, Feitelson MA, Gao H, Zhu M. Mutations in the C-terminus of the X protein of hepatitis B virus regulate Wnt-5a expression in hepatoma Huh7 cells: cDNA microarray and proteomic analyses. *Carcinogenesis* 2008; **29**: 1207-1214 [PMID: 18477650 DOI: 10.1093/carcin/bgn111]
39 **Liu X**, Zhang S, Lin J, Zhang S, Feitelson MA, Gao H, Zhu M. Hepatitis B virus X protein mutants exhibit distinct biological activities in hepatoma Huh7 cells. *Biochem Biophys Res Commun* 2008; **373**: 643-647 [PMID: 18602370 DOI: 10.1016/j.bbrc.2008.06.087]
40 **Liu F**, You X, Chi X, Wang T, Ye L, Niu J, Zhang X. Hepatitis B virus X protein mutant HBxΔ127 promotes proliferation of hepatoma cells through up-regulating miR-215 targeting PTPRT. *Biochem Biophys Res Commun* 2014; **444**: 128-134 [PMID: 24434140 DOI: 10.1016/j.bbrc.2014.01.004]
41 **Wang Q**, Zhang W, Liu Q, Zhang X, Lv N, Ye L, Zhang X. A mutant of hepatitis B virus X protein (HBxDelta127) promotes cell growth through a positive feedback loop involving 5-lipoxygenase and fatty acid synthase. *Neoplasia* 2010; **12**: 103-115 [PMID: 20126469]
42 **Fu XY**, Tan DM, Hou ZH, Hu ZL, Liu GZ, Ouyang Y, Liu F. [Effect of microRNA on proliferation caused by mutant HBx in human hepatocytes]. *Zhonghua Gan Zang Bing Zazhi* 2012; **20**: 598-604 [PMID: 23207154 DOI: 10.3760/cma.j.issn.1007-3418.2012.08.012]
43 **Liu LP**, Hu BG, Ye C, Ho RL, Chen GG, Lai PB. HBx mutants differentially affect the activation of hypoxia-inducible factor-1α in hepatocellular carcinoma. *Br J Cancer* 2014; **110**: 1066-1073 [PMID: 24346287 DOI: 10.1038/bjc.2013.787]
44 **Elmore LW**, Hancock AR, Chang SF, Wang XW, Chang S, Callahan CP, Geller DA, Will H, Harris CC. Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. *Proc Natl Acad Sci USA* 1997; **94**: 14707-14712 [PMID: 9405677]
45 **Xian L**, Zhao J, Wang J, Fang Z, Peng B, Wang W, Ji X, Yu L. p53 Promotes proteasome-dependent degradation of oncogenic protein HBx by transcription of MDM2. *Mol Biol Rep* 2010; **37**: 2935-2940 [PMID: 19842060 DOI: 10.1007/s11033-009-9855-1]
46 **Kew MC**. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 144-152 [PMID: 21199526 DOI: 10.1111/j.1440-1746.2010.06546.x]
47 **Jiang W**, Wang XW, Unger T, Forgues M, Kim JW, Hussain SP, Bowman E, Spillare EA, Lipsky MM, Meck JM, Cavalli LR, Haddad BR, Harris CC. Cooperation of tumor-derived HBx mutants and p53-249(ser) mutant in regulating cell proliferation, anchorage-independent growth and aneuploidy in a telomerase-immortalized normal human hepatocyte-derived cell line. *Int J Cancer* 2010; **127**: 1011-1020 [PMID: 20017137 DOI: 10.1002/ijc.25118]
48 **Yan J**, Yao Z, Hu K, Zhong Y, Li M, Xiong Z, Deng M. Hepatitis B Virus Core Promoter A1762T/G1764A (TA)/T1753A/T1768A Mutations Contribute to Hepatocarcinogenesis by Deregulating Skp2 and P53. *Dig Dis Sci* 2015; **60**: 1315-1324 [PMID: 25567052]
49 **Luo J**, Zhou H, Wang F, Xia X, Sun Q, Wang R, Cheng B. The hepatitis B virus X protein downregulates NF-κB signaling pathways through decreasing the Notch signaling pathway in HBx-transformed L02 cells. *Int J Oncol* 2013; **42**: 1636-1643 [PMID: 23450368 DOI: 10.3892/ijo.2013.1842]
50 **Lim KH**, Choi HS, Park YK, Park ES, Shin GC, Kim DH, Ahn SH, Kim KH. HBx-induced NF-κB signaling in liver cells is potentially mediated by the ternary complex of HBx with p22-FLIP and NEMO. *PLoS One* 2013; **8**: e57331 [PMID: 23483900 DOI: 10.1371/journal.pone.0057331]
51 **Lee JH**, Han KH, Lee JM, Park JH, Kim HS. Impact of hepatitis B virus (HBV) x gene mutations on hepatocellular carcinoma development in chronic HBV infection. *Clin Vaccine Immunol* 2011; **18**: 914-921 [PMID: 21490166 DOI: 10.1128/CVI.00474-10]
52 **Zhang F**, Wang Q, Ye L, Feng Y, Zhang X. Hepatitis B virus X protein upregulates expression of calpain small subunit 1 via nuclear factor-kappaB/p65 in hepatoma cells. *J Med Virol* 2010; **82**: 920-928 [PMID: 20419804 DOI: 10.1002/jmv.21753]
53 **Liu LP**, Liang HF, Chen XP, Zhang WG, Yang SL, Xu T, Ren L. The role of NF-kappaB in Hepatitis b virus X protein-mediated upregulation of VEGF and MMPs. *Cancer Invest* 2010; **28**: 443-451 [PMID: 20073580 DOI: 10.3109/07357900903405959]
54 **Han JM**, Kang JA, Han MH, Chung KH, Lee CR, Song WK, Jun Y, Park SG. Peroxisome-localized hepatitis Bx protein increases the invasion property of hepatocellular carcinoma cells. *Arch Virol* 2014; **159**: 2549-2557 [PMID: 24810099 DOI: 10.1007/s00705-014-2105-4]
55 **Peifer M**, Polakis P. Wnt signaling in oncogenesis and embryogenesis--a look outside the nucleus. *Science* 2000; **287**: 1606-1609 [PMID: 10733430]
56 **Kühl M**, Sheldahl LC, Park M, Miller JR, Moon RT. The Wnt/Ca2+ pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet* 2000; **16**: 279-283 [PMID: 10858654]
57 **Kühl M**, Sheldahl LC, Malbon CC, Moon RT. Ca(2+)/calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in Xenopus. *J Biol Chem* 2000; **275**: 12701-12711 [PMID: 10777564]
58 **Katoh M**. WNT/PCP signaling pathway and human cancer (review). *Oncol Rep* 2005; **14**: 1583-1588 [PMID: 16273260]
59 **Grigoryan T**, Wend P, Klaus A, Birchmeier W. Deciphering the

function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of beta-catenin in mice. *Genes Dev* 2008; **22**: 2308-2341 [PMID: 18765787 DOI: 10.1101/gad.1686208]

60 **Hsieh A**, Kim HS, Lim SO, Yu DY, Jung G. Hepatitis B viral X protein interacts with tumor suppressor adenomatous polyposis coli to activate Wnt/β-catenin signaling. *Cancer Lett* 2011; **300**: 162-172 [PMID: 20971552 DOI: 10.1016/j.canlet.2010.09.018]

61 **Liu XH**, Pan MH, Lu ZF, Wu B, Rao Q, Zhou ZY, Zhou XJ. Expression of Wnt-5a and its clinicopathological significance in hepatocellular carcinoma. *Dig Liver Dis* 2008; **40**: 560-567 [PMID: 18294932 DOI: 10.1016/j.dld.2007.12.011]

62 **Geng M**, Cao YC, Chen YJ, Jiang H, Bi LQ, Liu XH. Loss of Wnt5a and Ror2 protein in hepatocellular carcinoma associated with poor prognosis. *World J Gastroenterol* 2012; **18**: 1328-1338 [PMID: 22493546 DOI: 10.3748/wjg.v18.i12.1328]

63 **Lin X**, Wang Q, Cao Z, Geng M, Cao Y, Liu X. Differential Expression of Wnt Pathway Genes in Sporadic Hepatocellular Carcinomas Infected With Hepatitis B Virus Identified With OligoGE Arrays. *Hepat Mon* 2013; **13**: e6192 [PMID: 23483081 DOI: 10.5812/hepatmon.6192]

64 **Bi L**, Liu X, Wang C, Cao Y, Mao R, Li P, Geng M. Wnt5a involved in regulation of the biological behavior of hepatocellular carcinoma. *Int J Clin Exp Pathol* 2014; **7**: 987-995 [PMID: 24696716]

P- Reviewer: Troncoso MF **S- Editor**: Ma YJ **L- Editor**: Filipodia
E- Editor: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10739

World J Gastroenterol 2015 October 14; 21(38): 10739-10748
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

Immune and non-immune responses to hepatitis C virus infection

Jiaren Sun, Ricardo Rajsbaum, MinKyung Yi

Jiaren Sun, Ricardo Rajsbaum, MinKyung Yi, Department of Microbiology and Immunology, Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX 77555-1070, United States

Author contributions: Sun J, Rajsbaum R and Yi M analyzed the literature and wrote the manuscript.

Supported by NIH AI109100 (to Sun J), R01AI110358 (to Yi M); and UTMB Institute for Human Infection and Immunity and UT System Rising STAR Award (to Rajsbaum R).

Conflict-of-interest statement: The authors have no conflict of interest to report.

Correspondence to: Jiaren Sun, MD, PhD, Department of Microbiology and Immunology, Institute for Human Infections and Immunity, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-1070, United States. jisun@utmb.edu
Telephone: +1-409-7470186
Fax: +1-409-7476869

Received: May 18, 2015
Peer-review started: May 20, 2015
First decision: June 23, 2015
Revised: July 6, 2015
Accepted: September 14, 2015
Article in press: September 14, 2015
Published online: October 14, 2015

Abstract

The host innate and adaptive immune systems are involved in nearly every step of hepatitis C virus (HCV) infection. In patients, the outcome is determined by a series of complex host-virus interactions, whether it is a natural infection or results from clinical intervention. Strong and persistent $CD8^+$ and $CD4^+$ T-cell responses are critical in HCV clearance, as well as cytokine-induced factors that can directly inhibit virus replication. Newly available direct-acting antivirals (DAAs) are very effective in viral clearance in patients. DAA treatment may further result in the down-regulation of programmed death-1, leading to rapid restoration of HCV-specific $CD8^+$ T cell functions. In this review, we focus on recent studies that address the host responses critical for viral clearance and disease resolution. Additional discussion is devoted to the prophylactic vaccine development as well as to current efforts aimed at understanding the host innate responses against HCV infection. Current theories on how the ubiquitin system and interferon-stimulated genes may affect HCV replication are also discussed.

Key words: Hepatitis C virus; Hepatitis; T cell; Direct-acting antiviral; Innate immune response

Core tip: Hepatitis C virus (HCV) is an etiologic agent that can cause severe liver diseases, including chronic hepatitis, liver fibrosis, liver cirrhosis, and hepatocellular carcinoma. Although newly available direct-acting antivirals (DAAs) are very effective in viral clearance in patients, it remains unclear as to how many of the world's infected individuals will benefit from the new DAAs. In this review, we focus on recent studies that address the host responses critical for viral clearance and disease resolution. Additional discussion is devoted to the prophylactic vaccine development and innate responses against HCV infection. Current theories on how the ubiquitin system and interferon-stimulated genes may affect HCV replication are also discussed.

Sun J, Rajsbaum R, Yi M. Immune and non-immune responses to hepatitis C virus infection. *World J Gastroenterol* 2015; 21(38): 10739-10748 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10739.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10739

INTRODUCTION

Hepatitis C virus (HCV) is an etiologic agent that can cause severe liver diseases, including chronic hepatitis, liver fibrosis, liver cirrhosis, and hepatocellular carcinoma. Since the first discovery of this virus in 1989 by Choo *et al*[1], much has been learned about viral replication mechanisms and detailed functions of the viral proteins involved in these processes. Such knowledge has accelerated the development of direct-acting antivirals that could cure HCV infections, with much higher efficiency in shortening treatment duration compared to the traditional interferon-α-based therapy[2-6]. On the other hand, the vaccine development front seems to have lagged behind. This is certainly not due to the lack of effort to understand the HCV-induced immunity, but rather to the unique challenges in understanding effective immune responses against HCV.

The first challenge is the virus itself, which could successfully establish a chronic infection in about 80% of infected persons by effectively modulating innate and adaptive immune responses[7,8]. The second challenge is the difficulty in identifying and obtaining samples from acutely infected patients who successfully eliminated the virus due to the lack of distinct symptoms during acute phase of HCV infection. The third challenge is the lack of suitable small animal models that can recapitulate the HCV infection-mediated immune responses in humans. Although chimpanzee model has been the best immunocompetent animal model of HCV infection, with the recent National Institutes of Health (NIH) moratorium of usage of chimpanzee in HCV research, this challenge just got worse. Of note, there are continuing efforts to develop other immunocompetent animal model system[9,10]. Despite these difficulties, new information that could help us eventually control HCV-mediated immune dysregulation keeps emerging.

The goal of this review is to summarize the most up-to-date knowledge regarding both innate antiviral and adaptive immune responses that are available in the literature, to define the successful host responses that could contribute to HCV elimination. In addition, we discuss the implications of effective anti-HCV therapy on HCV-mediated immune modulation and vaccine development.

ADAPTIVE IMMUNE RESPONSES IN HEPATITIS C

Although HCV is capable of interfering with a wide range of host physiological processes, it is readily detected by the host sensing machinery, followed by the triggering of innate cellular responses[11,12], including the production of type Ⅰ interferons (IFN-Ⅰ) and the activation of downstream, antiviral target genes. However, despite these responses, HCV continues to replicate in the liver in the incubation phase. The adaptive immune response to HCV infection develops over several weeks[13]. Although the reasons for this delay are not understood, it is clear that the magnitude, diversity, and quality of the adaptive immune responses are the determinants of the outcome[13]. While this acute immune response has the potential to clear the viral infection, it is unsuccessful at least 50% of the time, and the virus has a very strong propensity to cause chronic infections. Development of chronicity is marked by a dramatic decrease in the activity of $CD8^+$ cytotoxic T lymphocytes (CTL) and $CD4^+$ Th cells in the liver without achieving viral clearance. Interestingly, the T cell dysfunction seems to be restricted to HCV-specific $CD8^+$ T cells, since influenza-specific $CD8^+$ cells were functional in chronic HCV patients[12].

T cell dysregulation in chronic infection

The effector functions of virus-specific $CD8^+$ and $CD4^+$ T cells are critical in viral clearance and disease resolution[14]. Interestingly, virus clearance and disease progression are seemingly mediated by phenotypically distinctive $CD8^+$ CTL populations. The $CD8^+$ CTLs participating in virus clearance expressed high levels of IFN-γ, but low levels of the activation marker CD38 (IFN-γ^{hi} $CD38^{lo}$)[12,13]. In contrast, the CTL involved in liver injury are commonly IFN-γ^{lo} $CD38^{hi}$, and the frequency of these cells tends to increase with the inflammation score (Figure 1, left panel)[15]. Although CTLs can exert limited antiviral activity, they are unable to keep pace with the evolution of HCV. As a result, HCV rapidly accumulates escape mutations in its genome, and persistent viremia ensues[16]. In studies with large cohorts of chronic subjects and spontaneous resolvers, adequate help from $CD4^+$ T cells was found to be essential to promoting immune protection[17]. Among resolvers, the HCV epitopes are presented by multiple alleles of major histocompatibility complex Ⅱ molecules, and nonstructural (NS) protein-directed $CD4^+$ T-cell responses are associated with high levels of IL-2 and IFN-γ[18]. On the other hand, HCV persistence is associated with a high frequency of $CD4^+$ regulatory T cells (Treg) that could directly suppress

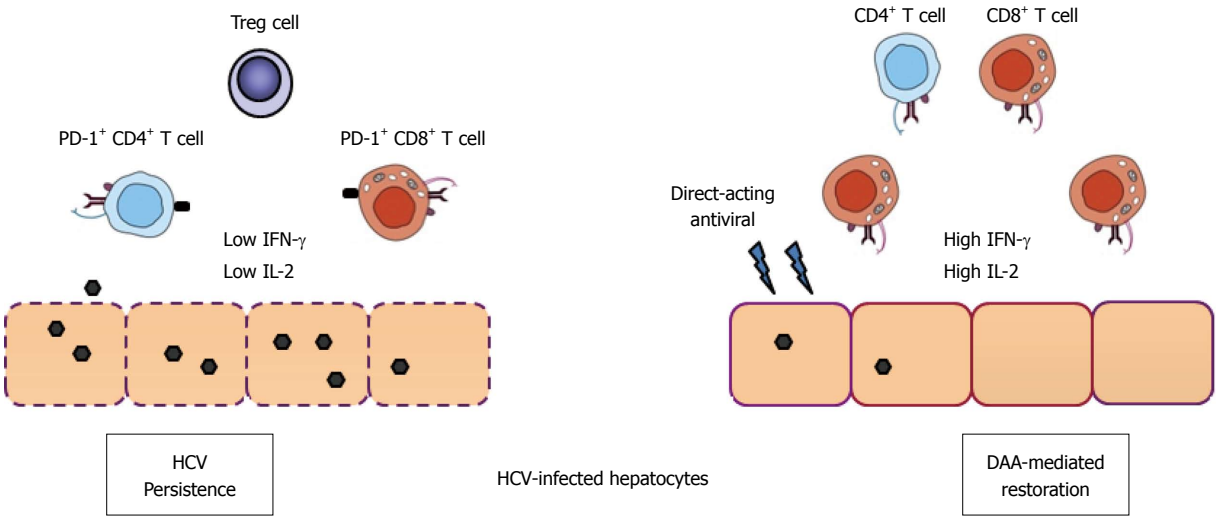


Figure 1 Direct-acting antiviral therapy and immune function restoration in hepatitis C virus infection. In chronic hepatitis C infection, T cells having a narrow repertoire of TCRs mount a weak response to HCV antigens, and their effector functions are often impaired. Many $CD8^+$ and $CD4^+$ T cells express low levels of IFN-γ and IL-2 accompanied by up-regulation of PD-1 molecules in the liver. Development of T regulatory cells and compromised dendritic cell functions also contribute to T cell functional impairment. Recent data suggest that IFN-free direct-acting antivirals not only clear HCV in the majority of patients, but also result in the down-regulation of PD-1, leading to rapid restoration of virus-specific $CD8^+$ T cell functions in patients[28]. HCV: Hepatitis C virus; IFN: Interferon; IL: Interleukin; PD-1: Program death-1; TCR: T cell receptor.

HCV-specific CTL in patients[19]. The resolution of disease is usually associated with the loss of expression of programmed death-1 (PD-1) and decreased functional suppression[17,20]. In addition to Treg cells, high expression of inhibitory receptor-PD-1 on $CD4^+$ and $CD8^+$ T cells also contributes to viral persistence as well as to failure of antiviral therapy (Figure 1, left panel)[21,22].

T cell responses in immunomodulatory and direct-acting antiviral therapies

Until recently, the mainstay of treatment for chronic HCV infection had been pegylated interferon and ribavirin for HCV genotype 1 infection. This treatment resulted in a sustained virologic response (SVR) in 50%-80% of patients with HCV genotype 1 infection (higher SVR among those with genotypes 2 or 3 infections). There has been a clear demonstration that IFN-α, even when it achieved viral clearance, was not able to rescue antiviral T cells from exhaustion due to its pleiotropic effects on T cells[23-25]. To improve the efficacy of hepatitis C therapy, one critical question is whether antiviral T cells are permanently blemished by the sustained expression of PD-1 or other tolerating mechanisms; or alternatively, the removal of IFN-α would promote host recovery and the expansion of T cells with antiviral functions[26,27]. In a recent study involving a clinical cohort undergoing IFN-free therapy, a combination of Faldaprevir (a protease inhibitor) and Deleobuvir (a non-nucleoside polymerase inhibitor) cleared HCV in the majority of patients[28]. Furthermore, viral antigen reduction in response to direct-acting antiviral (DAA) treatment resulted in the down-regulation of PD-1, leading to rapid restoration of HCV-specific $CD8^+$ T cell functions in patients (Figure 1, right panel)[28]. While this study unveils an unexpected benefit of these two DAAs, it is interesting to see whether other DAAs with or without ribavirin are also able to reverse exhaustion and restore full functions of HCV-specific T cells.

HCV PROPHYLACTIC VACCINES

HCV infection is a major public health problem in the world. Although DAAs are a significant advancement for an HCV treatment, it remains unclear as to how many of the world's infected individuals will benefit from the new DAAs. For instance, the high cost of these DAAs will undoubtedly deny their access to low income countries and may also result in selective use in middle and even high income counties[29]. Thus there will likely still be a substantial number of HCV cases, including those individuals who are not screened and unaware of their infection. Additionally, DAAs are not efficacious in those having already developed advanced hepatic cirrhosis, carcinoma and liver failures. Importantly, a recent study showed that persistently HCV-infected chimpanzees cured with DAA maintained narrowly focused stable $CD8^+$ T cell repertoires that were incapable of preventing persistent infection following HCV re-challenge[30]. Thus, vaccination may be necessary even for those individuals who have cleared virus following DAA treatment. For these reasons, an effective prophylactic HCV vaccine remains a critical instrument to halt the global HCV epidemic. Current human and chimpanzee studies suggest that a prophylactic vaccine inducing both protective T cells and broadly-neutralizing antibody (bnAb) responses is important for HCV control and thus highly desirable characteristics for the future vaccine candidates[29].

Circulating HCV is genetically diverse, and therefore a broadly effective vaccine must target conserved T- and B-cell epitopes of the virus. Several prophylactic vaccine candidates based on different strategies and viral targets have been developed in the last two decades. Vaccines aimed to target conserved T cell epitopes are shown to induce vigorous and broadly directed $CD4^+$ and $CD8^+$ T cell responses, and these are well underway in clinical development[31,32]. Despite the molecular trickeries employed by HCV, a number of bnAbs have been identified. The protective role of bnAbs against HCV infection has been demonstrated in chimpanzees, highlighting the possibility of developing a broadly effective vaccine by inducing bnAbs[33,34]. The HCV viral RNA genome encodes two structural envelope glycoproteins, E1 and E2. Although neutralizing antibodies (nAbs) to E1 have also been isolated, E2 is the main target of nAbs in HCV-infected patients[35]. In the last decade, significant discoveries of bnAbs and their structural analysis with antigenic epitopes have been made in the HIV-1 vaccine field; now a similar trend has begun to emerge in the HCV vaccine field. Recently, researchers have combined the latest findings in HCV structural biology and cutting-edge technologies in protein design and next-generation sequencing of Ab repertoires to facilitate HCV immunogen design for the induction of bnAbs in vaccination[35]. The linear HCV epitopes will be grated onto protein scaffolds, which allow epitope presentation in their bnAb-bound conformations. These studies demonstrate the feasibility of generating a highly potent antibody formulation against multiple, conserved neutralizing epitopes on HCV.

HCV AND INNATE IMMUNE RESPONSES

It is now clear that HCV infection induces innate responses capable of limiting virus replication to some extent. However, HCV is still able to establish chronic infections by escaping immune responses. Similar to other viruses, HCV encodes pathogen-associated molecular patterns (PAMPs), which are recognized by the host pattern-recognition receptors (PRRs). Members of the endosomal Toll-like receptor (TLR) family and the cytoplasmic Retinoic acid inducible gene (RIG-I)-like receptors (RLRs) can also recognize HCV PAMPs (Figure 2A). Pathogen recognition by PRRs results in activation of downstream signaling pathways leading to the production of pro-inflammatory cytokines, chemokines, IFN-I and type-Ⅲ IFN (IFN-λ)[36]. IFNs elicit their antiviral activity through the up-regulation of many IFN-I-stimulated genes (ISGs), which act as direct effectors of the antiviral response[37]. HCV-encoded PAMPs recognized by the RIG-I sensor include the polyuridine motif of the HCV genome 3′ non-translated region and its replication intermediate, which binds RIG-I through the 5′ terminal triphosphate on the viral RNA. This signaling induces IFN-I and antiviral ISGs in the liver *in vivo*[38]. HCV PAMPs that are recognized by RIG-I are also produced by cleavage of viral NS5B region of HCV RNA by the IFN-inducible host endoribonuclease RNase L, releasing small structured RNAs with 5′-hydroxyl (5′-OH) and 3′-monophosphoryl (3′-p) groups[39]. Binding of the HCV PAMP induces a conformational change in RIG-I and subsequent ubiquitination by the E3-ubiquitin ligase TRIM25 (Figure 2A). This process and interaction with the chaperone protein 14-3-3e promote recruitment of activated RIG-I to the adaptor protein mitochondria antiviral signaling protein (MAVS) that is anchored to the mitochondria and also located in an intracellular membrane network at the peroxisomes and on mitochondrial- associated membranes[40-43]. Subsequently, different signaling partners are known to be recruited to MAVS, resulting in the activation of the IκB (IKK) and IKK-related kinases[44,45], TBK1 and IKKε, which phosphorylate the transcription factors IRF3 and IRF7 required for IFN-I production as well as IFN-λ[46-48].

Deregulation of innate anti-viral signaling pathways during HCV replication

Both IFN-I and IFN-λ are produced upon innate recognition of viruses, although differential expression has been found in tissues such as the brain upon viral infection[49]. Although signaling occurs through different receptors, both IFN-I and IFN-λ can trigger downstream signaling through phosphorylation of signal transducers and activators of transcription 1 (STAT1) and STAT2, suggesting that both IFN-I and IFN-λ signaling result in induction of the same ISGs. Together, STAT1, STAT2 and IRF9 form the interferon-stimulated gene factor 3 (ISGF3) complex, which is essential for induction of ISGs (Figure 2B)[50]. The tyrosine kinases JAK1 and TYK2, which are both activated by IFN-I and IFN-λ, phosphorylate tyrosine 701 (Y701) on STAT1[51]. In addition, phosphorylation of S708 on STAT1 by the IKKε kinase is also required for the efficient induction of all ISGs in response to IFN-I[52]; however, it is currently unknown whether IFN-λ stimulation also results in activation of IKKε and STAT1-S708 phosphorylation. Furthermore, activation of IKKε during IFN-I signaling also requires binding to unanchored lysine-48 (K48)-linked polyubiquitin chains, which are not covalently attached to any protein[53]. Some evidence suggests that HCV inhibits STAT1 function. For example, by using a microRNA array in human hepatocytes infected with HCV, it was shown that miR-373 is up-regulated in HCV-infected cells (Figure 2B). This microRNA targeted JAK1 and IRF9 and reduced phosphorylation of STAT1. Consistent with this observation, knockdown of miR-373 resulted in the reduction of HCV RNA replication[54]. Furthermore, the core protein of HCV associates with STAT1 and promotes its degradation[55]. Immune cell populations and hepatocytes from HCV^+ patients have reduced STAT1 and STAT3 proteins.

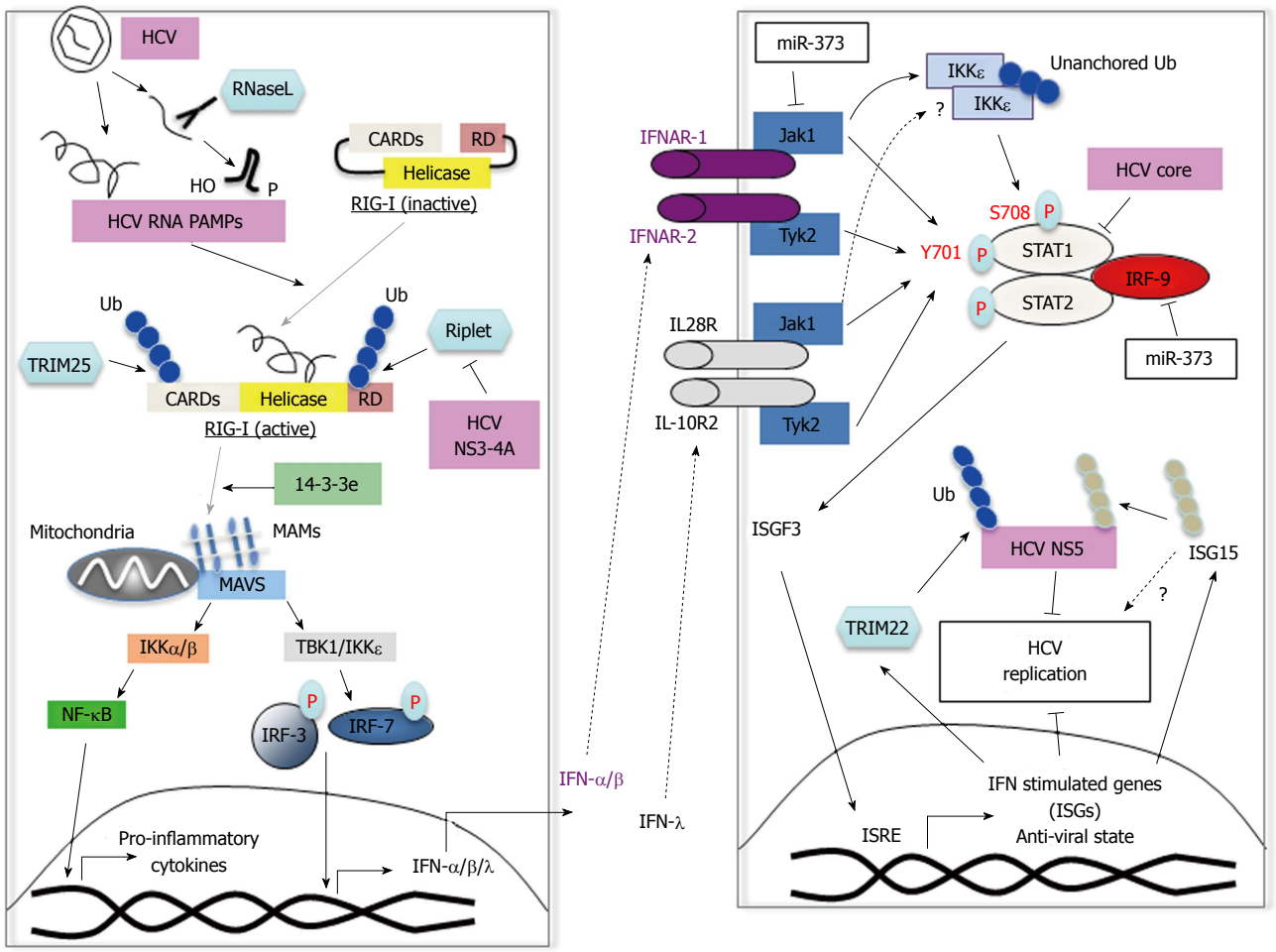


Figure 2 Innate immune response to hepatitis C virus and viral escape mechanisms. A: RIG-I recognizes HCV PAMPs including HCV 5'-triphosphate RNA and small structured RNAs with 5'-hydroxyl (5'-OH) and 3'-monophosphoryl (3'-p) groups, which are cleaved by the host endoribonuclease RNase L. Binding of the HCV PAMP induces a conformational change in RIG-I and subsequent ubiquitination by TRIM25 and Riplet. RIG-I is then recruited to MAVS *via* mitochondria associated membranes (MAMs) and the chaperone protein 14-3-3e. Subsequently, TBK1 and IKKε phosphorylate IRF3 and IRF7 for IFN-I production as well as IFN-λ, and pro-inflammatory cytokines *via* NF-κB activation. The NS3-4A of HCV inhibits Riplet-dependent activation of RIG-I; B: IFN-I and IFN-λ are recognized by the IFNA receptor and IL28/IL-10 receptor respectively. Both trigger downstream signaling through phosphorylation of STAT1 and STAT2. Together, STAT1, STAT2 and IRF9 form the ISGF3 complex, which translocates to the nucleus for induction of antiviral ISGs. The tyrosine kinases JAK1 and TYK2, phosphorylate tyrosine 701 (Y701) on STAT1. In addition, phosphorylation of S708 on STAT1 by the IKKε kinase (activated by unanchored polyubiquitin chains) is also required for ISG induction. It is currently unknown whether IFN-λ stimulation also results in IKKε activation. miR-373 is up-regulated in HCV-infected cells and inhibits JAK1 and IRF9 function resulting in reduced STAT1 phosphorylation. TRIM22, which is induced by IFN-I, inhibits HCV replication probably by a mechanism involving ubiquitination of viral NS5. ISG15, another IFN-I inducible protein can inhibit HCV replication by ISGylation of viral NS5 rendering unstable. ISG15 has also been proposed to have pro-viral roles. The core protein of HCV associates with STAT1 and promotes its degradation. RIG-I: Retinoic acid inducible gene; HCV: Hepatitis C virus; PAMP: Pathogen-associated molecular pattern; IFN: Interferon; IL: Interleukin; ISGF: Interferon-stimulated gene factor; STAT: Signal transducers and activators of transcription; NF-κB: Nuclear factor-kappa B; IKK: Inhibitor of NF-κB kinase.

Furthermore, STAT3 was preferentially ubiquitinated and targeted for proteosomal degradation in the presence of HCV[56]. These observations may explain in part the hypo-responsiveness observed in some patients to IFN-α treatment.

Since IFN-α/β and IFN-λ trigger similar signaling pathways, they are predicted to induce the same group of ISGs. However, in contrast to the widely expressed IFN-I receptor in different cell types, the expression of the IL-10R2 subunit of the IFN-λ receptor appears to be restricted to cell types and tissues, particularly on epithelial cells[49]. This raised the possibility that IFN-λ administration may be a better option to the common therapy for treating HCV infection with pegylated IFN-α (Peg-IFN-α) and ribavirin. It is now known that Peg-IFN-α treatment is only effective in a fraction of HCV-infected individuals with the presence of some side effects. Thus far Peg-IFN-λ has shown promising results[57,58]. Besides the tissue/cell type-specific expression of the IL-10R2 receptor, Peg-IFN-λ may be more effective since, unlike IFN-α treatment[59], Peg-IFN-λ treatment did not lead to refractoriness of JAK-STAT signaling following multiple stimulations[60]. IFN-λ treatment also results in a faster reduction in viral load as compared to those with IFN-α[61]. In addition, HCV infection appears to down-regulate the expression of IFN-I receptor, in contrast to sustained expression of the IL-28 receptor subunit. Global

transcriptome analysis in hepatocytes indicated that IFN-λ stimulation prolonged the expression of various ISGs that are potentially beneficial to antiviral defense mechanisms[62].

In addition to inhibition of STAT-dependent innate immune responses, HCV has also been shown to inhibit TLR3 signaling and IFN-λ production in human hepatoma cell lines, with subsequent reduction in the antiviral ISG56, MxA and OAS-1. NS3/4A, NS5A and NS5B had the ability to inhibit poly I:C-induced IFN-λ1 expression in Huh7 cells[63,64].

Role of ubiquitin and ubiquitin-like molecules in HCV replication

Ubiquitination of proteins is a post-translational process, which has been demonstrated to regulate not only protein stability but also various steps of the signaling pathways in immune regulation and cytokine production[65]. Viruses have adapted to antagonize innate immune responses by using different mechanisms including manipulating the ubiquitin system for their own advantage[66]. For example, it was recently proposed that the Influenza virion carries unanchored polyubiquitin chains that promote virus uncoating by utilizing the host aggresome machinery[67]. At the same time these polyubiquitin chains that are released in the cytoplasm may function as a mechanism to alert the innate cellular response early upon virus entry to the cell[68]. Whether HCV utilizes unanchored polyubiquitin chains for replication remains to be tested. Nevertheless, HCV also has been shown to target the ubiquitin system to escape the innate immune response. HCV NS3-4A proteases inhibit the E3-ubiquitin ligase Riplet, which, together with TRIM25, is required for efficient ubiquitination and activation of RIG-I (Figure 2A)[69]. The ubiquitin system is also utilized by the host to restrict virus replication, and, in fact, may be one of the mechanisms by which IFN therapy limits virus replication in patients. For example the E3-ubiquitin ligase TRIM22, which is highly induced by IFN-I[70-72], may be associated with responsiveness to Peg-IFN-α-2a/RBV combination therapy[73]. A possible mechanistic explanation for these findings is supported by data showing that TRIM22 overexpression inhibits HCV replication and knockdown reduces IFN-induced anti-HCV activity. In addition, TRIM22 appears to promote ubiquitination of HCV-NS5A[74]. Although this study did not elucidate the functional effects of NS5A ubiquitanation, ubiquitin ligases as well as de-ubiquitinating enzymes are emerging as important proteins in controlling HCV replication. In particular, a recent study using an RNA interference (RNAi) screen found a few ubiquitin ligases to be important in HCV replication; the E2-conjugating enzyme UbE2J1, is involved in viral RNA replication; USP11, a de-ubiquitinating enzyme is involved in HCV IRES-mediated translation. In addition, TRIM42, another member of the E3-ubiquitin ligase family of proteins, and UbE2M, another E2 ubiquitin-conjugating enzyme, are also involved at early stages of viral post-entry[75].

Similar to posttranslational covalent modification by ubiquitin, the small ubiquitin-like modifier (SUMO) can also be covalently attached to protein lysines post-translationally, and regulates many different cellular processes. HCV also utilizes this cellular process for its own advantage; the NS5A protein of HCV is SUMOylated, resulting in increased protein stability by inhibiting ubiquitination[76].

ISG15, another ubiquitin-like molecule that can be covalently attached to lysine residues of proteins post-translationally, has been shown to play both positive and negative roles during HCV infection. ISG15 is a highly IFN-inducible gene that has antiviral functions against many viruses[77]. Accordingly, overexpression of ISG15- and ISG15-conjugation enzymes resulted in inhibition of HCV replication. Furthermore, HCV-NS5A protein was ISGylated, and this appeared to decrease NS5A stability[78]. However, in contrast to this study, other studies identified ISG15 as a pro-HCV host factor promoting HCV replication[79,80]. ISG15 may have different effects depending on the cell type or tissue expression. If indeed ISG15 acts as a pro-viral factor, its high induction by IFN treatment may help explain why in some patients IFN-based treatment results in persistent HCV infection. Additional evidence that ISG15 plays a role in HCV replication comes from studies on USP18, which specifically cleaves ISG15 from its cellular targets[81]. USP18 knockout mice are hypersensitive to IFN, with prolonged Jak/Stat signaling[82]. Expression of USP18 is increased in the liver biopsy specimens of patients who do not respond to IFN-α therapy, and siRNA knockdown of USP18 in human cells increases the ability of IFN to inhibit HCV replication as well as to increase cellular protein ISGylation and prolonged STAT1 phosphorylation, and a general enhancement of IFN-stimulated gene expression[83].

To demonstrate that *in vivo* knockdown of ISG15 may be used therapeutically to inhibit HCV replication, Real *et al*[84] used lipid nanoparticles to deliver siRNA specific to ISG15. The treatment resulted in specific reduction of ISG15 expression in the liver *in vivo*, resulting in reduced responses to IFN treatment. This also resulted in a reduction in HCV replication, supporting the role of ISG15 as a pro-viral factor. In addition, ISG15 knockdown revealed five potential candidates as pro-viral factors that depend on ISG15 expression. In particular, knockdown of the ISG15-dependent heterogeneous nuclear ribonucleoprotein K (HnrnpK) also resulted in decreased levels of HCV replication[84].

HCV infection is also known to induce the ubiquitin-dependent degradation of some cellular proteins including the retinoblastoma tumor suppressor protein by viral NS5B[85,86], and the suppressor of cytokine

signaling 3 (SOCS3), which is a negative regulator of the JAK-STAT pathway[87]. HCV viral proteins have also been described to be ubiquitinated and degraded by the proteasome through both ubiquitin-dependent and independent mechanisms (review by Shoji *et al*[88]).

IFN-stimulated genes as antiviral factors to HCV

For decades Peg-IFN-α has been one of the most important therapeutics to control HCV infection in patients, although the exact mechanisms of viral inhibition have remained unclear. It is well established that IFN treatment will induce a large number of ISGs with known antiviral functions, but which ISGs and how they act against HCV remained largely unknown until recently. Some of these ISGs have direct or indirect anti-HCV functions and have been reviewed recently (see Horner *et al*[43]). These include *ADAR*, *DDIT4*, *DDX58* (*RIG-I*), *DDX60*, *EIF2AK2* (*PKR*), *GBP1*, *IFI44L*, *IFI6*, *IFIT1*, *IFIT3*, *IFITM3*, *IRF1*, *IRF7*, *ISG12*, *ISG20*, *MAP3K14* (*NIK*), *MOV10*, *MS4A4A*, *MX1* (*MxA*), *NOS2*, *NT5C3*, *OAS1*, *OASL*, *PLSCR1*, *RNASEL*, *RSAD2* (*viperin*), *SSBP3*, and *TRIM14*[43]. Many of these genes were found by using lentiviral vectors expressing 389 selected ISGs in Huh-7.5 cells, a RIG-I-defective derivative of Huh-7 cells. Although most of the ISGs showed some degree of inhibition of HCV replication, RIG-I, MDA5, IRF1 and IRF7, which are genes involved in signaling to produce IFN-I, were the strongest inhibitors of HCV[37]. Other studies showed the IFN-induced transmembrane protein 1 (IFITM1) as an inhibitor of HCV[89,90]. In addition, another screen identified several antiviral ISGs induced by IFN-α and IFN-γ using an RNAi[91]. IFITM1 is highly induced by both IFN-I and IFN-γ has been shown to inhibit different viruses including West Nile virus, Influenza, HIV and HCV[89,92]. IFITM1 accumulates at hepatic tight junctions in HCV-infected human patient liver during IFN therapy and interacts with the HCV co-receptors CD81 and occludin, blocking viral entry[90]. ISG56 was also shown to inhibit HCV replication[89]. Additional ISGs have been reported to inhibit HCV. ISG20, and PKR are reported to inhibit HCV RNA synthesis[93]. Recently, the Cholesterol-25-hydroxylase (CH25H), a 31.6-kDa endoplasmic reticulum-associated enzyme that catalyzes oxidation of cholesterol to 25-hydroxycholesterol (25HC), was also shown to inhibit HCV. CH25H is an ISG that is induced in many tissues upon *in vivo* exposure to TLR ligands and IFN stimulations[94]. 25HC has also been reported to possess anti-HCV activity[95]. CH25H can interact with the NS5A protein of HCV and inhibit its dimer formation, which is essential for HCV replication[96].

In summary, although in recent years there have been great advances in our understanding of the anti-HCV functions of ISGs, many of these studies still fail to take into consideration the physiological conditions in which the virus replicates, as well as relevant immune cell types that are localized in the liver. Furthermore, it remains unclear as to how to induce these genes with exogenous treatments, or how to deliver lentiviral vectors containing specific ISG as potential antiviral treatments. Thus, additional studies are required using novel *in vivo* models combined with biochemical methods to identify the molecular mechanisms of antiviral functions.

CONCLUSION

The recent availability of highly effective DAAs against HCV infection brings hope for HCV eradication. However, initial reports suggest that DAA alone may not be enough to achieve this goal. Instead, HCV eradication will ultimately require the boosting of favorable innate and adaptive immune responses and ultimately vaccine development. Based on our knowledge of antiviral immune responses, the raising of effective antiviral responses against HCV will require agents or vaccine candidates that promote innate antiviral signaling and enhance both CD4 and CD8 responses effectively without inducing exhausted phenotypes and bnAb that could neutralize multiple genotypes of HCV. There is no doubt that effective immune-modulators against HCV infection will be available someday as a result of our continued efforts to understand HCV-induced immune regulation.

ACKNOWLEDGMENTS

The authors wish to express gratitude to their trainees, as well as Ms. Mardelle Susman for assistance with manuscript preparation.

REFERENCES

1 **Choo QL**, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562]
2 **Feld JJ**. Interferon-free strategies with a nucleoside/nucleotide analogue. *Semin Liver Dis* 2014; **34**: 37-46 [PMID: 24782257]
3 **Rupp D**, Bartenschlager R. Targets for antiviral therapy of hepatitis C. *Semin Liver Dis* 2014; **34**: 9-21 [PMID: 24782254]
4 **Scheel TK**, Rice CM. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. *Nat Med* 2013; **19**: 837-849 [PMID: 23836234]
5 **Welzel TM**, Dultz G, Zeuzem S. Interferon-free antiviral combination therapies without nucleosidic polymerase inhibitors. *J Hepatol* 2014; **61**: S98-S107 [PMID: 25443350]
6 **Yau AH**, Yoshida EM. Hepatitis C drugs: the end of the pegylated interferon era and the emergence of all-oral interferon-free antiviral regimens: a concise review. *Can J Gastroenterol Hepatol* 2014; **28**: 445-451 [PMID: 25229466]
7 **Abdel-Hakeem MS**, Shoukry NH. Protective immunity against hepatitis C: many shades of gray. *Front Immunol* 2014; **5**: 274 [PMID: 24982656]
8 **Rehermann B**. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. *J Clin Invest* 2009; **119**: 1745-1754 [PMID: 19587449]
9 **Billerbeck E**, de Jong Y, Dorner M, de la Fuente C, Ploss A. Animal models for hepatitis C. *Curr Top Microbiol Immunol* 2013; **369**: 49-86 [PMID: 23463197]

10 **Scull MA**, Shi C, de Jong YP, Gerold G, Ries M, von Schaewen M, Donovan BM, Labitt RN, Horwitz JA, Gaska JM, Hrebikova G, Xiao JW, Flatley B, Fung C, Chiriboga L, Walker CM, Evans DT, Rice CM, Ploss A. Hepatitis C virus infects rhesus macaque hepatocytes and simianized mice. *Hepatology* 2015; **62**: 57-67 [PMID: 25820364]

11 **Su AI**, Pezacki JP, Wodicka L, Brideau AD, Supekova L, Thimme R, Wieland S, Bukh J, Purcell RH, Schultz PG, Chisari FV. Genomic analysis of the host response to hepatitis C virus infection. *Proc Natl Acad Sci USA* 2002; **99**: 15669-15674 [PMID: 12441396]

12 **Thimme R**, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, Govindarajan S, Purcell RH, Chisari FV. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci USA* 2002; **99**: 15661-15668 [PMID: 12441397]

13 **Thimme R**, Chang KM, Pemberton J, Sette A, Chisari FV. Degenerate immunogenicity of an HLA-A2-restricted hepatitis B virus nucleocapsid cytotoxic T-lymphocyte epitope that is also presented by HLA-B51. *J Virol* 2001; **75**: 3984-3987 [PMID: 11264388]

14 **Thimme R**, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, Chisari FV. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J Virol* 2003; **77**: 68-76 [PMID: 12477811]

15 **Wang J**, Holmes TH, Cheung R, Greenberg HB, He XS. Expression of chemokine receptors on intrahepatic and peripheral lymphocytes in chronic hepatitis C infection: its relationship to liver inflammation. *J Infect Dis* 2004; **190**: 989-997 [PMID: 15295707]

16 **Grakoui A**, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghrayeb J, Murthy KK, Rice CM, Walker CM. HCV persistence and immune evasion in the absence of memory T cell help. *Science* 2003; **302**: 659-662 [PMID: 14576438]

17 **Smyk-Pearson S**, Golden-Mason L, Klarquist J, Burton JR, Tester IA, Wang CC, Culbertson N, Vandenbark AA, Rosen HR. Functional suppression by FoxP3+CD4+CD25(high) regulatory T cells during acute hepatitis C virus infection. *J Infect Dis* 2008; **197**: 46-57 [PMID: 18171284]

18 **Schulze zur Wiesch J**, Lauer GM, Day CL, Kim AY, Ouchi K, Duncan JE, Wurcel AG, Timm J, Jones AM, Mothe B, Allen TM, McGovern B, Lewis-Ximenez L, Sidney J, Sette A, Chung RT, Walker BD. Broad repertoire of the CD4+ Th cell response in spontaneously controlled hepatitis C virus infection includes dominant and highly promiscuous epitopes. *J Immunol* 2005; **175**: 3603-3613 [PMID: 16148104]

19 **Sugimoto K**, Ikeda F, Stadanlick J, Nunes FA, Alter HJ, Chang KM. Suppression of HCV-specific T cells without differential hierarchy demonstrated ex vivo in persistent HCV infection. *Hepatology* 2003; **38**: 1437-1448 [PMID: 14647055]

20 **Bowen DG**, Shoukry NH, Grakoui A, Fuller MJ, Cawthon AG, Dong C, Hasselschwert DL, Brasky KM, Freeman GJ, Seth NP, Wucherpfennig KW, Houghton M, Walker CM. Variable patterns of programmed death-1 expression on fully functional memory T cells after spontaneous resolution of hepatitis C virus infection. *J Virol* 2008; **82**: 5109-5114 [PMID: 18337576]

21 **Golden-Mason L**, Palmer B, Klarquist J, Mengshol JA, Castelblanco N, Rosen HR. Upregulation of PD-1 expression on circulating and intrahepatic hepatitis C virus-specific CD8+ T cells associated with reversible immune dysfunction. *J Virol* 2007; **81**: 9249-9258 [PMID: 17567698]

22 **Golden-Mason L**, Klarquist J, Wahed AS, Rosen HR. Cutting edge: programmed death-1 expression is increased on immunocytes in chronic hepatitis C virus and predicts failure of response to antiviral therapy: race-dependent differences. *J Immunol* 2008; **180**: 3637-3641 [PMID: 18322167]

23 **Abdel-Hakeem MS**, Bédard N, Badr G, Ostrowski M, Sékaly RP, Bruneau J, Willems B, Heathcote EJ, Shoukry NH. Comparison of immune restoration in early versus late alpha interferon therapy against hepatitis C virus. *J Virol* 2010; **84**: 10429-10435 [PMID: 20668076 DOI: 10.1128/JVI.01094-10]

24 **Barnes E**, Gelderblom HC, Humphreys I, Semmo N, Reesink HW, Beld MG, van Lier RA, Klenerman P. Cellular immune responses during high-dose interferon-alpha induction therapy for hepatitis C virus infection. *J Infect Dis* 2009; **199**: 819-828 [PMID: 19434929 DOI: 10.1086/597072]

25 **Missale G**, Pilli M, Zerbini A, Penna A, Ravanetti L, Barili V, Orlandini A, Molinari A, Fasano M, Santantonio T, Ferrari C. Lack of full CD8 functional restoration after antiviral treatment for acute and chronic hepatitis C virus infection. *Gut* 2012; **61**: 1076-1084 [PMID: 22337949 DOI: 10.1136/gutjnl-2011-300515]

26 **Odorizzi PM**, Wherry EJ. Immunology. An interferon paradox. *Science* 2013; **340**: 155-156 [PMID: 23580520 DOI: 10.1126/science.1237568]

27 **Welsh RM**, Bahl K, Marshall HD, Urban SL. Type 1 interferons and antiviral CD8 T-cell responses. *PLoS Pathog* 2012; **8**: e1002352 [PMID: 22241987 DOI: 10.1371/journal.ppat.1002352]

28 **Martin B**, Hennecke N, Lohmann V, Kayser A, Neumann-Haefelin C, Kukolj G, Böcher WO, Thimme R. Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. *J Hepatol* 2014; **61**: 538-543 [PMID: 24905492]

29 **Baumert TF**, Fauvelle C, Chen DY, Lauer GM. A prophylactic hepatitis C virus vaccine: a distant peak still worth climbing. *J Hepatol* 2014; **61**: S34-S44 [PMID: 25443345 DOI: 10.1016/j.jhep.2014.09.009]

30 **Tarr AW**, Urbanowicz RA, Ball JK. The role of humoral innate immunity in hepatitis C virus infection. *Viruses* 2012; **4**: 1-27 [PMID: 22355450 DOI: 10.3390/v4010001]

31 **Folgori A**, Capone S, Ruggeri L, Meola A, Sporeno E, Ercole BB, Pezzanera M, Tafi R, Arcuri M, Fattori E, Lahm A, Luzzago A, Vitelli A, Colloca S, Cortese R, Nicosia A. A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees. *Nat Med* 2006; **12**: 190-197 [PMID: 16462801]

32 **Barnes E**, Folgori A, Capone S, Swadling L, Aston S, Kurioka A, Meyer J, Huddart R, Smith K, Townsend R, Brown A, Antrobus R, Ammendola V, Naddeo M, O'Hara G, Willberg C, Harrison A, Grazioli F, Esposito ML, Siani L, Traboni C, Oo Y, Adams D, Hill A, Colloca S, Nicosia A, Cortese R, Klenerman P. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. *Sci Transl Med* 2012; **4**: 115ra1 [PMID: 22218690 DOI: 10.1126/scitranslmed.3003155]

33 **Ray R**, Meyer K, Banerjee A, Basu A, Coates S, Abrignani S, Houghton M, Frey SE, Belshe RB. Characterization of antibodies induced by vaccination with hepatitis C virus envelope glycoproteins. *J Infect Dis* 2010; **202**: 862-866 [PMID: 20677942 DOI: 10.1086/655902]

34 **de Jong YP**, Dorner M, Mommersteeg MC, Xiao JW, Balazs AB, Robbins JB, Winer BY, Gerges S, Vega K, Labitt RN, Donovan BM, Giang E, Krishnan A, Chiriboga L, Charlton MR, Burton DR, Baltimore D, Law M, Rice CM, Ploss A. Broadly neutralizing antibodies abrogate established hepatitis C virus infection. *Sci Transl Med* 2014; **6**: 254ra129 [PMID: 25232181 DOI: 10.1126/scitranslmed.3009512]

35 **Kong L**, Giang E, Nieusma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M. Hepatitis C virus E2 envelope glycoprotein core structure. *Science* 2013; **342**: 1090-1094 [PMID: 24288331 DOI: 10.1126/science.1243876]

36 **Thomson EC**, Smith JA, Klenerman P. The natural history of early hepatitis C virus evolution; lessons from a global outbreak in human immunodeficiency virus-1-infected individuals. *J Gen Virol* 2011; **92**: 2227-2236 [PMID: 21775583 DOI: 10.1099/vir.0.033910-0]

37 **Schoggins JW**, Rice CM. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol* 2011; **1**: 519-525 [PMID: 22328912 DOI: 10.1016/j.coviro.2011.10.008]

38 **Saito T**, Owen DM, Jiang F, Marcotrigiano J, Gale M. Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. *Nature* 2008; **454**: 523-527 [PMID: 18548002 DOI: 10.1038/nature07106]

39 **Malathi K**, Saito T, Crochet N, Barton DJ, Gale M, Silverman RH. RNase L releases a small RNA from HCV RNA that refolds into a potent PAMP. *RNA* 2010; **16**: 2108-2119 [PMID: 20833746 DOI:

10.1261/rna.2244210]
40 **Liu HM**, Loo YM, Horner SM, Zornetzer GA, Katze MG, Gale M. The mitochondrial targeting chaperone 14-3-3ε regulates a RIG-I translocon that mediates membrane association and innate antiviral immunity. *Cell Host Microbe* 2012; **11**: 528-537 [PMID: 22607805 DOI: 10.1016/j.chom.2012.04.006]
41 **Saito T**, Hirai R, Loo YM, Owen D, Johnson CL, Sinha SC, Akira S, Fujita T, Gale M. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. *Proc Natl Acad Sci USA* 2007; **104**: 582-587 [PMID: 17190814]
42 **Gack MU**, Shin YC, Joo CH, Urano T, Liang C, Sun L, Takeuchi O, Akira S, Chen Z, Inoue S, Jung JU. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature* 2007; **446**: 916-920 [PMID: 17392790]
43 **Horner SM**, Gale M. Regulation of hepatic innate immunity by hepatitis C virus. *Nat Med* 2013; **19**: 879-888 [PMID: 23836238 DOI: 10.1038/nm.3253]
44 **Kawai T**, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O, Akira S. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat Immunol* 2005; **6**: 981-988 [PMID: 16127453]
45 **Yamamoto M**, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* 2003; **301**: 640-643 [PMID: 12855817]
46 **Hemmi H**, Takeuchi O, Sato S, Yamamoto M, Kaisho T, Sanjo H, Kawai T, Hoshino K, Takeda K, Akira S. The roles of two IkappaB kinase-related kinases in lipopolysaccharide and double stranded RNA signaling and viral infection. *J Exp Med* 2004; **199**: 1641-1650 [PMID: 15210742]
47 **Lee HC**, Narayanan S, Park SJ, Seong SY, Hahn YS. Transcriptional regulation of IFN-λ genes in hepatitis C virus-infected hepatocytes via IRF-3·IRF-7·NF-κB complex. *J Biol Chem* 2014; **289**: 5310-5319 [PMID: 24385435 DOI: 10.1074/jbc.M113.536102]
48 **Sharma S**, tenOever BR, Grandvaux N, Zhou GP, Lin R, Hiscott J. Triggering the interferon antiviral response through an IKK-related pathway. *Science* 2003; **300**: 1148-1151 [PMID: 12702806]
49 **Sommereyns C**, Paul S, Staeheli P, Michiels T. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathog* 2008; **4**: e1000017 [PMID: 18369468 DOI: 10.1371/journal.ppat.1000017]
50 **Platanias LC**. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* 2005; **5**: 375-386 [PMID: 15864272]
51 **Shuai K**, Ziemiecki A, Wilks AF, Harpur AG, Sadowski HB, Gilman MZ, Darnell JE. Polypeptide signalling to the nucleus through tyrosine phosphorylation of Jak and Stat proteins. *Nature* 1993; **366**: 580-583 [PMID: 7504784]
52 **Tenoever BR**, Ng SL, Chua MA, McWhirter SM, García-Sastre A, Maniatis T. Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. *Science* 2007; **315**: 1274-1278 [PMID: 17332413]
53 **Rajsbaum R**, Versteeg GA, Schmid S, Maestre AM, Belicha-Villanueva A, Martínez-Romero C, Patel JR, Morrison J, Pisanelli G, Miorin L, Laurent-Rolle M, Moulton HM, Stein DA, Fernandez-Sesma A, tenOever BR, García-Sastre A. Unanchored K48-linked polyubiquitin synthesized by the E3-ubiquitin ligase TRIM6 stimulates the interferon-IKKε kinase-mediated antiviral response. *Immunity* 2014; **40**: 880-895 [PMID: 24882218 DOI: 10.1016/j.immuni.2014.04.018]
54 **Mukherjee A**, Di Bisceglie AM, Ray RB. Hepatitis C virus-mediated enhancement of microRNA miR-373 impairs the JAK/STAT signaling pathway. *J Virol* 2015; **89**: 3356-3365 [PMID: 25589644 DOI: 10.1128/JVI.03085-14]
55 **Lin W**, Choe WH, Hiasa Y, Kamegaya Y, Blackard JT, Schmidt EV, Chung RT. Hepatitis C virus expression suppresses interferon signaling by degrading STAT1. *Gastroenterology* 2005; **128**: 1034-1041 [PMID: 15825084]
56 **Stevenson NJ**, Bourke NM, Ryan EJ, Binder M, Fanning L, Johnston JA, Hegarty JE, Long A, O'Farrelly C. Hepatitis C virus targets the interferon-α JAK/STAT pathway by promoting proteasomal degradation in immune cells and hepatocytes. *FEBS Lett* 2013; **587**: 1571-1578 [PMID: 23587486]
57 **Muir AJ**, Shiffman ML, Zaman A, Yoffe B, de la Torre A, Flamm S, Gordon SC, Marotta P, Vierling JM, Lopez-Talavera JC, Byrnes-Blake K, Fontana D, Freeman J, Gray T, Hausman D, Hunder NN, Lawitz E. Phase 1b study of pegylated interferon lambda 1 with or without ribavirin in patients with chronic genotype 1 hepatitis C virus infection. *Hepatology* 2010; **52**: 822-832 [PMID: 20564352 DOI: 10.1002/hep.23743]
58 **Ramos EL**. Preclinical and clinical development of pegylated interferon-lambda 1 in chronic hepatitis C. *J Interferon Cytokine Res* 2010; **30**: 591-595 [PMID: 20645873]
59 **Sarasin-Filipowicz M**, Wang X, Yan M, Duong FH, Poli V, Hilton DJ, Zhang DE, Heim MH. Alpha interferon induces long-lasting refractoriness of JAK-STAT signaling in the mouse liver through induction of USP18/UBP43. *Mol Cell Biol* 2009; **29**: 4841-4851 [PMID: 19564419 DOI: 10.1128/MCB.00224-09]
60 **Makowska Z**, Duong FH, Trincucci G, Tough DF, Heim MH. Interferon-β and interferon-λ signaling is not affected by interferon-induced refractoriness to interferon-α in vivo. *Hepatology* 2011; **53**: 1154-1163 [PMID: 21480323 DOI: 10.1002/hep.24189]
61 **Muir AJ**, Arora S, Everson G, Flisiak R, George J, Ghalib R, Gordon SC, Gray T, Greenbloom S, Hassanein T, Hillson J, Horga MA, Jacobson IM, Jeffers L, Kowdley KV, Lawitz E, Lueth S, Rodriguez-Torres M, Rustgi V, Shemanski L, Shiffman ML, Srinivasan S, Vargas HE, Vierling JM, Xu D, Lopez-Talavera JC, Zeuzem S. A randomized phase 2b study of peginterferon lambda-1a for the treatment of chronic HCV infection. *J Hepatol* 2014; **61**: 1238-1246 [PMID: 25064437 DOI: 10.1016/j.jhep.2014.07.022]
62 **Friborg J**, Ross-Macdonald P, Cao J, Willard R, Lin B, Eggers B, McPhee F. Impairment of type I but not type III IFN signaling by hepatitis C virus infection influences antiviral responses in primary human hepatocytes. *PLoS One* 2015; **10**: e0121734 [PMID: 25826356 DOI: 10.1371/journal.pone.0121734]
63 **Li K**, Foy E, Ferreon JC, Nakamura M, Ferreon AC, Ikeda M, Ray SC, Gale M, Lemon SM. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proc Natl Acad Sci USA* 2005; **102**: 2992-2997 [PMID: 15710891]
64 **Wang Y**, Li J, Wang X, Ye L, Zhou Y, Thomas RM, Ho W. Hepatitis C virus impairs TLR3 signaling and inhibits IFN-λ 1 expression in human hepatoma cell line. *Innate Immun* 2014; **20**: 3-11 [PMID: 23529855 DOI: 10.1177/1753425913478991]
65 **Oudshoorn D**, Versteeg GA, Kikkert M. Regulation of the innate immune system by ubiquitin and ubiquitin-like modifiers. *Cytokine Growth Factor Rev* 2012; **23**: 273-282 [PMID: 22964110 DOI: 10.1016/j.cytogfr.2012.08.003]
66 **Rajsbaum R**, García-Sastre A. Viral evasion mechanisms of early antiviral responses involving regulation of ubiquitin pathways. *Trends Microbiol* 2013; **21**: 421-429 [PMID: 23850008 DOI: 10.1016/j.tim.2013.06.006]
67 **Banerjee I**, Miyake Y, Nobs SP, Schneider C, Horvath P, Kopf M, Matthias P, Helenius A, Yamauchi Y. Influenza A virus uses the aggresome processing machinery for host cell entry. *Science* 2014; **346**: 473-477 [PMID: 25342804 DOI: 10.1126/science.1257037]
68 **Rajsbaum R**, García-Sastre A. Virology. Unanchored ubiquitin in virus uncoating. *Science* 2014; **346**: 427-428 [PMID: 25342790 DOI: 10.1126/science.1261509]
69 **Oshiumi H**, Miyashita M, Matsumoto M, Seya T. A distinct role of Riplet-mediated K63-Linked polyubiquitination of the RIG-I repressor domain in human antiviral innate immune responses. *PLoS Pathog* 2013; **9**: e1003533 [PMID: 23950712 DOI: 10.1371/journal.ppat.1003533]
70 **Barr SD**, Smiley JR, Bushman FD. The interferon response inhibits HIV particle production by induction of TRIM22. *PLoS Pathog* 2008; **4**: e1000007 [PMID: 18389079 DOI: 10.1371/journal.ppat.1000007]
71 **Carthagena L**, Bergamaschi A, Luna JM, David A, Uchil PD, Margottin-Goguet F, Mothes W, Hazan U, Transy C, Pancino G,

Nisole S. Human TRIM gene expression in response to interferons. *PLoS One* 2009; **4**: e4894 [PMID: 19290053 DOI: 10.1371/journal.pone.0004894]
72 **Tissot C**, Mechti N. Molecular cloning of a new interferon-induced factor that represses human immunodeficiency virus type 1 long terminal repeat expression. *J Biol Chem* 1995; **270**: 14891-14898 [PMID: 7797467]
73 **Sadeghi F**, Bokharaei-Salim F, Salehi-Vaziri M, Monavari SH, Alavian SM, Salimi S, Vahabpour R, Keyvani H. Associations between human TRIM22 gene expression and the response to combination therapy with Peg-IFNα-2a and ribavirin in Iranian patients with chronic hepatitis C. *J Med Virol* 2014; **86**: 1499-1506 [PMID: 24889558 DOI: 10.1002/jmv.23985]
74 **Yang C**, Zhao X, Sun D, Yang L, Chong C, Pan Y, Chi X, Gao Y, Wang M, Shi X, Sun H, Lv J, Gao Y, Zhong J, Niu J, Sun B6. Interferon alpha (IFNα)-induced TRIM22 interrupts HCV replication by ubiquitinating NS5A. *Cell Mol Immunol* 2015; Epub ahead of print [PMID: 25683609 DOI: 10.1038/cmi.2014.131]
75 **Li Q**, Zhang YY, Chiu S, Hu Z, Lan KH, Cha H, Sodroski C, Zhang F, Hsu CS, Thomas E, Liang TJ. Integrative functional genomics of hepatitis C virus infection identifies host dependencies in complete viral replication cycle. *PLoS Pathog* 2014; **10**: e1004163 [PMID: 24852294 DOI: 10.1371/journal.ppat.1004163]
76 **Lee HS**, Lim YS, Park EM, Baek SH, Hwang SB. SUMOylation of nonstructural 5A protein regulates hepatitis C virus replication. *J Viral Hepat* 2014; **21**: e108-e117 [PMID: 24602294 DOI: 10.1111/jvh.12241]
77 **Morales DJ**, Lenschow DJ. The antiviral activities of ISG15. *J Mol Biol* 2013; **425**: 4995-5008 [PMID: 24095857 DOI: 10.1016/j.jmb.2013.09.041]
78 **Kim MJ**, Yoo JY. Inhibition of hepatitis C virus replication by IFN-mediated ISGylation of HCV-NS5A. *J Immunol* 2010; **185**: 4311-4318 [PMID: 20810994 DOI: 10.4049/jimmunol.1000098]
79 **Chen L**, Sun J, Meng L, Heathcote J, Edwards AM, McGilvray ID. ISG15, a ubiquitin-like interferon-stimulated gene, promotes hepatitis C virus production in vitro: implications for chronic infection and response to treatment. *J Gen Virol* 2010; **91**: 382-388 [PMID: 19846672 DOI: 10.1099/vir.0.015388-0]
80 **Broering R**, Zhang X, Kottilil S, Trippler M, Jiang M, Lu M, Gerken G, Schlaak JF. The interferon stimulated gene 15 functions as a proviral factor for the hepatitis C virus and as a regulator of the IFN response. *Gut* 2010; **59**: 1111-1119 [PMID: 20639253 DOI: 10.1136/gut.2009.195545]
81 **Malakhov MP**, Malakhova OA, Kim KI, Ritchie KJ, Zhang DE. UBP43 (USP18) specifically removes ISG15 from conjugated proteins. *J Biol Chem* 2002; **277**: 9976-9981 [PMID: 11788588]
82 **Malakhova OA**, Yan M, Malakhov MP, Yuan Y, Ritchie KJ, Kim KI, Peterson LF, Shuai K, Zhang DE. Protein ISGylation modulates the JAK-STAT signaling pathway. *Genes Dev* 2003; **17**: 455-460 [PMID: 12600939]
83 **Randall G**, Chen L, Panis M, Fischer AK, Lindenbach BD, Sun J, Heathcote J, Rice CM, Edwards AM, McGilvray ID. Silencing of USP18 potentiates the antiviral activity of interferon against hepatitis C virus infection. *Gastroenterology* 2006; **131**: 1584-1591 [PMID: 17101330]
84 **Real CI**, Megger DA, Sitek B, Jahn-Hofmann K, Ickenstein LM, John MJ, Walker A, Timm J, Kuhlmann K, Eisenacher M, Meyer HE, Gerken G, Broering R, Schlaak JF. Identification of proteins that mediate the pro-viral functions of the interferon stimulated gene 15 in hepatitis C virus replication. *Antiviral Res* 2013; **100**: 654-661 [PMID: 24416772]
85 **Munakata T**, Liang Y, Kim S, McGivern DR, Huibregtse J, Nomoto A, Lemon SM. Hepatitis C virus induces E6AP-dependent degradation of the retinoblastoma protein. *PLoS Pathog* 2007; **3**: 1335-1347 [PMID: 17907805]
86 **Munakata T**, Nakamura M, Liang Y, Li K, Lemon SM. Down-regulation of the retinoblastoma tumor suppressor by the hepatitis C virus NS5B RNA-dependent RNA polymerase. *Proc Natl Acad Sci USA* 2005; **102**: 18159-18164 [PMID: 16332962]
87 **Starr R**, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA, Hilton DJ. A family of cytokine-inducible inhibitors of signalling. *Nature* 1997; **387**: 917-921 [PMID: 9202125]
88 **Shoji I**. Roles of the two distinct proteasome pathways in hepatitis C virus infection. *World J Virol* 2012; **1**: 44-50 [PMID: 24175210 DOI: 10.5501/wjv.v1.i2.44]
89 **Raychoudhuri A**, Shrivastava S, Steele R, Kim H, Ray R, Ray RB. ISG56 and IFITM1 proteins inhibit hepatitis C virus replication. *J Virol* 2011; **85**: 12881-12889 [PMID: 21976647 DOI: 10.1128/JVI.05633-11]
90 **Wilkins C**, Woodward J, Lau DT, Barnes A, Joyce M, McFarlane N, McKeating JA, Tyrrell DL, Gale M. IFITM1 is a tight junction protein that inhibits hepatitis C virus entry. *Hepatology* 2013; **57**: 461-469 [PMID: 22996292 DOI: 10.1002/hep.26066]
91 **Metz P**, Dazert E, Ruggieri A, Mazur J, Kaderali L, Kaul A, Zeuge U, Windisch MP, Trippler M, Lohmann V, Binder M, Frese M, Bartenschlager R. Identification of type I and type II interferon-induced effectors controlling hepatitis C virus replication. *Hepatology* 2012; **56**: 2082-2093 [PMID: 22711689 DOI: 10.1002/hep.25908]
92 **Brass AL**, Huang IC, Benita Y, John SP, Krishnan MN, Feeley EM, Ryan BJ, Weyer JL, van der Weyden L, Fikrig E, Adams DJ, Xavier RJ, Farzan M, Elledge SJ. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. *Cell* 2009; **139**: 1243-1254 [PMID: 20064371 DOI: 10.1016/j.cell.2009.12.017]
93 **Jiang D**, Guo H, Xu C, Chang J, Gu B, Wang L, Block TM, Guo JT. Identification of three interferon-inducible cellular enzymes that inhibit the replication of hepatitis C virus. *J Virol* 2008; **82**: 1665-1678 [PMID: 18077728]
94 **Park K**, Scott AL. Cholesterol 25-hydroxylase production by dendritic cells and macrophages is regulated by type I interferons. *J Leukoc Biol* 2010; **88**: 1081-1087 [PMID: 20699362]
95 **Ye J**, Wang C, Sumpter R, Brown MS, Goldstein JL, Gale M. Disruption of hepatitis C virus RNA replication through inhibition of host protein geranylgeranylation. *Proc Natl Acad Sci USA* 2003; **100**: 15865-15870 [PMID: 14668447]
96 **Chen Y**, Wang S, Yi Z, Tian H, Aliyari R, Li Y, Chen G, Liu P, Zhong J, Chen X, Du P, Su L, Qin FX, Deng H, Cheng G. Interferon-inducible cholesterol-25-hydroxylase inhibits hepatitis C virus replication via distinct mechanisms. *Sci Rep* 2014; **4**: 7242 [PMID: 25467815 DOI: 10.1038/srep07242]

P- Reviewer: Takahashi T **S- Editor**: Ma YJ **L- Editor**: A
E- Editor: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10749

World J Gastroenterol 2015 October 14; 21(38): 10749-10759
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

Hepatitis C virus markers in infection by hepatitis C virus: In the era of directly acting antivirals

Nicola Coppola, Mariantonietta Pisaturo, Rosa Zampino, Margherita Macera, Caterina Sagnelli, Evangelista Sagnelli

Nicola Coppola, Mariantonietta Pisaturo, Margherita Macera, Evangelista Sagnelli, Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, 80131 Naples, Italy

Mariantonietta Pisaturo, Division of Infectious Diseases, AORN Sant'Anna e San Sebastiano di Caserta, 81100 Caserta, Italy

Rosa Zampino, Internal Medicine and Hepatology, Second University of Naples, 80131 Naples, Italy

Caterina Sagnelli, Department of Clinical and Experimental Medicine and Surgery "F. Magrassi e A. Lanzara", Second University of Naples, 80131 Naples, Italy

Author contributions: All authors substantial contributed to conception and design, acquisition of data, or analysis and interpretation of data; Coppola N contributed to drafting the article or revising it critically for important intellectual content; Pisaturo M analysis of "HCV markers in Diagnosis of chronic HCV infection and in Assessment of the severity of chronic hepatitis C"; Zampino R analysis of "HCV-RNA kinetics and clearance as markers of remission"; Macera M analysis of "Assessment of factors associated with the response to anti-viral treatment"; Sagnelli C analysis of "HCV markers in acute hepatitis C"; Sagnelli E contributed to drafting the article and revising it critically for important intellectual content; and all authors approved the final version to be published.

Conflict-of-interest statement: All the authors of the manuscript declare that they have no conflict of interest in connection with this paper.

Correspondence to: Nicola Coppola, MD, PhD, Assistant Professor of Infectious Diseases, Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, Via L. Armanni 5, 80131 Naples, Italy. nicola.coppola@unina2.it
Telephone: +39-815-666719
Fax: +39-815-666013

Received: April 26, 2015
Peer-review started: April 28, 2015
First decision: June 2, 2015
Revised: July 4, 2015
Accepted: September 2, 2015
Article in press: September 2, 2015
Published online: October 14, 2015

Abstract

About 130-170 million people are infected with the hepatitis C virus (HCV) worldwide and more than 350000 people die each year of HCV-related liver diseases. The combination of pegylated interferon (Peg-IFN) and ribavirin (RBV) was recommended as the treatment of choice for chronic hepatitis C for nearly a decade. In 2011 the directly acting antivirals (DAA) HCV NS3/4A protease inhibitors, telaprevir and boceprevir, were approved to treat HCV-genotype-1 infection, each in triple combination with Peg-IFN and RBV. These treatments allowed higher rates of SVR than the double Peg-IFN + RBV, but the low tolerability and high pill burden of these triple regimes were responsible for reduced adherence and early treatment discontinuation. The second and third wave DAAs introduced in 2013-2014 enhanced the efficacy and tolerability of anti-HCV treatment. Consequently, the traditional indicators for disease management and predictors of treatment response should be revised in light of these new therapeutic options. This review article will focus on the use of the markers of HCV infection and replication, of laboratory and

instrumental data to define the stage of the disease and of predictors, if any, of response to therapy in the DAA era. The article is addressed particularly to physicians who have patients with hepatitis C in care in their everyday clinical practice.

Key words: Chronic hepatitis C; Hepatitis C virus replication; Directly acting antivirals; Staging; Hepatitis C virus infection

Core tip: The second and third wave directly acting antivirals introduced in 2013-2014 enhanced the efficacy and tolerability of anti-hepatitis C virus (HCV) treatment. Consequently, the traditional indicators for disease management and predictors of treatment response should be revised in light of these new therapeutic options. This review article analyzes the modern use of the markers of HCV infection in: (1) the diagnosis of acute hepatitis C; (2) the diagnosis of chronic HCV infection; (3) the assessment of the severity of chronic hepatitis C; (4) the assessment of factors associated with response to anti-viral treatment; and (5) HCV-RNA kinetics and clearance as markers of remission.

Coppola N, Pisaturo M, Zampino R, Macera M, Sagnelli C, Sagnelli E. Hepatitis C virus markers in infection by hepatitis C virus: In the era of directly acting antivirals. *World J Gastroenterol* 2015; 21(38): 10749-10759 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10749.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10749

INTRODUCTION

The World Health Organization (WHO) estimates that 130-170 million people are infected with hepatitis C virus (HCV) worldwide and that more than 350000 people die each year of HCV-related liver diseases[1]. Primary infection causes acute hepatitis C (AHC), which is asymptomatic in the majority of cases, but progresses to chronicity in about two-thirds of the cases and spontaneously remits in the remaining one-third[2-7]. Patients with chronic hepatitis C (CHC) frequently show increasing severity of liver fibrosis over time, which leads to liver cirrhosis in nearly a quarter of cases. Hepatocellular carcinoma (HCC) develops in HCV-related liver cirrhosis with a yearly rate around 3%[8-16].

The combination of pegylated interferon (Peg-IFN) and ribavirin (RBV) was recommended as the treatment of choice for CHC for nearly a decade[17-22]. This treatment, although poorly tolerated, provided a sustained clearance of circulating HCV (sustained viral response - SVR) in half of the patients with CHC due to HCV genotype 1 and in nearly 70% of those with HCV genotype 2 or 3. Several predictors of an unfavorable response to this treatment have been identified: viral (HCV genotype 1 or 4 and a slow decline in serum HCV RNA during treatment), host factors (male sex, older age), a co-pathology (insulin resistance, diabetes), Afro-American ethnicity, severe fibrosis and/or steatosis, high body mass index and interleukin (IL) 28-B non-CC genotype[23]. In 2011 the directly acting antivirals (DAAs) NS3/4A protease inhibitors telaprevir and boceprevir were approved to treat HCV-genotype-1 infection, each in triple combination with Peg-IFN and RBV[24-27]. These treatments allowed higher rates of SVR than the double Peg-IFN + RBV[18,28-33], but the low tolerability and high pill burden of these triple regimes[34] were responsible for reduced adherence and early treatment discontinuation. The second and third wave DAAs introduced in 2013-2014 enhanced the efficacy and tolerability of anti-HCV treatment[35,36]. In fact the second and third generation DAAs afford SVR rates above 90%, regardless of HCV genotype, better tolerability and adherence used either in IFN-free regimens or in combination with interferon and ribavirin[37-40]. Consequently, the traditional indicators for disease management and predictors of treatment response should be revised in light of these new therapeutic options.

This review article will focus on the use of the markers of HCV infection and replication, of laboratory and instrumental data to define the stage of the disease and of predictors, if any, of response to therapy in the DAA era (Table 1). The article is addressed particularly to physicians who have patients with hepatitis C in care in their everyday clinical practice.

HCV MARKERS IN AHC

In its symptomatic form AHC is characterized by nausea, malaise, abdominal pain, jaundice and by the typical biochemical abnormalities[41-43]. The HCV etiology is usually established on the basis of a documented seroconversion to anti-HCV and/or HCV-RNA positivity during the natural course of the illness[19,39,44,45], but it is impossible to establish for patients first observed when seroconversion has already occurred. In addition, AHC remains frequently undiagnosed because asymptomatic in the majority of the cases[41]. Despite its typically mild clinical course, AHC progresses to chronicity in nearly 70% of the cases. Treatment with a 3- or 6-mo course of Peg-IFN has been shown to be effective in eradicating acute HCV infection in most cases, but to date no standardized treatment schedule has been defined. Delaying the treatment to 8-12 wk after the beginning of the illness allows the identification of cases that resolve spontaneously and does not compromise the efficacy. The use of IFN-free treatment regimens for AHC patients awaits assessment.

Table 1 Viral and host markers useful for the management or treatment of chronic hepatitis C

	Roles in the Peg-IFN era	Roles in the DAA era
Anti-HCV assay	Diagnosis/screening	Diagnosis/screening
HCV-RNA assay	Diagnosis/active replication Pre-treatment predictor of response to antiviral treatments Monitoring antiviral treatment Assessment of response to treatment	Diagnosis/active replication Assessment of response to treatment
HCV genotype	Pre-treatment predictor of response to antiviral treatments	Choosing the most appropriate DAA regimen
HCV Q80K polymorphism	None	Selecting patients with HCV-genotype 1a for the simeprevir plus Peg-IFN regimen
Markers of liver fibrosis (liver biopsy/non-invasive methods)	Staging of liver disease Pre-treatment predictors of response to antiviral treatments	Staging of liver disease Selecting the patients with urgency for DAA-based treatment
IL-28B polymorphism	Pre-treatment predictor of response to antiviral treatments	Pre-treatment predictor of response only in Peg-IFN-based regimens
ITPA polymorphism	Pre-treatment predictive factor of hemolytic anemia during ribavirin-based regimen	Indicator of risk/benefit of using ribavirin in a DAA-based regimen

Peg-IFN: Pegylated interferon; DAAs: Directly acting antivirals; HCV: Hepatitis C virus; IL: Interleukin.

New strategies for an early diagnosis of AHC have been investigated[46,47]. In addition, attempts have been made to distinguish this clinical form from an acute exacerbation of CHC, a clinical event characterized by a substantial increase in serum alanine aminotransferase (ALT) levels above the previous values in patients with CHC[6,7,48-50]. A combined use in serial serum samples of the rise in the anti-HCV titers and of the changes in antibody positivity in a recombinant immunoblot assay was found to be of some use by Lu *et al*[46]. Araujo *et al*[47], using a flow-cytometric microsphere immunoassay to measure anti-HCV IgG reactivity to the core NS3, NS4 and NS5 HCV recombinant proteins, correctly classified serum samples of AHC and CHC with a cross-validation of 90.8% for the AHC group and 97.2% for the CHC group. The role of anti-HCV IgG avidity and anti-HCV IgM titers to diagnose AHC have been extensively investigated[5,51-54]. A successful attempt to distinguish between AHC and a reactivation of CHC was made by Sagnelli *et al*[4] by carrying out a serial determination of anti-HCV IgM at two or three checking points within the third week from the disease onset. In another study by the same group, Coppola *et al*[5] successfully explored the distinction between AHC and a reactivation of CHC using the avidity of anti-HCV IgG to diagnose AHC. When both methods (anti-HCV IgM titer and anti-HCV IgG avidity) were applied to serial serum samples obtained during the illness, the distinction between the two clinical forms reached a level of sensitivity and specificity approaching 95%. The IgG avidity assay showed the highest efficacy during the initial two weeks of the illness and the IgM titer assay during the subsequent two weeks[53].

The diagnosis of AHC remains important even in the DAA era, since, although not yet assessed, it seems reasonable that all or nearly all patients with AHC can be cured with a short DAA-based regimen.

HCV MARKERS IN CHC

Diagnosis of chronic HCV infection

The diagnosis of CHC is based on the detection of serum anti-HCV and HCV RNA, elevated serum values of aminotransferases for at least six months and necroinflammation and fibrosis in liver tissue[8,19,39]. Screening to detect anti-HCV in serum is indicated for persons with a history of intravenous drug use, or sharing paraphernalia for intranasal drug use, acupuncture, body piercing or tattooing, persons who received blood, blood products or solid organs before 1992, hemodialysis patients, children born of HCV-infected mothers, patients with hepatitis B virus (HBV) infection and those with human immunodeficiency virus (HIV) infection[19,55-61]. Anti-HCV-positive subjects should be tested for serum HCV RNA, the confirmatory test of an ongoing HCV infection[62-65].

Anti-HCV can be detected by an enzyme-linked immunosorbent assay, three generations of which have been developed since 1989. The first generation assay, incorporating the recombinant c100-3 epitope from the NS4 region was used until 1992, when it was replaced with the second generation assay incorporating the epitopes c22-3 and c33c from the HCV core and NS3 regions, respectively. The third generation assay used at present contains reconfigured core and NS3 antigens and a newly incorporated antigen from the NS5 region[62,66,67], is more sensitive than the previous assays and has a diagnostic specificity of over 99%[62]. However, the third generation enzyme immunoassays can, albeit rarely, yield false-negative results in immunocompromised patients and in those undergoing hemodialysis[19].

More recently an assay for a rapid detection of anti-HCV in fingerstick capillary blood, venipuncture whole blood or saliva has been developed[68]. This assay, easy

to perform and time-saving, has a good sensitivity and specificity and is particularly indicated for screening large populations.

The recombinant immunoblot assay, used in the past as a confirmatory assay of HCV infection, has not been recommended since 2013[69]. Subjects found to be anti-HCV-positive at screening should be tested for HCV RNA, the serum marker of HCV replication and current infection. Real-time PCR technologies can quantify HCV RNA during the exponential phase of amplification, with great sensitivity and a broad linear dynamic range (about 10 to 108 IU/mL). The majority of the commercial HCV RNA assays used by the clinical laboratories are based on the WHO international standard for HCV-RNA nucleic acid technology[70] and have an excellent specificity (98%-99%)[19].

Testing for HCV RNA should be considered for all anti-HCV-positive subjects and among the anti-HCV-negative for immunocompromised patients and for individuals exposed to HCV in the past 6 mo.

Concluding on this point, the high rate of SVR obtained by the DAA-based treatments is a further stimulus to screen all subjects exposed to HCV infection using a sensitive, specific, easy-to-perform and time-saving assay.

Assessment of the severity of CHC

The severity of CHC is variable among patients and over time in single patients. In most cases the disease shows a benign indolent course, but in some cases there is a rapid progression to liver cirrhosis, hepatocellular carcinoma and to an end-stage liver disease[8]. Liver cirrhosis is found in approximately 20% of patients with HCV-related chronic liver disease, associated in its advanced stages with life-threatening complications such as ascites, esophageal varice hemorrhage and liver failure. Hepatocellular carcinoma (HCC) occurs mostly in cirrhotic patients at a rate of 3%-5% per year[8].

The extent of liver necroinflammation and the degree of fibrosis in liver biopsy are considered reliable predictors of disease progression[71]. Several other investigators considered the stage of fibrosis detected in liver biopsy as a key point for the clinical management of CHC[72].

Although the liver histology is still considered the gold standard to assess the stage of liver fibrosis, because of the sides effects of liver biopsy[73-79] several surrogate non-invasive methods have been introduced. The measurement of liver stiffness by transient elastography offers an accredited method for the assessment of liver fibrosis[80]. This technique involves the use of a transducer on the end of an ultrasound probe that transmits 50 MHz pressure waves through the liver tissue. The velocity of the resulting "shear wave" is measured by ultrasound. The shear-wave velocity correlates with liver stiffness, thus providing an estimate of liver fibrosis[81,82]. Tsochatzis *et al*[82] performed a meta-analysis including 40 studies on numerous patients with chronic hepatitis of various etiologies (HBV, HCV, alcohol and other etiologic agents) and showed that transient elastography had a pooled sensitivity and specificity in diagnosing liver cirrhosis of 83% and 89%, respectively.

The ultrasound assay is another well-established non-invasive method to diagnose liver cirrhosis. The transition to cirrhosis is documented by the development of the characteristic coarse or nodular patterns in the liver parenchyma, hepatomegaly and caudate lobe hypertrophy[83]. Ultrasound can also detect the development of portal hypertension by measuring the portal vein diameter, velocity of flow and flow reversal, ascites and splenomegaly[84], but the sensitivity of ultrasound in assessing liver fibrosis is low.

There is no single surrogate test able to predict reliably the progression to cirrhosis in each single patient. However, high serum ALT levels have been associated with a higher risk of fibrosis progression[85-87], which, instead, is an uncommon event in patients with persistently normal serum ALT[88-91].

Several other non-invasive surrogate biomarkers or a combination of biomarkers may be of some help in assessing liver fibrosis, such as platelet count, INR index, aspartate aminotransferase (AST) serum levels and albumin serum concentration. One well-known combination of biomarkers that has been extensively validated in CHC[92,93] and in non-alcoholic fatty liver disease[94] is the so-called APRI test, an acronym for AST-platelet ratio index[95]. Also of some interest is the Fibrotest (Fibrosure in the United States), which includes five biomarkers and 2 clinical parameters[96]: α-2 macroglobulin, haptoglobin, total bilirubin, apolipoprotien-A, γ-glutamyl transferase, age and gender. Using a patented formula, a numerical value from 0.0 to 1.0 is obtained, a score correlated with the METAVIR fibrosis score in chronic hepatitis of different etiologies[92,97,98]. Combining the ALT serum value with the panel of biomarkers included in the Fibrotest, a new surrogate method to measure liver fibrosis was obtained, named Actitest and validated to diagnose liver cirrhosis in CHC patients[99]. FIB4 is a biomarker panel using age, AST, ALT and platelet count[100] validated in HIV/HCV co-infected[101] and HCV-monoinfected patients[102].

Concluding on this point, the assessment of liver fibrosis is still essential, even in the DAA era, since it allows the high-cost DAA treatment to be applied on the basis of the severity of liver damage and of the presumed speed of disease progression.

Assessment of factors associated with the response to anti-viral treatment

In the DAA era, HCV genotypes and subtypes remain cornerstones in the management of chronic HCV infection, since the rate of response and the consequent duration of treatment differ for the various

genotypes and subtypes[103]. In fact, considering patients with HCV genotype 1 or 4, whether therapy-naïve or -experienced, the combination of sofosbuvir and simeprevir (± ribavirin in non-responders to previous treatment) is the regimen of choice for subjects with METAVIR fibrosis scores 3 or 4, whereas for patients with fibrosis 0-2, optimal results were obtained with the combination of Peg-IFN, ribavirin and simeprevir[39,40]. Sofosbuvir plus ribavirin has been demonstrated to be an optimal combination for patients with HCV-genotype 2 or 3, whether therapy-naïve or -experienced, and the combination sofosbuvir plus daclatasvir for patients with HCV genotype 3[39,40]. In addition, in the simeprevir plus Peg-IFN-based regimen, it is essential to distinguish between patients with HCV sub-genotype 1a and 1b, since subtype 1a at times showed a Q80K substitution in the NS3 protease sequence, thus entailing a higher rate of treatment failure[39,40]. Currently, HCV genotyping can be performed by direct DNA sequencing by a bi-directional sequence where genotype and subtype characterization is determined by two fluorescently labeled DNA primers or by a commercial line probe assay[103].

In the DAA era, the detection of HCV viral load at baseline is now of no value in the treatment choice, since the anti-viral potency of these drugs controls even the highest level of HCV replication. The use of this test to monitor treated patients during the follow-up in order to detect possible reactivation seems good clinical practice.

The impact of staging in choosing a treatment schedule has decreased in proportion to the increase in the antiviral potency of the DAAs. In fact, the treatment regimens based on the third-wave DAAs achieve HCV eradication in almost all patients, regardless of the presence of liver cirrhosis[39,40].

The polymorphisms in the *IL28B* gene have been strongly associated with the spontaneous clearance of acute HCV infection and with the response to Peg-IFN and RBV combination therapy[104-107]. Their predictive value was more evident in difficult-to-treat HCV-genotype 1 and genotype 4 patients than in those with HCV-genotype 2 or 3 infection[108]. The distribution of *IL28B* polymorphisms varies among different populations, accounting, at least in part, for the ethnic and racial differences in the response to Peg-IFN plus RBV[107], the CC genotype being a predictor of a favorable response. At present, IL-28 genotyping has no predictive role in the high-efficacy DAA-based regimens[39,40], but might be of some value in settings where a Peg-IFN-based regimen might still be used.

Hemolytic anemia is a common side effect of RBV-based therapy that, although reversible and dose-related, induced a RBV dose reduction or premature treatment withdrawal in more than 15% of the cases[109,110]. Fellay *et al*[111] identified two variants (rs1127354 and rs7270101) in the *ITPA* gene that were functionally responsible for ITPA deficiency and correlated with the risk of RBV-induced anemia in European and American populations. The rs1127354 variant was associated with protection against anemia in other investigations[112-115]. The single nucleotide polymorphism (SNP) ITPA has never been associated with the treatment outcome[111-115], and in the DAA era it can be used only to evaluate the risk/benefit of adding ribavirin in some DAA-based regimens for patients with a lower rate of SVR, such as cirrhotics or previous non-responders.

Concluding on this point, DAA treatment eradicates HCV infection in nearly all treated patients, greatly reducing the clinical importance of markers previously used to predict the response to therapy. In fact, the HCV load and the degree of fibrosis do not predict the response to DAA therapy, and the polymorphisms in the *IL28B* gene may be useful only for patients with a METAVIR score F0-F2 treated with Peg-IFN, ribavirin and simeprevir, and the two SNPs in the *ITPA* gene only for those receiving a DAA plus ribavirin.

Instead, the determination of HCV genotype and subtype is of clinical value even in the DAA era, mandatory to choose the type and duration of therapy.

HCV-RNA kinetics and clearance as markers of remission

HCV-RNA clearance persisting 6 mo after therapy (SVR) remains a marker of the eradication of chronic HCV infection also in the DAA era.

International treatment guidelines[116-118] identified some virological predictors of SVR to Peg-IFN + RBV treatment: a rapid virological response *i.e.*, HCV-RNA clearance after 1 mo of therapy, and an early virological response, *i.e.*, HCV-RNA clearance after 3 mo of therapy. Subsequently, a very early predictor of SVR to Peg-IFN + RBV was suggested[119,120], *i.e.*, a decrease in the HCV load 2 d after the start of therapy. These predictors have been used to distinguish with good accuracy the patients with a good chance of achieving an SVR from those with a very low chance, who should discontinue treatment[121].

Compared to Peg-IFN + RBV treatments, the DAA-based therapies are more effective and better tolerated, but more expensive. The HCV-RNA kinetics during DAA treatment have been investigated in a limited number of patients and for short periods. Simeprevir given alone achieved a median HCV-RNA reduction of 3.9-$\log_{10}$ IU/mL over the first 3 d of treatment, independently of previous treatments and HCV genotype[122]. The administration of a single dose of 100-mg daclatasvir generated a decline in the HCV load of nearly 2-$\log_{10}$ in six hours and of 3.3-$\log_{10}$ in 24 h[123,124]. In addition, sofosbuvir obtained HCV-RNA clearance in 88%-94% of patients within the fourth week of treatment[125]. These data suggest that the determination of the HCV-RNA kinetics is of limited value in predicting the SVR in the DAA-based

IFN-free treatments, since the majority of treated patients[126] achieve this favorable outcome. Several studies assessed serum HCV RNA at weeks 2 and 4 of treatment[37,127-129] and found that the persistence of HCV RNA in serum at these check-points is predictive of treatment failure[130].

In Peg-IFN + RBV regimens, the normalization of serum aminotransferases has been used as a parameter to evaluate the biochemical response[131], often associated with an SVR. In recent studies on DAA-based treatments, serum aminotransferases were no longer used to evaluate the response to treatment[37,127-129], since, for reasons unrelated to HCV replication (presence of liver steatosis or consumption of alcohol or other drugs known to be hepatotoxic), they may remain elevated even in SVR patients. In addition, an increase in the aminotransferase serum values may occur in some patients during treatment, an event to be monitored carefully because therapy discontinuation may be necessary[128]. At present, no other biochemical parameter has been associated with the SVR or with the need to discontinue therapy[132].

Concluding on this point, monitoring the HCV-RNA kinetics during DAA treatment seems good clinical practice and may help to identify early on the patients with a lesser chance of eradicating HCV chronic infection. Due to the ability of HCV to replicate not only in hepatocytes, but also in lymphocytes and possibly in other cell subsets, a reactivation of HCV replication in patients who had achieved an SVR cannot be excluded, and monitoring the HCV-RNA kinetics during the post-treatment follow-up can identify these cases.

CONCLUSION

The eradication of HCV infection in nearly all patients treated with the second- or third-wave DAAs and a more extensive use of these treatments in the near future will significantly contribute to curbing the spread of HCV infection and to reducing its related morbidity and mortality. At present, there is a strong stimulus for an early diagnosis of AHC, which can almost certainly be cured with a short-term DAA-based regimen, and for screening subjects with a history of previous exposure to HCV. Because of the high efficacy of the DAA treatments, the majority of the predictors of response to therapy will become obsolete. In particular, the degree of liver fibrosis does not predict the response to DAA therapy and its determination remains essential only to assess the priority for the high-cost DAA treatments based on disease severity and progression. Instead, the determination of HCV genotype and subtype remain essential in order to choose the type and duration of DAA treatment.

Monitoring the HCV-RNA kinetics during DAA treatment and post-treatment follow-up seems good inexpensive clinical practice, useful for an early identification of patients with a lesser chance of HCV eradication and of those prone to reactivation.

REFERENCES

1 **Sagnelli E**, Santantonio T, Coppola N, Fasano M, Pisaturo M, Sagnelli C. Acute hepatitis C: clinical and laboratory diagnosis, course of the disease, treatment. *Infection* 2014; **42**: 601-610 [PMID: 24619833 DOI: 10.1007/s15010-014-0608-2]

2 **Sagnelli E**, Tonziello G, Pisaturo M, Sagnelli C, Coppola N. Clinical applications of antibody avidity and immunoglobulin M testing in acute HCV infection. *Antivir Ther* 2012; **17**: 1453-1458 [PMID: 23322703 DOI: 10.3851/IMP2471]

3 **Sagnelli E**, Coppola N, Marrocco C, Coviello G, Rossi G, Battaglia M, Sagnelli C, Messina V, Tonziello A, Scolastico C, Filippini P. Diagnosis of HCV related acute hepatitis by serial determination of IgM to HCV: a preliminary observation. *J Biol Regul Homeost Agents* 2003; **17**: 207-210 [PMID: 14518726]

4 **Sagnelli E**, Coppola N, Marrocco C, Coviello G, Battaglia M, Messina V, Rossi G, Sagnelli C, Scolastico C, Filippini P. Diagnosis of hepatitis C virus related acute hepatitis by serial determination of IgM anti-HCV titres. *J Hepatol* 2005; **42**: 646-651 [PMID: 15826712]

5 **Coppola N**, Pisapia R, Marrocco C, Martini S, Vatiero LM, Messina V, Tonziello G, Sagnelli C, Filippini P, Piccinino F, Sagnelli E. Anti-HCV IgG avidity index in acute hepatitis C. *J Clin Virol* 2007; **40**: 110-115 [PMID: 17720621]

6 **Coppola N**, Vatiero LM, Sagnelli E. HCV genotype 2 as a risk factor for reactivation of chronic HCV infection. *Gut* 2005; **54**: 1207 [PMID: 16009701]

7 **Sagnelli E**, Pisaturo M, Stanzione M, Messina V, Alessio L, Sagnelli C, Starace M, Pasquale G, Coppola N. Clinical presentation, outcome, and response to therapy among patients with acute exacerbation of chronic hepatitis C. *Clin Gastroenterol Hepatol* 2013; **11**: 1174-1180.e11 [PMID: 23591280]

8 **Seeff LB**. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35-S46 [PMID: 12407575]

9 **Aghemo A**, Colombo M. Hepatocellular carcinoma in chronic hepatitis C: from bench to bedside. *Semin Immunopathol* 2013; **35**: 111-120 [PMID: 23010890 DOI: 10.1007/s00281-012-0330-z]

10 **Dohmen K**, Kawano A, Takahashi K, Shigematsu H, Tanaka H, Haruno M, Yanagita K, Ichiki Y, Mori T, Hayashida K, Shimoda S, Ishibashi H, Nomura H. The incidence and risk factors for the development of hepatocellular carcinoma after peginterferon plus ribavirin therapy for chronic hepatitis C. *Hepatogastroenterology* 2013; **60**: 2034-2038 [PMID: 24719946]

11 **Harada N**, Hiramatsu N, Oze T, Morishita N, Yamada R, Hikita H, Miyazaki M, Yakushijin T, Miyagi T, Yoshida Y, Tatsumi T, Kanto T, Kasahara A, Oshita M, Mita E, Hagiwara H, Inui Y, Katayama K, Tamura S, Yoshihara H, Imai Y, Inoue A, Hayashi N, Takehara T. Risk factors for hepatocellular carcinoma in hepatitis C patients with normal alanine aminotransferase treated with pegylated interferon and ribavirin. *J Viral Hepat* 2014; **21**: 357-365 [PMID: 24716638 DOI: 10.1111/jvh.12151]

12 **Ishikawa T**. Strategy for improving survival and reducing recurrence of HCV-related hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 6127-6130 [PMID: 24115808 DOI: 10.3748/wjg.v19.i37.6127]

13 **Kim MN**, Kim BK, Han KH. Hepatocellular carcinoma in patients with chronic hepatitis C virus infection in the Asia-Pacific region. *J Gastroenterol* 2013; **48**: 681-688 [PMID: 23463401 DOI: 10.1007/s00535-013-0770-9]

14 **Asia-Pacific Working Party on Prevention of Hepatocellular Carcinoma**. Prevention of hepatocellular carcinoma in the Asia-Pacific region: consensus statements. *J Gastroenterol Hepatol* 2010; **25**: 657-663 [PMID: 20492323 DOI: 10.1111/j.1440-1746.2009.06167.x]

15 **Kanda T**, Yokosuka O, Omata M. Hepatitis C virus and hepatocellular carcinoma. *Biology* (Basel) 2013; **2**: 304-316 [PMID: 24832662 DOI: 10.3390/biology2010304]

16 **Tomoda T**, Nouso K, Sakai A, Ouchida M, Kobayashi S, Miyahara K, Onishi H, Nakamura S, Yamamoto K, Shimizu K. Genetic risk of hepatocellular carcinoma in patients with hepatitis C virus: a

case control study. *J Gastroenterol Hepatol* 2012; **27**: 797-804 [PMID: 22004425 DOI: 10.1111/j.1440-1746.2011.06948.x]
17 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]
18 **Coppola N**, Pisaturo M, Tonziello G, Sagnelli C, Sagnelli E, Angelillo IF. Efficacy of Pegylated interferon α-2a and α-2b in patients with genotype 1 chronic hepatitis C: a meta-analysis. *BMC Infect Dis* 2012; **12**: 357 [PMID: 23245594 DOI: 10.1186/1471-2334-12-357]
19 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]
20 **Yee HS**, Chang MF, Pocha C, Lim J, Ross D, Morgan TR, Monto A. Update on the management and treatment of hepatitis C virus infection: recommendations from the Department of Veterans Affairs Hepatitis C Resource Center Program and the National Hepatitis C Program Office. *Am J Gastroenterol* 2012; **107**: 669-689; quiz 690 [PMID: 22525303 DOI: 10.1038/ajg.2012.48]
21 **European Association of the Study of the Liver**. 2011 European Association of the Study of the Liver hepatitis C virus clinical practice guidelines. *Liver Int* 2012; **32** Suppl 1: 2-8 [PMID: 22212565 DOI: 10.1111/j.1478-3231.2011.02703.x]
22 **Sagnelli E**, Pisaturo M, Martini S, Sagnelli C, Filippini P, Coppola N. Advances in the treatment of hepatitis B virus/hepatitis C virus coinfection. *Expert Opin Pharmacother* 2014; **15**: 1337-1349 [PMID: 24773464 DOI: 10.1517/14656566.2014.913571]
23 **Coppola N**, Pisaturo M, Sagnelli C, Sagnelli E, Angelillo IF. Peg-interferon plus ribavirin with or without boceprevir or telaprevir for HCV genotype 1: a meta-analysis on the role of response predictors. *PLoS One* 2014; **9**: e94542 [PMID: 24728219 DOI: 10.1371/journal.pone.0094542]
24 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
25 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
26 **Zeuzem S**, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]
27 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
28 **Dieterich DT**, Soriano V, Sherman K, Girard PM, Rockstroh J, Adiwijaya B, McCallister S, Adda N, Mahnke L, Sulkowski MS; on behalf of the Study 110 Team. Telaprevir in combination with pegylated interferon-a-2a RBV in HCV/HIV-co-infected patients: a 24-week treatment interim analysis. March 5-8-2012, [abstract #46]. In: Conference on Retroviruses and Other Opportunistic Infections. Seattle: WA, 2012
29 **Sulkowski MS**, Pol S, Cooper C, Fainboim H, Slim J, Rivero A, Laguno M, Thompson S, Wahl J, Greaves W. Boceprevir pegylated interferon ribavirin for the treatment of HCV/HIV-coinfected patients: end of treatment (week 48) interim results. March 5-8 2012,[Abstract #47]. In: Conference on Retroviruses and Other Opportunistic Infections. Seattle: WA, 2012
30 **Montes M**, Nelson M, Girard PM, Sasadeusz J, Horban A, Grinsztejn B, Zakharova N, Rivero A, Lathouwers E, Janssen K, Ouwerkerk-Mahadevan S, Witek J. Telaprevir combination therapy in HCV/HIV co-infected patients (INSIGHT study): sustained virologic response at 12 weeks final analysis. *J Int AIDS Soc* 2014; **17**: 19626 [PMID: 25394130 DOI: 10.7448/IAS.17.4.19626]
31 **Kumada H**, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2012; **56**: 78-84 [PMID: 21827730 DOI: 10.1016/j.jhep.2011.07.016]
32 **Bruno S**, Vierling JM, Esteban R, Nyberg LM, Tanno H, Goodman Z, Poordad F, Bacon B, Gottesdiener K, Pedicone LD, Albrecht JK, Brass CA, Thompson S, Burroughs MH. Efficacy and safety of boceprevir plus peginterferon-ribavirin in patients with HCV G1 infection and advanced fibrosis/cirrhosis. *J Hepatol* 2013; **58**: 479-487 [PMID: 23183529 DOI: 10.1016/j.jhep.2012.11.020]
33 **Coppola N**, Martini S, Pisaturo M, Sagnelli C, Filippini P, Sagnelli E. Treatment of chronic hepatitis C in patients with HIV/HCV coinfection. *World J Virol* 2015; **4**: 1-12 [PMID: 25674512 DOI: 10.5501/wjv.v4.i1.1]
34 **Cotte L**, Barrail-Tran A, Vincent C, Valantin MA, Fournier I, Lacombe K, Chevaliez S, Aboulker JP, Taburet AM, Molina JM; ANRS HC26 study group. Telaprevir enhances ribavirin-induced anaemia through renal function impairment. *Antivir Ther* 2015; Epub ahead of print [PMID: 25560644 DOI: 10.3851/IMP2929]
35 **Koff RS**. Review article: the efficacy and safety of sofosbuvir, a novel, oral nucleotide NS5B polymerase inhibitor, in the treatment of chronic hepatitis C virus infection. *Aliment Pharmacol Ther* 2014; **39**: 478-487 [PMID: 24387618 DOI: 10.1111/apt.12601]
36 **Gentile I**, Borgia F, Buonomo AR, Castaldo G, Borgia G. A novel promising therapeutic option against hepatitis C virus: an oral nucleotide NS5B polymerase inhibitor sofosbuvir. *Curr Med Chem* 2013; **20**: 3733-3742 [PMID: 23848533]
37 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
38 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
39 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]
40 Documento di indirizzo dell'Associazione Italiana per lo studio del Fegato per l'uso razionale di antivirali diretti di seconda generazione nelle categorie di pazienti affetti da epatite C cronica ammesse alla rimborsabilità in Italia. AISF 24/02/2015. Available from: URL: http://www.webaisf.org
41 **Orland JR**, Wright TL, Cooper S. Acute hepatitis C. *Hepatology* 2001; **33**: 321-327 [PMID: 11172332]
42 **Blackard JT**, Shata MT, Shire NJ, Sherman KE. Acute hepatitis C virus infection: a chronic problem. *Hepatology* 2008; **47**: 321-331 [PMID: 18161707]
43 **Loomba R**, Rivera MM, McBurney R, Park Y, Haynes-Williams V, Rehermann B, Alter HJ, Herrine SK, Liang TJ, Hoofnagle JH, Heller T. The natural history of acute hepatitis C: clinical presentation, laboratory findings and treatment outcomes. *Aliment Pharmacol Ther* 2011; **33**: 559-565 [PMID: 21198704 DOI:

10.1111/j.1365-2036.2010.04549]
44 **Pawlotsky JM**. Use and interpretation of virological tests for hepatitis C. *Hepatology* 2002; **36**: S65-S73 [PMID: 12407578]
45 **Alter HJ**, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo QL, Kuo G. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med* 1989; **321**: 1494-1500 [PMID: 2509915]
46 **Lu SN**, Tung HD, Chen TM, Lee CM, Wang JH, Hung CH, Chen CH, Changchien CS. Is it possible to diagnose acute hepatitis C virus (HCV) infection by a rising anti-HCV titre rather than by seroconversion? *J Viral Hepat* 2004; **11**: 563-570 [PMID: 15500558]
47 **Araujo AC**, Astrakhantseva IV, Fields HA, Kamili S. Distinguishing acute from chronic hepatitis C virus (HCV) infection based on antibody reactivities to specific HCV structural and nonstructural proteins. *J Clin Microbiol* 2011; **49**: 54-57 [PMID: 21084519 DOI: 10.1128/JCM.01064-10]
48 **Sheen IS**, Liaw YF, Lin DY, Chu CM. Acute exacerbations in chronic hepatitis C: a clinicopathological and prognostic study. *J Hepatol* 1996; **24**: 525-531 [PMID: 8773906]
49 **Hiraga N**, Suzuki F, Akuta N, Suzuki Y, Sezaki H, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Matsuda M, Watabiki S, Satoh J, Kumada H. Clinical and virological characteristics of untreated patients with chronic hepatitis C who develop serum alanine aminotransferase flare-up. *J Med Virol* 2005; **75**: 240-248 [PMID: 15602722]
50 **Rumi MG**, De Filippi F, La Vecchia C, Donato MF, Gallus S, Del Ninno E, Colombo M. Hepatitis C reactivation in patients with chronic infection with genotypes 1b and 2c: a retrospective cohort study of 206 untreated patients. *Gut* 2005; **54**: 402-406 [PMID: 15710990]
51 **Ward KN**, Dhaliwal W, Ashworth KL, Clutterbuck EJ, Teo CG. Measurement of antibody avidity for hepatitis C virus distinguishes primary antibody responses from passively acquired antibody. *J Med Virol* 1994; **43**: 367-372 [PMID: 7525865]
52 **Kanno A**, Kazuyama Y. Immunoglobulin G antibody avidity assay for serodiagnosis of hepatitis C virus infection. *J Med Virol* 2002; **68**: 229-233 [PMID: 12210412]
53 **Coppola N**, Pisapia R, Tonziello G, Masiello A, Martini S, Pisaturo M, Messina V, Sagnelli C, Macera M, Signoriello G, Sagnelli E. Improvement in the aetiological diagnosis of acute hepatitis C: a diagnostic protocol based on the anti-HCV-IgM titre and IgG Avidity Index. *J Clin Virol* 2009; **46**: 222-229 [PMID: 19758839 DOI: 10.1016/j.jcv.2009.08.009]
54 **Gaudy-Graffin C**, Lesage G, Kousignian I, Laperche S, Girault A, Dubois F, Goudeau A, Barin F. Use of an anti-hepatitis C virus (HCV) IgG avidity assay to identify recent HCV infection. *J Clin Microbiol* 2010; **48**: 3281-3287 [PMID: 20610669 DOI: 10.1128/JCM.00303-10]
55 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714 [PMID: 16702586]
56 **Wasley A**, Miller JT, Finelli L. Surveillance for acute viral hepatitis--United States, 2005. *MMWR Surveill Summ* 2007; **56**: 1-24 [PMID: 17363893]
57 **Alter MJ**, Seeff LB, Bacon BR, Thomas DL, Rigsby MO, Di Bisceglie AM. Testing for hepatitis C virus infection should be routine for persons at increased risk for infection. *Ann Intern Med* 2004; **141**: 715-717 [PMID: 15520428]
58 **Hosein SR**, Wilson DP. HIV, HCV, and drug use in men who have sex with men. *Lancet* 2013; **382**: 1095-1096 [PMID: 24075047 DOI: 10.1016/S0140-6736(13)62020-6]
59 **van de Laar TJ**, Matthews GV, Prins M, Danta M. Acute hepatitis C in HIV-infected men who have sex with men: an emerging sexually transmitted infection. *AIDS* 2010; **24**: 1799-1812 [PMID: 20601854 DOI: 10.1097/QAD.0b013e32833c11a5]
60 **Urbanus AT**, Van De Laar TJ, Geskus R, Vanhommerig JW, Van Rooijen MS, Schinkel J, Heijman T, Coutinho RA, Prins M. Trends in hepatitis C virus infections among MSM attending a sexually transmitted infection clinic; 1995-2010. *AIDS* 2014; **28**: 781-790 [PMID: 24832014 DOI: 10.1097/QAD.0000000000000126]
61 **Wiessing L**, Likatavicius G, Hedrich D, Guarita B, van de Laar MJ, Vicente J. Trends in HIV and hepatitis C virus infections among injecting drug users in Europe, 2005 to 2010. *Euro Surveill* 2011; **16**: pii: 20031 [PMID: 22172300]
62 **Colin C**, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. *J Viral Hepat* 2001; **8**: 87-95 [PMID: 11264728]
63 **Dufour DR**, Talastas M, Fernandez MD, Harris B. Chemiluminescence assay improves specificity of hepatitis C antibody detection. *Clin Chem* 2003; **49**: 940-944 [PMID: 12765991]
64 **Alter MJ**, Kuhnert WL, Finelli L. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. *MMWR Recomm Rep* 2003; **52**: 1-13, 15; quiz CE1-CE4 [PMID: 12585742]
65 **Pawlotsky JM**, Lonjon I, Hezode C, Raynard B, Darthuy F, Remire J, Soussy CJ, Dhumeaux D. What strategy should be used for diagnosis of hepatitis C virus infection in clinical laboratories? *Hepatology* 1998; **27**: 1700-1702 [PMID: 9620345]
66 **Barrera JM**, Francis B, Ercilla G, Nelles M, Achord D, Darner J, Lee SR. Improved detection of anti-HCV in post-transfusion hepatitis by a third-generation ELISA. *Vox Sang* 1995; **68**: 15-18 [PMID: 7536987]
67 **Morishima C**, Gretch DR. Clinical use of hepatitis C virus tests for diagnosis and monitoring during therapy. *Clin Liver Dis* 1999; **3**: 717-740 [PMID: 11291247]
68 **Shivkumar S**, Peeling R, Jafari Y, Joseph L, Pant Pai N. Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis. *Ann Intern Med* 2012; **157**: 558-566 [PMID: 23070489 DOI: 10.7326/0003-4819-157-8-201210160-00006]
69 **Centers for Disease Control and Prevention (CDC)**. Testing for HCV infection: an update of guidance for clinicians and laboratorians. *MMWR Morb Mortal Wkly Rep* 2013; **62**: 362-365 [PMID: 23657112]
70 **Saldanha J**. Sensitivity of PCR assays for the determination of hepatitis A virus RNA in plasma pools. A collaborative study. *Vox Sang* 1999; **76**: 163-165 [PMID: 10341331]
71 **Yano M**, Kumada H, Kage M, Ikeda K, Shimamatsu K, Inoue O, Hashimoto E, Lefkowitch JH, Ludwig J, Okuda K. The long-term pathological evolution of chronic hepatitis C. *Hepatology* 1996; **23**: 1334-1340 [PMID: 8675148]
72 **Smith BD**, Morgan RL, Beckett GA, Falck-Ytter Y, Holtzman D, Teo CG, Jewett A, Baack B, Rein DB, Patel N, Alter M, Yartel A, Ward JW. Recommendations for the identification of chronic hepatitis C virus infection among persons born during 1945-1965. *MMWR Recomm Rep* 2012; **61**: 1-32 [PMID: 22895429]
73 **Sagnelli E**, Sagnelli C, Pisaturo MA, Coppola N, Pasquale G, Piccinino F. Liver biopsy in chronic hepatitis C: the experience of 15 Italian wards of infectious diseases. *Infez Med* 2012; **20**: 31-36 [PMID: 22475658]
74 **Pasquale G**, Sagnelli E, Coppola N, Onofrio M, Scarano F, Scolastico C, Bellomo PF, Lettieri A, Mogavero AR, Caprio N, Sagnelli C, Piccinino F. [An attempt to improve classification of HCV-correlated chronic hepatitis]. *Infez Med* 2005; **13**: 16-22 [PMID: 15888977]
75 **Sagnelli E**, Pasquale G, Coppola N, Marrocco C, Scarano F, Imparato M, Sagnelli C, Scolastico C, Piccinino F. Liver histology in patients with HBsAg negative anti-HBc and anti-HCV positive chronic hepatitis. *J Med Virol* 2005; **75**: 222-226 [PMID: 15602732]
76 **Sagnelli E**, Pasquale G, Coppola N, Scarano F, Marrocco C, Scolastico C, Santantonio T, Gentile A, Piccinino F. Influence of chronic coinfection with hepatitis B and C virus on liver histology. *Infection* 2004; **32**: 144-148 [PMID: 15188073]
77 **Sagnelli E**, Coppola N, Scolastico C, Filippini P, Piccinino F. [Virological and clinical aspects of multiple hepatitis virus

infections: preliminary data of an italian multicentre study] *Infez Med* 1999; **7**: 90-95 [PMID: 12759587]
78 **Pasquale G**, Sagnelli E, Coppola N, Scarano F, Scolastico C, Sagnelli C, Bellomo PF, Lettieri A, Filippini P, Piccinino F. Uselessness of liver biopsy in patients with hepatitis C virus chronic infection and persistently normal aminotransferase levels. *Infez Med* 2003; **11**: 11-17 [PMID: 12719665]
79 **Sagnelli E**, Coppola N, Scolastico C, Mogavero AR, Filippini P, Piccinino F. HCV genotype and "silent" HBV coinfection: two main risk factors for a more severe liver disease. *J Med Virol* 2001; **64**: 350-355 [PMID: 11424125]
80 **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]
81 **Chon YE**, Choi EH, Song KJ, Park JY, Kim do Y, Han KH, Chon CY, Ahn SH, Kim SU. Performance of transient elastography for the staging of liver fibrosis in patients with chronic hepatitis B: a meta-analysis. *PLoS One* 2012; **7**: e44930 [PMID: 23049764 DOI: 10.1371/journal.pone.0044930]
82 **Tsochatzis EA**, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; **54**: 650-659 [PMID: 21146892 DOI: 10.1016/j.jhep.2010.07.033]
83 **Di Lelio A**, Cestari C, Lomazzi A, Beretta L. Cirrhosis: diagnosis with sonographic study of the liver surface. *Radiology* 1989; **172**: 389-392 [PMID: 2526349 DOI: 10.1148/radiology]
84 **Aubé C**, Oberti F, Korali N, Namour MA, Loisel D, Tanguy JY, Valsesia E, Pilette C, Rousselet MC, Bedossa P, Rifflet H, Maïga MY, Penneau-Fontbonne D, Caron C, Calès P. Ultrasonographic diagnosis of hepatic fibrosis or cirrhosis. *J Hepatol* 1999; **30**: 472-478 [PMID: 10190731]
85 **Marcellin P**, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* 2002; **36**: S47-S56 [PMID: 12407576]
86 **Marcellin P**, Akre´mi R, Cazals D, Boyer N, Aupe´rin A, Vidaud D, Degott C. Genotype 1 is associated with a slower progression of fibrosis in un- treated patients with mild chronic hepatitis C. *J Hepatol* 2001; **34** (Suppl 1): 159
87 **Ghany MG**, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K, Herion D, Park Y, Liang TJ, Hoofnagle JH. Progression of fibrosis in chronic hepatitis C. *Gastroenterology* 2003; **124**: 97-104 [PMID: 12512034]
88 **Martinot-Peignoux M**, Boyer N, Cazals-Hatem D, Pham BN, Gervais A, Le Breton V, Levy S, Degott C, Valla DC, Marcellin P. Prospective study on anti-hepatitis C virus-positive patients with persistently normal serum alanine transaminase with or without detectable serum hepatitis C virus RNA. *Hepatology* 2001; **34**: 1000-1005 [PMID: 11679971]
89 **Mathurin P**, Moussalli J, Cadranel JF, Thibault V, Charlotte F, Dumouchel P, Cazier A, Huraux JM, Devergie B, Vidaud M, Opolon P, Poynard T. Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transaminase activity. *Hepatology* 1998; **27**: 868-872 [PMID: 9500720]
90 **Persico M**, Persico E, Suozzo R, Conte S, De Seta M, Coppola L, Palmentieri B, Sasso FC, Torella R. Natural history of hepatitis C virus carriers with persistently normal aminotransferase levels. *Gastroenterology* 2000; **118**: 760-764 [PMID: 10734027]
91 **Pasquale G**, Sagnelli E, Coppola N, Scarano F, Scolastico C, Bellomo PF, Lettieri A, Piccinino F. Is liver biopsy necessary for hepatitis C virus carriers with persistently normal aminotransferase levels? *Eur J Gastroenterol Hepatol* 2003; **15**: 831-833 [PMID: 12811317]
92 **Chou R**, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. *Ann Intern Med* 2013; **159**: 372 [PMID: 24026329 DOI: 10.7326/0003-4819-159-5-201309030-00021]
93 **Lin ZH**, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, Sun Y, Xuan SY. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* 2011; **53**: 726-736 [PMID: 21319189 DOI: 10.1002/hep.24105]
94 **Festi D**, Schiumerini R, Marzi L, Di Biase AR, Mandolesi D, Montrone L, Scaioli E, Bonato G, Marchesini-Reggiani G, Colecchia A. Review article: the diagnosis of non-alcoholic fatty liver disease -- availability and accuracy of non-invasive methods. *Aliment Pharmacol Ther* 2013; **37**: 392-400 [PMID: 23278163 DOI: 10.1111/apt.12186]
95 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]
96 **Imbert-Bismut F**, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069-1075 [PMID: 11297957 DOI: 10.1016/S0140-6736(00)04258-6]
97 **Kim BK**, Kim SU, Kim HS, Park JY, Ahn SH, Chon CY, Cho IR, Joh DH, Park YN, Han KH, Kim do Y. Prospective validation of FibroTest in comparison with liver stiffness for predicting liver fibrosis in Asian subjects with chronic hepatitis B. *PLoS One* 2012; **7**: e35825 [PMID: 22536445 DOI: 10.1371/journal.pone.0035825]
98 **Poynard T**, Morra R, Halfon P, Castera L, Ratziu V, Imbert-Bismut F, Naveau S, Thabut D, Lebrec D, Zoulim F, Bourliere M, Cacoub P, Messous D, Munteanu M, de Ledinghen V. Meta-analyses of FibroTest diagnostic value in chronic liver disease. *BMC Gastroenterol* 2007; **7**: 40 [PMID: 17937811 DOI: 10.1186/1471-230X-7-40]
99 **Poynard T**, Munteanu M, Ngo Y, Castera L, Halfon P, Ratziu V, Imbert-Bismut F, Thabut D, Bourliere M, Cacoub P, Messous D, de Ledinghen V. ActiTest accuracy for the assessment of histological activity grades in patients with chronic hepatitis C, an overview using Obuchowski measure. *Gastroenterol Clin Biol* 2010; **34**: 388-396 [PMID: 20580175 DOI: 10.1016/j.gcb.2010.05.001]
100 **McPherson S**, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 1265-1269 [PMID: 20801772 DOI: 10.1136/gut.2010.216077]
101 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]
102 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]
103 **Bouchardeau F**, Cantaloube JF, Chevaliez S, Portal C, Razer A, Lefrère JJ, Pawlotsky JM, De Micco P, Laperche S. Improvement of hepatitis C virus (HCV) genotype determination with the new version of the INNO-LiPA HCV assay. *J Clin Microbiol* 2007; **45**: 1140-1145 [PMID: 17251399]
104 **Coppola N**, Marrone A, Pisaturo M, Starace M, Signoriello G, Gentile I, Adinolfi LE, Sagnelli E, Zampino R. Role of interleukin 28-B in the spontaneous and treatment-related clearance of HCV infection in patients with chronic HBV/HCV dual infection. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 559-567 [PMID: 24081499 DOI: 10.1007/s10096-013-1985-7]
105 **Coppola N**, Rosa Z, Cirillo G, Stanzione M, Macera M, Boemio A, Grandone A, Pisaturo M, Marrone A, Adinolfi LE, Sagnelli E, Miraglia Del Giudice E. TM6SF2 E167K variant is associated with severe steatosis in chronic hepatitis C, regardless of PNPLA3 polymorphism. *Liver Int* 2015; **35**: 1959-1963 [PMID: 25581573 DOI: 10.1111/liv.12781]
106 **Zampino R**, Coppola N, Cirillo G, Boemio A, Minichini C, Marrone A, Stanzione M, Starace M, Durante-Mangoni E, Sagnelli E, Restivo L, Salzillo G, Fascione MC, Nevola R, Del Giudice EM, Adinolfi LE. Insulin resistance and steatosis in HBV-HCV co-

infected patients: Role of PNPLA3 polymorphisms and impact on liver fibrosis progression. *World J Hepatol* 2014; **6**: 677-684 [PMID: 25276284 DOI: 10.4254/wjh.v6.i9.677]

107 **Suppiah V**, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]

108 **Thompson AJ**, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology* 2010; **139**: 120-129.e18 [PMID: 20399780 DOI: 10.1053/j.gastro.2010.04.013]

109 **Fried MW**. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002; **36**: S237-S244 [PMID: 12407599]

110 **Zampino R**, Alessio L, Marrone A, Stanzione M, Boemio A, Grandone A, Minichini C, Pisaturo M, Starace M, Adinolfi LE, Sagnelli E, Coppola N. Role of ITPA and IL28B variants in the management of chronic hepatitis C treatment. *Infez Med* 2015; **23**: 134-139 [PMID: 26110293]

111 **Fellay J**, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, Little LD, Qiu P, Bertelsen AH, Watson M, Warner A, Muir AJ, Brass C, Albrecht J, Sulkowski M, McHutchison JG, Goldstein DB. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 2010; **464**: 405-408 [PMID: 20173735 DOI: 10.1038/nature08825]

112 **Thompson AJ**, Santoro R, Piazzolla V, Clark PJ, Naggie S, Tillmann HL, Patel K, Muir AJ, Shianna KV, Mottola L, Petruzzellis D, Romano M, Sogari F, Facciorusso D, Goldstein DB, McHutchison JG, Mangia A. Inosine triphosphatase genetic variants are protective against anemia during antiviral therapy for HCV2/3 but do not decrease dose reductions of RBV or increase SVR. *Hepatology* 2011; **53**: 389-395 [PMID: 21274861 DOI: 10.1002/hep.24068]

113 **Kurosaki M**, Tanaka Y, Tanaka K, Suzuki Y, Hoshioka Y, Tamaki N, Kato T, Yasui Y, Hosokawa T, Ueda K, Tsuchiya K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Asahina Y, Matsuura K, Sugauchi F, Enomoto N, Nishida N, Tokunaga K, Mizokami M, Izumi N. Relationship between polymorphisms of the inosine triphosphatase gene and anaemia or outcome after treatment with pegylated interferon and ribavirin. *Antivir Ther* 2011; **16**: 685-694 [PMID: 21817190 DOI: 10.3851/IMP1796]

114 **Naggie S**, Rallon NI, Benito JM, Morello J, Rodriguez-Novoa S, Clark PJ, Thompson AJ, Shianna KV, Vispo E, McHutchison JG, Goldstein DB, Soriano V. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia in HIV/HCV-coinfected patients with all HCV genotypes. *J Infect Dis* 2012; **205**: 376-383 [PMID: 22158703 DOI: 10.1093/infdis/jir754]

115 **Fujino T**, Aoyagi Y, Takahashi M, Yada R, Yamamoto N, Ohishi Y, Nishiura A, Kohjima M, Yoshimoto T, Fukuizumi K, Nakashima M, Kato M, Kotoh K, Nakamuta M, Enjoji M. Association of ITPA polymorphism with outcomes of peginterferon-α plus ribavirin combination therapy. *World J Gastrointest Pharmacol Ther* 2013; **4**: 54-60 [PMID: 23919217 DOI: 10.4292/wjgpt.v4.i3.54]

116 **Italian Association for the Study of the Liver**; Italian Society of Infectious, Tropical Diseases; Italian Society for the Study of Sexually Transmitted Diseases. Practice guidelines for the treatment of hepatitis C: recommendations from an AISF/SIMIT/SIMAST Expert Opinion Meeting. *Dig Liver Dis* 2010; **42**: 81-91 [PMID: 19748329 DOI: 10.1016/j.dld.2009.08.001]

117 **Ghany MG**, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 1433-1444 [PMID: 21898493 DOI: 10.1002/hep.24641]

118 **WHO Guidelines Approved by the Guidelines Review Committee**. Guidelines for the Screening, Care and Treatment of Persons with Hepatitis C Infection. Geneva: World Health Organization, 2014 [PMID: 25535634]

119 **Kronenberger B**, Herrmann E, Micol F, von Wagner M, Zeuzem S. Viral kinetics during antiviral therapy in patients with chronic hepatitis C and persistently normal ALT levels. *Hepatology* 2004; **40**: 1442-1449 [PMID: 15565603]

120 **Carlsson T**, Reichard O, Norkrans G, Bläckberg J, Sangfelt P, Wallmark E, Weiland O. Hepatitis C virus RNA kinetics during the initial 12 weeks treatment with pegylated interferon-alpha 2a and ribavirin according to virological response. *J Viral Hepat* 2005; **12**: 473-480 [PMID: 16108761]

121 **Durante-Mangoni E**, Zampino R, Portella G, Adinolfi LE, Utili R, Ruggiero G. Correlates and prognostic value of the first-phase hepatitis C virus RNA kinetics during treatment. *Clin Infect Dis* 2009; **49**: 498-506 [PMID: 19591593 DOI: 10.1086/600887]

122 **Reesink HW**, Fanning GC, Farha KA, Weegink C, Van Vliet A, Van 't Klooster G, Lenz O, Aharchi F, Mariën K, Van Remoortere P, de Kock H, Broeckaert F, Meyvisch P, Van Beirendonck E, Simmen K, Verloes R. Rapid HCV-RNA decline with once daily TMC435: a phase I study in healthy volunteers and hepatitis C patients. *Gastroenterology* 2010; **138**: 913-921 [PMID: 19852962 DOI: 10.1053/j.gastro.2009.10.033]

123 **Gao M**, Nettles RE, Belema M, Snyder LB, Nguyen VN, Fridell RA, Serrano-Wu MH, Langley DR, Sun JH, O'Boyle DR, Lemm JA, Wang C, Knipe JO, Chien C, Colonno RJ, Grasela DM, Meanwell NA, Hamann LG. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010; **465**: 96-100 [PMID: 20410884 DOI: 10.1038/nature08960]

124 **Guedj J**, Dahari H, Rong L, Sansone ND, Nettles RE, Cotler SJ, Layden TJ, Uprichard SL, Perelson AS. Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life. *Proc Natl Acad Sci USA* 2013; **110**: 3991-3996 [PMID: 23431163 DOI: 10.1073/pnas.1203110110]

125 **Rodriguez-Torres M**, Lawitz E, Kowdley KV, Nelson DR, Dejesus E, McHutchison JG, Cornpropst MT, Mader M, Albanis E, Jiang D, Hebner CM, Symonds WT, Berrey MM, Lalezari J. Sofosbuvir (GS-7977) plus peginterferon/ribavirin in treatment-naïve patients with HCV genotype 1: a randomized, 28-day, dose-ranging trial. *J Hepatol* 2013; **58**: 663-668 [PMID: 23183528 DOI: 10.1016/j.jhep.2012.11.018]

126 **Sarrazin C**, Wedemeyer H, Cloherty G, Cohen DE, Chevaliez S, Herman C, Bernstein B, Pawlotsky JM. Importance of very early HCV RNA kinetics for prediction of treatment outcome of highly effective all oral direct acting antiviral combination therapy. *J Virol Methods* 2015; **214**: 29-32 [PMID: 25528998 DOI: 10.1016/j.jviromet.2014.11.027]

127 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]

128 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]

129 **Fried MW**, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, Marcellin P, Manns M, Nikitin I, Poordad F, Sherman M, Zeuzem S, Scott J, Gilles L, Lenz O, Peeters M, Sekar V, De Smedt G, Beumont-Mauviel M. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology* 2013; **58**:

1918-1929 [PMID: 23907700 DOI: 10.1002/hep.26641]
130 **Andreone P**, Colombo MG, Enejosa JV, Koksal I, Ferenci P, Maieron A, Müllhaupt B, Horsmans Y, Weiland O, Reesink HW, Rodrigues L, Hu YB, Podsadecki T, Bernstein B. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. *Gastroenterology* 2014; **147**: 359-365.e1 [PMID: 24818763 DOI: 10.1053/j.gastro.2014.04.045]
131 **Sezaki H**, Suzuki F, Kawamura Y, Yatsuji H, Hosaka T, Akuta N, Kobayashi M, Suzuki Y, Arase Y, Ikeda K, Kumada H. Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. *Hepatol Res* 2007; **37**: 787-792 [PMID: 17573943]
132 **Everson GT**, Sims KD, Rodriguez-Torres M, Hézode C, Lawitz E, Bourlière M, Loustaud-Ratti V, Rustgi V, Schwartz H, Tatum H, Marcellin P, Pol S, Thuluvath PJ, Eley T, Wang X, Huang SP, McPhee F, Wind-Rotolo M, Chung E, Pasquinelli C, Grasela DM, Gardiner DF. Efficacy of an interferon- and ribavirin-free regimen of daclatasvir, asunaprevir, and BMS-791325 in treatment-naive patients with HCV genotype 1 infection. *Gastroenterology* 2014; **146**: 420-429 [PMID: 24184132 DOI: 10.1053/j.gastro.2013.10.057]

P- Reviewer: Cao GW **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10760

World J Gastroenterol 2015 October 14; 21(38): 10760-10775
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

Impact of new treatment options for hepatitis C virus infection in liver transplantation

Elda Righi, Angela Londero, Alessia Carnelutti, Umberto Baccarani, Matteo Bassetti

Elda Righi, Angela Londero, Alessia Carnelutti, Matteo Bassetti, Infectious Diseases Division, Santa Maria della Misericordia University Hospital, 33100 Udine, Italy

Umberto Baccarani, Liver Transplant Unit, Santa Maria della Misericordia University Hospital, 33100 Udine, Italy

Author contributions: Righi E analyzed the literature and wrote the manuscript; Baccarani U, Carnelutti A, Londero A and Bassetti M reviewed the literature.

Conflict-of-interest statement: The authors have no conflict of interest to report.

Correspondence to: Elda Righi, MD, PhD, Infectious Diseases Division, Santa Maria della Misericordia University Hospital, 50 Colugna Street, 33100 Udine, Italy. elda.righi@libero.it
Telephone: +39-0432-559355
Fax: +39-0432-559360

Received: April 30, 2015
Peer-review started: May 7, 2015
First decision: June 23, 2015
Revised: July 12, 2015
Accepted: September 15, 2015
Article in press: September 15, 2015
Published online: October 14, 2015

Abstract

Liver transplant candidates and recipients with hepatitis C virus (HCV)-related liver disease greatly benefit from an effective antiviral therapy. The achievement of a sustained virological response before transplantation can prevent the recurrence of post-transplant HCV disease that occurs universally and correlates with enhanced progression to graft cirrhosis. Previous standard-of-care regimens (*e.g.*, pegylated-interferon plus ribavirin with or without first generation protease inhibitors, boceprevir and telaprevir) displayed suboptimal results and poor tolerance in liver transplant recipients. A new class of potent direct-acting antiviral agents (DAA) characterized by all-oral regimens with minimal side effects has been approved and included in the recent guidelines for the treatment of liver transplant recipients with recurrent HCV disease. Association of sofosbuvir with ribavirin and/or ledipasvir is recommended in liver transplant recipients and patients with decompensated cirrhosis. Other regimens include simeprevir, daclatasvir, and combination of other DAA. Possible interactions should be monitored, especially in coinfected human immunodeficiency virus/HCV patients receiving antiretrovirals.

Key words: Hepatitis C virus; Direct antiviral agents; Liver transplantation

Core tip: Until recently, a well-tolerated and effective treatment protocol to address the recurrence of hepatitis C virus (HCV) infection following liver transplantation has been an important unmet clinical need. Safe and effective treatment options are now available thanks to the approval of new classes of direct antiviral agents. The aim of this review was to summarize the outcome of previous treatments and discuss the impact of current options for the treatment of HCV among liver transplantation candidates and recipients, including coinfected human immunodeficiency virus/HCV patients.

Righi E, Londero A, Carnelutti A, Baccarani U, Bassetti M. Impact of new treatment options for hepatitis c virus infection in liver transplantation. *World J Gastroenterol* 2015; 21(38): 10760-10775 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10760.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10760

INTRODUCTION

An estimated 130 to 170 million people are infected with hepatitis C virus (HCV) worldwide, and an additional 3 to 4 million are newly infected each year[1]. The epidemiology and burden of HCV infection vary geographically, with prevalence rates ranging from < 1% to > 10%[2]. Overall, around 25% of all cases of cirrhosis and HCC are related to HCV, with significantly higher rates among countries that have a high prevalence of the disease[3]. Chronic HCV infection is associated with substantial mortality, with over 350000 deaths per year attributed to HCV-related cirrhosis and hepatocellular carcinoma (HCC)[4-6]. The development of cirrhosis and HCC due to HCV infection represents the most common indication for liver transplantation (LT) in the United States, accounting for around 40% of all cases on the United States waiting list[7]. Furthermore, projections have identified a constant increase in the number of patients with HCV-related end-stage liver disease (ESLD) who will be listed for LT over the next 10 years[8,9]. In this patient population, transplantation is an effective treatment to reduce morbidity and mortality. HCV recurrence, however, is universal in liver transplant recipients (LTR). Since HCV disease is associated with accelerated graft loss and diminished patient survival, the availability of a safe and efficacious therapy is essential among LTR[10]. For this group of patients, the real challenge for HCV treatment starts after LT.

In the past, the use of HCV treatments including pegylated interferon (Peg-INF) and ribavirin (RBV), either alone or in association with first generation protease inhibitors (PI) such as telaprevir or boceprevir, was limited by suboptimal viral responses, drug-drug interactions, and the occurrence of severe side effects, some of which have caused graft loss or have been fatal[11]. The approval of highly effective new molecules (*i.e.*, new wave NS3-4A PI, nucleotide analogues, NS5A inhibitors) has revolutionized the scenario for the treatment of HCV infection. Goals of the new anti-HCV drugs include outcome improval, reduction of side effects and drug-drug interactions, and regimen simplification. As summarized in Table 1, newly anti-HCV drugs are expected to optimize the treatment before LT, allowing patients to undergo transplantation with undetectable HCV viral load, and after LT, offering safe and broadly effective options to prevent recurrence of HCV infection.

To keep pace with the newest discoveries in the field of HCV treatment, the Infectious Diseases Society of America (IDSA) and the American Association for the Study of Liver Diseases (AASLD), in collaboration with the International Antiviral Society-United States (IAS-United States), created a website that allows to access updated, evidence-based recommendations for the management of HCV[12].

ANTI-HCV DRUGS: OLDER AND NEWER OPTIONS FOR PATIENTS WITH ADVANCED LIVER DISEASE

The goal of treatment in HCV infected individuals is the achievement of virologic cure (or sustained virological response, SVR), defined as the absence of detectable levels of HCV RNA (*e.g.,* $\leqslant$ 25 IU/mL with an FDA approved nucleic acid test) at least 12 wk after completion of therapy (SVR12). In more than 99% of patients, SVR12 has been shown to be durable for 5 years or more[13]. Successful HCV treatment dramatically decreases hepatic decompensation events, HCC incidence, and liver-related mortality[14]. Furthermore, it has been demonstrated that patients with advanced fibrosis who achieve SVR have a decreased need for LT compared with patients who do not attain SVR[15]. Thus, prompt HCV treatment is prioritized for advanced liver disease, and urgent initiation is advocated in patients with severe extrahepatic HCV disease, significant fibrosis (Metavir F3-F4), decompensated cirrhosis (Child-Turcotte-Pugh B and C), and candidates or recipients of LT[16].

Interferon-ribavirin combination

Until recently, the combination of IFN or Peg-IFN and RBV has been considered the treatment of choice for patients with chronic HCV, including those progressing to cirrhosis. With this regimen, SVR can be achieved in 30%-40% and 70%-90% of patients with HCV genotype 1 *vs* genotypes 2 or 3, respectively[17-19]. Over the past two decades, modest efficacy along with a high incidence of serious adverse events (SAE) have characterized this treatment; furthermore, Peg-INF/RBV optimal timing, dose, and duration in difficult-to-treat populations requiring urgent treatment, such as patients with ESLD, have never been clearly defined.

Boceprevir and telaprevir

In 2011, the first generation of direct-acting antivirals (DAA), boceprevir (BOC) and telaprevir (TVR), was approved for patients with genotype 1 HCV disease. BOC is a keto-amide serine PI that reversibly binds to the HCV nonstructural 3 (NS3) active site; TVR inhibits the NS3/4A HCV protease[20,21]. SVR with PI-based triple therapy (*e.g.,* association of a PI with Peg-IFN/RBV) reached 68%-75% in naïve and 59%-88% in experienced patients[22-25]. Due to the improved rate of SVR attainment for genotype 1 patients, the use of

Table 1 Expected benefits of new treatments for hepatitis C virus infection

Target population	Main objectives	Outcome
General population with chronic HCV infection	Achieve excellent SVR rates for all genotypes, reduce side effects, shorten treatment duration, simplify regimen schedules	Reduced ESLD incidence and indication for LT
Patients on LT waiting list	Achieve pre-transplant undetectable HCV-RNA; improve MELD scores	Reduced post-LT HCV recurrence; improved clinical conditions
Recipients of LT with HCV recurrence	Increase SVR rates, reduce side effects and dropouts, decrease drug-drug interactions, simplify regimen schedules	Increased patients and grafts survival
HIV/HCV-coinfected patients and coinfected LT recipients	Increase SVR rates, reduce side effects and dropouts, decrease drug-drug interactions, simplify regimen schedules	Increased patients and grafts survival

HCV: Hepatitis C virus; SVR: Sustained virological response; ESLD: End stage liver disease; LT: Liver transplant; MELD: Model for end-stage liver disease; HIV: Human immunodeficiency virus.

BOC and TVR was initially included as standard-of-care for HCV infection[26]. However, these drugs still had to be associated with Peg-IFN/RBV and required long treatment duration (24-48 wk), causing an increase in treatment burden and side effects. For these reasons, BOC use is no more recommended and TVR has been removed from the market due to the development of more effective compounds[27,28].

DAA

More recently, clinical trials have shown revolutionary results in the treatment of HCV with the use of new DAA and their combination products, with and without Peg-IFN. Due to elevated SVR, good safety profiles, and once to twice daily administration, these compounds have now been incorporated into the AASLD/IDSA recommendations[12].

In December 2013, the United States Food and Drug Administration (FDA) approved sofosbuvir (SOF), a nucleotide polymerase inhibitor of NS5B targeting HCV-RNA replication[29]. SOF is metabolized in its active form that competes with the uridine triphosphate for incorporation into the growing HCV-RNA by the non-structural protein 5B (NS5B) polymerase, acting as a chain terminator[30]. Since the NS5B active site is highly conserved across HCV genotypes, SOF displays a pan-genotypic efficacy[31]. The administration of SOF 400 mg once daily (OD) for 12 wk has been associated with rapid decrease of HCV-RNA and SVR above 85%, either in combination with Peg-IFN/RBV or with RBV alone as part of an IFN-free regimen[32,33]. Safety data has been promising also in advanced, decompensated cirrhosis showing discontinuation rates below 2% and few SAE[34]. Furthermore, low drug-drug interactions have been observed and no dose adjustments were required in patients with hepatic impairment[35]. Simeprevir (SMV, 150 mg OD), a second wave NS3/4A protease inhibitor, has been approved for use in combination with Peg-IFN/RBV in 2013 and, in November 2014, for the treatment of HCV genotype 1 in combination with SOF. IFN-free regimens containing SMV were also well-tolerated and showed overall SVR12 above 90%[36]. The association of ledipasvir (LDV, 90 mg OD), a NS5A inhibitor, with SOF was approved by the FDA in November 2014 based on the results of large phase 3 multicenter, open-label, randomized clinical trials showing SVR between 93% and 99%[37,38]. A four-drug, twice daily combination regimen, consisting of 75 mg of paritaprevir (a NS3/4A protease inhibitor), 50 mg of ritonavir (a CYP3A inhibitor, used as a pharmacologic booster), and 12.5 mg of ombitasvir (a NS5A inhibitor), packaged with two 250 mg dasabuvir (a non-nucleoside NS5B polymerase inhibitor) tablets has also been approved by the FDA and studied in combination with RBV for genotype 1 patients[39-41]. Daclatasvir (DCV, 60 mg OD), a pan-genotypic NS5A inhibitor, was approved in Europe in August 2014 and is currently used in combination with other DAA in various countries[16].

DAA therapy in patients with cirrhosis

Although characterized by ground-breaking results, recent trials have underrepresented the populations traditionally associated with poorer treatment outcomes, including patients with advanced liver fibrosis. Nevertheless, encouraging results seem to emerge from reports comprising "real world" data collected from several institutions. Table 2 summarizes the outcome of the most representative clinical trials including cirrhotic patients treated with DAA.

SVR > 50% have been reported among cirrhotic patients treated with SOF/RBV although, in genotype 3 patients receiving 12-wk regimens, cirrhosis was associated with limited responses[33,42]. LDV/SOF, with or without RBV (± RBV), has shown excellent SVR and low adverse effects in patients with cirrhosis[43,44]. A post-hoc analysis of data from seven clinical trials including 513 patients with genotype 1 HCV and compensated cirrhosis receiving LDV/SOF for 12 or 24 wk ± RBV showed SVR12 of 98% and 95% for treatment-naïve and previously treated patients, respectively. Results were similar in patients receiving RBV compared to RBV-free regimens, except among previously treated patients who showed the lowest SVR (90%) in the arm without RBV. SAE and discontinuation rates were in the range of 1%-2%[45]. Recently, the results of SOF/SMV ± RBV regimens in a heterogeneous cohort of 995 patients including

Table 2 Sustained virological response among recent clinical trials of new treatment regimens for hepatitis C virus including patients with cirrhosis

Ref.	Trial	Population	Drug	Overall SVR12	SVR12 in cirrhosis
Jacobson *et al*[143], 2014	Fusion	G2, G3 experienced 34% cirrhotic	SOF/RBV 12 *vs* 16 wk	G2 86% *vs* 94% G3 62% *vs* 30%	G2 60% *vs* 78% G3 19% *vs* 61%
Lawitz *et al*[33], 2015	Fission	G2, G3 naïve 20% cirrhosis	SOF/RBV 12 wk *vs* Peg-IFN/RBV 24 wk	G2 97% *vs* 78% G3 56% *vs* 63%	G2 92% *vs* 62% G3 30% *vs* 34%
Jacobson *et al*[143], 2014	Positron	G2, G3 naïve and experienced IFN ineligible	SOF/RBV	G2 93%, G3 61%	G2 92%, G3 21%
Zeuzem *et al*[144], 2014	Valence	G3 extended 24 wk 21% cirrhosis	SOF/RBV	G2 94%, G3 91%	G2 82%, G3 68%
Lawitz *et al*[42], 2015	Lonestar-2	G 2 and 3	SOF/RBV/Peg-IFN	G2 96%, G3 83%	G2 93%, G3 83%
Bourliere *et al*[43], 2015	Sirius	G1 with compensated cirrhosis, NR previous treatment	SOF/LDV 24 wk *vs* SOF/LDV/RBV 12 wk	N/A	97% *vs* 96%
Lawitz *et al*[36], 2014	Cosmos	G1 NR, 52% F3-F4	SOF/SMV ± RBV 12 or 24 wk	92%	94%
Gane *et al*[114], 2014	Electron Ⅱ	G1 naïve, experienced and decompensated, G3 naïve, 15% cirrhosis	LDV/RBV 12 wk	G1 100%, G3 64%	G1 65%

Peg-IFN: Pegylated interferon; RBV: Ribavirin; SVR12: Sustained virological response; G: Genotype; LDV: Ledipasvir; SOF: Sofosbuvir; SMV: Simeprevir; NR: Non responder.

30% of patients with cirrhosis were compared with SOF/PEG/RBV and SOF/RBV[46]. In the group of patients with genotype 1 and previously treated for HCV, a significant difference in SVR was noted between patients without cirrhosis *vs* patients with cirrhosis, with better results for SOF/SIM ± RBV (84% *vs* 65%, respectively) compared to SOF/Peg-IFN/RBV (94% *vs* 80%, respectively). Overall, discontinuation rates around 5% were noted. Other promising DAA combinations include grazoprevir (MK-5172) and elbasvir (MK-8742), showing high SVR12 at 12 wk among patients with genotype 1 and cirrhosis with and without RBV (90% and 97%, respectively)[47]. MK-5172/MK-8742 combination has recently also been tested among patients with advanced chronic kidney disease, showing SVR12 of 99%[48]. The 3DAA combination of DCV with asunaprevir (NS3 protease inhibitor) and BMS-791325 (non-nucleoside NS5B inhibitor) was studied in patients with HCV genotype 1 infection and compensated cirrhosis. SVR were 87% and 93% in experienced patients treated with and without RBV, respectively[49].

IMPACT OF RECURRENT HCV INFECTION AFTER LIVER TRANSPLANTATION

Patients showing detectable HCV-RNA levels at transplantation universally experience recurrent postoperative HCV infection[50]. Reinfection likely occurs during graft reperfusion *via* circulating virions or infected mononuclear cells, and it is documented as detection of HCV-RNA in serum or in the allograft itself. HCV-RNA can be present as early as 48 h post-LT, with expression of HCV antigens on the hepatocytes from postoperative day 10[51-53]. Post-transplant HCV kinetics has shown that serum HCV-RNA levels reach pre-LT titers usually within day 4, then increase and peak around month 3, attaining levels 10- to 100-fold greater than the mean pre-LT months around one year after LT[54]. Histologic progression of HCV during immunosuppressive therapy is more rapid than that in nontransplant patients, probably due to a compromised virus-specific T-helper subtype 1 (TH1) CD4 immune response[55]. Liver biopsies are currently the most effective method to diagnose and differentiate HCV disease, showing good sensitivity starting from 3 mo after LT[51]. In earlier stages, histological differentiation between HCV disease, reperfusion injury, and rejection can be challenging. A small proportion of patients (4%-7%) develop fibrosing cholestatic hepatitis (FCH), an accelerated course of liver injury associated with very high levels of viremia, rapid allograft failure, and poor response to therapy due to direct cytotoxic damage favored by a lack of specific anti-HCV response along with increased TH2 cytokine expression[56]. Following graft infection, chronic HCV disease develops in 75% to 90% of patients. Evolution towards cirrhosis is reported 5% to 30% of cases within 5 years and up to 40% within 10 years compared to 20 years in the nontransplantation setting[57-59]. HCV-associated graft failure represents the most common cause of graft loss and patient mortality in HCV-infected recipients, occurring in approximately 10% of LT recipients within 5 years[60]. Overall, survival of patients and grafts with recurrent post-LT HCV infection is lower compared to patients receiving LT for other indications[57,61]. Various risk factors have been associated with unfavorable outcomes in HCV-infected recipients. Some of them, such as prolonged cold ischemia time, advanced donor age, CMV hepatitis, treatment for acute rejection (*e.g.*, steroid bolus or monoclonal antibody OKT3), development of postoperative insulin resistance diabetes mellitus or metabolic syndrome are potentially modifiable and should be either carefully evaluated in the process of donor selection or monitored in the post-LT[10,62-64].

Table 3 Pros and cons of hepatitis C virus treatment before and after liver transplant

	Before LT	After LT
Aim	Prevention of HCV recurrence	Treatment of HCV recurrence
Advantages	Undetectable HCV-RNA at transplantation correlates with low rates of post-LT HCV recurrence	Increased tolerance to treatment
Disadvantages	Low eligibility due to compromised baseline conditions High rates of serious side effects and discontinuation rates Low SVR rates	High rates of adverse effects Moderate SVR rates Drug-drug interactions

HCV: Hepatitis C virus; LT: Liver transplant; SVR: Sustained virological response.

Other risk factors include high preoperative model for end-stage liver disease (MELD) score, fibrosis stage ⩾ 2 at 12-mo biopsy, recipient IL28B TT genotype, and history of HCC[10,50,65-68]. Marked, transient hyperbilirubinemia has been associated with allograft cirrhosis in HCV-infected LT recipients[69]. Among virological factors, high pretransplantation HCV-RNA titers (> 1 mEq/mL) have been strongly related with severe recurrent HCV. Patients with lower pretransplantation HCV RNA had 5-year survival of 84% compared to 57% of patients with higher HCV RNA titer ($P < 0.0001$)[70]. Interestingly, neither viral genotype nor elevated post-LT viral titers have been found to be reliable predictors of outcome. At best, the most effective way to prevent HCV recurrence is the eradication of HCV prior to LT.

ANTIVIRAL THERAPY IN RECURRENT HCV INFECTION

HCV infection treatment: Before or after liver transplantation?

The likelihood of SVR diminishes with increasing severity of liver disease. In patients with cirrhosis, SVR rates are reduced compared to non-cirrhotic patients, ranging between 40%-50% for Child-Turcotte-Pugh (CTP) class A and being as low as 7%-26% for CTP class C patients treated with Peg-IFN/RBV[17-19,71]. Genotype 1 and 4 patients with cirrhosis showed lower treatment responses compared with genotype 2 and 3 patients (33% *vs* 57%, respectively)[71]. Factors such as poor tolerability, dose reductions, and therapy discontinuation have a significant impact on therapy outcomes in this patient population[72]. IFN-based treatment is generally poorly tolerated and can be associated with severe infections and liver decompensation; overall, up to a third of patients is reported to discontinue the treatment because of adverse events[72]. Nevertheless, the evidence that high HCV-RNA levels at transplantation correlate with rapid, clinically evident recurrence of post-transplantation HCV disease supports the attempt of an aggressive pre-transplantation treatment[10]. IFN is contraindicated in patients with decompensated cirrhosis; selected patients listed for LT showing compensated or mildly decompensated liver disease, however, have been previously considered for treatment with Peg-IFN/RBV ± TPV or BOC. A significant portion of LT candidate often present advanced ESLD or absolute contraindications to IFN-based therapy, requiring to delay HCV treatment after transplant. With the recent introduction of new DAA, successful treatment of patients on transplant waiting list seems possible. In this group, a reduction in MELD score caused by the positive impact of the treatment on liver decompensation can potentially lead to patient delisting, therefore lowering the proportion of waiting list registrants for transplantation due to HCV-related ESLD.

Post-LT treatment is generally started following the 12-mo liver biopsy if histologic severity reaches grade 3 or 4 inflammation or stage 2 or higher of fibrosis. Irrespective of grade and stage, cholestatic hepatitis is usually an indication for treatment[10]. Treatment of post-LT recurrent HCV disease is limited by moderate SVR, potential drug-drug interactions, and toxicity. In this cohort, as in the pre-transplant group, new anti-HCV therapies can provide substantial improvements in terms of efficacy and safety. Aims, advantages and disadvantages of the pre-LT and post-LT approaches are reported in Table 3.

Treatment before liver transplantation

The treatment of patients with decompensated cirrhosis is problematic due to coexisting leukopenia, thrombocytopenia, and other manifestations of ESLD that cause poor drug tolerance, often requiring the use of grow factors and transfusions[73]. In the registration trials for Peg-IFN/RBV, SVR rates were 5% to 15% lower in patients with advanced fibrosis or cirrhosis compared to patients who did not present advanced liver disease[17,18]. Various non-randomized studies have investigated the efficacy of diverse IFN or Peg-IFN-based regimens in HCV-infected patients candidate to LT (Table 4). A study using increasing doses of IFN and RBV based on tolerability demonstrated SVR only in 13% of patients with HCV genotype 1. Predictors of SVR were non-1 genotype, CTP class A for patients with genotype 1, and ability to tolerate full dose and treatment completion[74]. Other reports showed rates of HCV-RNA suppression in patients with advanced liver disease around 20%-30%[75-78]. More recently, Everson *et al*[79] conducted a randomized, controlled trial to test the efficacy and safety of Peg-IFN/RBV, both escalated as tolerated, to prevent post-transplant

Table 4 Outcome of pre-transplant hepatitis C virus therapy in studies with different regimens

Ref.	Population	*n*	Treatment regimen	Outcome	Adverse effects
Everson *et al*[74], 2005	63% decompensated cirrhosis (MELD 11 ± 3.7)	124	IFN (5 MU 3/wk) or Peg-IFN (0.75 μg/kg per week)/RBV (600 mg/d escalated)	SVR 13% (G1), 50% (other genotypes) 53% relapse 29% completed course	13% discontinuations and SAE (2 deaths)
Crippin *et al*[75], 2002	LT waiting list	15	IFN (3 MU 3/wk or 1 MU/d) ± RBV 400 bid	SVR 33%	1.3 SAE/patient (one death)
Forns *et al*[145], 2003	LT waiting list	30	IFN (3 MU/d)/RBV 800 mg/d	SVR 20% (3 relapse after LT)	63% dose reduction
Thomas *et al*[76], 2003	LT waiting list	21	IFN (5 MU/d)	SVR 20% (8 relapse after LT)	No SAE
Carrión *et al*[78], 2009	LT waiting list	51	Peg-IFN/RBV	SVR 20%	39% bacterial infections
Everson *et al*[79], 2013	LT waiting list	59	Peg-IFN/RBV (from 0.75 μg/kg per week and 600 mg/d escalated)	SVR12 22% (G 1-4), 29% (G 2-3), 50% if > 16 wk	68% (2.7 SAE/patient)
Verna *et al*[11], 2015	LT waiting list	29	PI-based triple therapy (93% TVR, 7% BOC)	SVR 52%	31% SAE; one death 28% hospitalizations
Curry *et al*[81], 2015	LT waiting list for HCC (CTP < 7)	43	Sofosbuvir 400/d plus RBV 1000-1200 up to 48 wk	SVR pre-LT maintained in 69% LT	18% SAE 2 discontinuation
Charlton *et al*[82], 2015	Decompensated cirrhosis	108	LDV/SOF/RBV (600 mg/d escalating) 12 *vs* 24 wk	SVR 87% *vs* 89%, CTP B 87% *vs* 89%, CTP C 86% *vs* 87%	26% SAE 3 discontinuation
Poordad *et al*[85], 2015	Advanced cirrhosis (70% CTP B-C)	60	DCV/SOF/RBV 12 wk	SVR 83%, CTP A 91%, CTP B 92%, CTP C 50%	No SAE

LT: Liver transplant; HCC: Hepatocellular carcinoma; CTP: Child-Turcotte-Pugh; IFN: Interferon; Peg-IFN: Pegylated interferon; RBV: Ribavirin; SVR: Sustained virological response; G: Genotype; SAE: Serious adverse effects; MELD: Model for End-Stage Liver Disease; PI: Protease inhibitor; TVR: Telaprevir; BOC: Boceprevir; LDV: Ledipasvir; SOF: Sofosbuvir; DCV: Daclatasvir.

HCV recurrence in patients listed for LT. Overall, 22% of patients with genotype 1, 4 or 6 and 29% of patients with genotype 2 or 3 obtained SVR12. Among patients completing at least 16 wk of treatment, SVR rates reached 50%. In conclusion, IFN-based regimens obtained poor SVR among patients listed for LT, mainly due to an intrinsic reduced response along with a low rate of treatment completion. DAA triple therapy showed increased SVR in a study including 29 patients with low MELD scores but high rates (66%) of prior non-responders. The majority of patients were treated with Peg-IFN/RBV/TVR. Patients on waiting list had SVR of 41% and patients undergoing LT showed SVR of 67%. Despite demonstrating considerably higher SVR rates compared to Peg-IFN/RBV, the use of BOC or TPV was associated with increased SAE and a high pill burden[11]. As shown in Table 4, encouraging results have been displayed by IFN-free HCV regimens. Osinusi *et al*[80] administered SOF in combination with either weight-based (*n* = 24) or low-dose (600 mg daily) RBV for 24 wk to 28 genotype 1 patients, including those with advanced fibrosis. SOF/RBV combination resulted in 50% and 29% SVR in weight-based and low-dose RBV groups, respectively (difference not significant). Advanced liver fibrosis and high HCV RNA at baseline were identified as predictors of relapse. Neither discontinuation nor SAE were registered. SOF/RBV combination was also used in a phase 2 study to treat 61 patients (73% with genotype 1 and 75% previously treated for HCV) waitlisted to undergo LT for HCC. Overall, 49% of treated patients has post-LT SVR; among those who had undetectable HCV-RNA at transplantation, 70% achieve SVR[81]. A number of days of undetectable HCV RNA level pretransplant > 30 was significantly associated with SVR12.

IFN-free, DAA combination therapies have shown the highest rates of SVR among patients with advanced liver disease previously treated for HCV. Cure rates close to 90% in patients with decompensated cirrhosis were reported among 108 patients receiving LDV/SOF/RBV for 12 or 24 wk[82]. Of note, a substantial improvement of liver synthesis function of the patients with successful HCV therapy was documented by an improvement in MELD score. Nevertheless, despite achieving SVR, liver disease continued to progress in some patients. Although no current data is available in patients with decompensated cirrhosis treated with LDV/SOF without RBV, promising results have been achieved in patients with compensated cirrhosis, including those previously treated with SOF[83,84].

Various IFN-free, DAA combination trials are currently ongoing in patients with decompensated cirrhosis[85,86]. A recent trial included patients with advanced cirrhosis and post-liver transplant HCV recurrence treated with DCV/SOF/RBV for 12 wk. In the cirrhosis cohort, genotype 1 patients achieved overall SVR of 82% (92%, 91% and 50% in CTP A, B, and C, respectively[87].

Current recommendations for the treatment of LT candidates with decompensated cirrhosis include LDV/SOF/RBV for genotype 1 administered for 12 wk

Table 5 Anti-hepatitis C virus therapy in liver transplant recipients with recurrent hepatitis C virus infection: Outcome of main studies from the past 10 years

Ref.	Population	*n*	Treatment regimen	SVR	Adverse effects
Interferon (IFN) or pegylated interferon (Peg-IFN) plus ribavirin (RBV) regimens					
Fernández *et al*[95], 2006	LTR with recurrent HCV	47	Peg-IFN/RBV	23%	21% SAE
Carrión *et al*[77], 2008	LTR with mild recurrence (F0-F2)	27	Peg-IFN/RBV	48%	56% discontinuation
Berenguer *et al*[92], 2008	LTR with recurrent HCV	89	IFN/RBV *vs* Peg-IFN/RBV	16% *vs* 48%	20% decompensation; 15% deaths
Hanouneh *et al*[93], 2008	LTR with recurrent HCV	53	Peg-IFN/RBV	35%	23% SAE
Ueda *et al*[146], 2010	LTR with recurrent HCV (G1)	34	Peg-IFN alfa-2b + RBV	50%	18% discontinuation
DAA triple therapy with Peg-IFN/RBV plus boceprevir (BOC) or telaprevir (TVR)					
Verna *et al*[109], 2015	Advanced fibrosis (F > 3) and 9 FCH	49	Peg-IFN/RBV/TVR or BOC	51% AF 44% CH	22% AF and 33% CH decompensation
Pungpapong *et al*[108], 2013	LTR with recurrent HCV	60	Peg-IFN/RBV/TVR (35) or BOC (25)	67% TVR 45% BOC	12% decompensation, 2 deaths
Coilly *et al*[107], 2014	LTR with recurrent HCV	37	Peg-IFN/RBV/TVR (19) or BOC (18)	20% TVR 71% BOC	14% SAE, 27% infection, 3 deaths
IFN-free DAA regimens					
Forns *et al*[111], 2015	Post-LT decompensated cirrhosis and FCH	92	SOF/RBV ± Peg-IFN 24-48 wk	59%	46% SAE
Charlton *et al*[110], 2015	LTR with recurrent HCV	40	SOF/RBV 24 wk	70%	No SAE
Reddy *et al*[44], 2015	Post LT recurrence (121 CPT B and C)	223	LDV/SOF/RBV 12 *vs* 24 wk	94% (60% CTP C)	4% SAE, 3% discontinuation
Gutierrez *et al*[118], 2015	Post LT recurrence	61	SOF/SMV ± RBV	93%	No SAE
Pungpapong *et al*[119], 2015	Post LT recurrence	123	SOF/SMV ± RBV	90%	1 death possibly related to treatment
Kwo *et al*[103], 2014	Post LT recurrence (G1)	34	Paritaprevir/r/Ombitasvir and Dasabuvir/RBV	97%	1 discontinuation
Poordad *et al*[85], 2015	Post LT recurrence	53	DCV/SOF/RBV 12 wk	94%	1 discontinuation (SVR); no SAE

LTR: Liver transplant recipients; SVR: Sustained virological response; CTP: Child-Turcotte-Pugh; SAE: Serious adverse event; FCH: Fibrosing cholestatic hepatitis; SOF: Sofosbuvir; SMV: Simeprevir; LDV: Ledipasvir; r: Ritonavir; DCV: Daclatasvir.

(or 24 wk if RBV intolerant or previous SOF therapy), SOF/RBV for 48 wk in genotypes 2 and 3 and DCV/SOF/RBV for 12 wk for all genotypes[16,85].

HCV treatment after LT

The achievement of SVR in recurrent HCV infection after LT is associated with stabilization of fibrosis and improved graft survival. In this setting, however, poor therapy tolerability represents an important limitation. Some studies have explored the effects of early or pre-emptive treatment, starting anti-HCV therapy immediately after LT in patients who may tolerate it, such as HCC patients with low MELD[88]. The rationale for this approach is to act at a time when HCV-RNA is low and histologic damage is virtually absent[89]. Among living donor recipients, in particular, the treatment could be easily planned and has shown encouraging results[88]. Overall, the success of this strategy was limited by low SVR and high rates of discontinuation, while the effective impact on patients' survival has not been clearly proven[90,91]. In the treatment of clinically evident disease, non-controlled studies including patients with recurrent HCV infection showed SVR rates ranging from 26% to 50% for Peg-IFN/RBV therapy (Table 5)[92-101]. When initiated at the early stages of HCV recurrence (F0-F2), an advantage of Peg-IFN/RBV treatment was demonstrated, showing SVR around 50%; however, the possible increased risk of rejection was not defined[77]. Similarly to nontransplant patients, factors associated with SVR among LTR included low pretreatment HCV RNA levels, absence of advanced cirrhosis, having a genotype other than 1, and early virological response[93]. A systematic review encompassing 38 studies showed overall SVR of 24% for standard IFN and 27% for Peg-IFN/RBV, with discontinuation rates of 24% and 26%, respectively[102]. Similarly to LT candidates, PI-based triple therapy in HCV-infected LT recipients was initially deemed as a combination that would have drastically increased the rates of SVR. Nevertheless, this treatment did not meet the expectations, showing suboptimal efficacy counterbalanced by high SAE rates and challenges in managing drug-drug interactions between PI and calcineurin inhibitors (CNI), particularly tacrolimus[103-106]. Overall, anemia, infection rates, and liver decompensation have significantly limited this therapeutic approach in LTR[107-109].

2014 AASLD recommendations

A multicenter study has shown SVR of 70% among 40 LTR with compensated HCV disease treated with SOF/RBV for 24 wk[110]. There were no deaths, graft losses or episodes of liver decompensation among post-liver transplantation patients, and no drug-drug interactions were reported between SOF and immunosuppressive agents. Among 92 patients with

Table 6 American Association for the Study of Liver Diseases 2014 recommendations for therapy in recurrent hepatitis C virus post liver transplant

Rating	Population	CPT B and C	Regimen	Daily Dose
Ⅰ B-recommended	G 1, 4 experienced and naïve	RBV 600 mg, increased as tolerated[1]	LDV/SOF/RBV 12 wk	90 mg/400 mg/weight-based[2]
Ⅰ B-alternative	G 1, 4 naïve, RBV intolerant	Not recommended	LDV/SOF 24 wk	90 mg/400 mg
Ⅰ B-alternative	G1	Not recommended	SOF/SMV ± RBV 12 wk	400 mg + 150 mg ± weight-based[2]
Ⅰ B-alternative	G1	Recommended only for non-cirrhosis	Paritaprevir/r/rombitasvir/dasabuvir + RBV for 24 wk	150 mg/100 mg/25 mg/250 mg bid/weight-based[2]
Ⅱ B-recommended	G2 experienced and naïve	600 mg/d, increased as tolerated[1]	SOF/RBV 24 wk	400 mg/weight-based[2]
Ⅰ B-recommended	G3 experienced and naïve	600 mg, increased as tolerated[1]	SOF/RBV 24 wk	400 mg/weight-based[2]
Ⅲ A Not recommended: Regimens containing PEG-IFN, monotherapy with PEG-IFN, RBV, or a DAA; TVR or BOC-based regimens				

[1]*e.g.*, increased monthly by 200 mg/d; [2]1000 mg < 75 kg, 1200 mg > 75 kg. Recommendations are graded according the level of the evidence and strength of the recommendation. G: Genotype; RBV: Ribavirin; LDV: Ledipasvir; SOF: Sofosbuvir; RBV: Ribavirin; r: Ritonavir; DCV: Daclatasvir; DAA: Direct active antiviral; TVR: Telaprevir; BOC: Boceprevir.

severe HCV disease, including liver decompensation, SOF compassionate use program (in association with RBV ± Peg-IFN) showed SVR12 of 59%; higher SVR (73%) were shown in patients treated for early severe recurrence[111]. Based on these results, combination treatments containing SOF are currently included in the 2014 AASLD recommendations for patients who develop recurrent HCV infection post-LT (Table 6)[112]. DAA combination therapy with LDP/SOF/RBV is indicated for patients with genotype 1 and 4, including those previously treated for HCV and patients with decompensated cirrhosis (with reduced RBV dose). The efficacy of this regimen was assessed in a large, multicenter, randomized controlled trial showing high rates of SVR irrespective of the treatment duration (12 wk *vs* 24 wk) along with improvements in MELD score, albumin and bilirubin[113]. The study included 223 LTR with a wide spectrum of histologic and clinical severity of HCV recurrence. Thirty-seven/44 (84%) CTP B and 5/8 (63%) CTP C patients achieved SVR12, compared to 97% of patients with F0-F2 and compensated cirrhosis. Overall, 8 treatment-related SAE were documented. CTP C patients appeared to have lower SVR compared to the other groups, although the number of patients in this group was limited. Although its importance cannot be ascertained, the addition of RBV could have been responsible for the high SVR12 rates observed. According to the AASLD guidelines, a 24-wk course of LDP/SOF is recommended in LTR that are intolerant or ineligible to receive RBV. Patients with genotype 3 including cirrhotic patients, however, have shown suboptimal responses, especially with 12-wk regimens (Table 2). A 24-wk course of SOF/RBV is recommended in patients with genotype 3 with recurrent post-LT HCV disease (Table 6). Indications on the use of LDP/SOF for genotype 3 LTR are not made due to a lack of data in the post-LT setting and limited data among patients with cirrhosis. Nevertheless, a phase Ⅱ study has reported SVR 12 of 100% for LDP/SOF/RBV compared to 64% for LDP/SOF in a cohort of patients with G3 infection (including 15% cirrhotic), potentially suggesting that LDV could even shorten the treatment duration in this group[114]. A limitation in the use of LDV regards the concomitant use of proton pump inhibitors, that attenuate its absorption by > 90%. Promising results in LTR were also shown with the pan-genotypic combination of DCV/SOF/RBV. Analysis from a small group of 12 LTR showed SVR of 75% along with absence of drug-drug interactions and SAE[115]. A study presented at the 2014 AASLD meeting including patients from the same cohort showed CTP score improvements in 20 patients (from 7.3 to 5.8, $P = 0.004$)[116]. More recently, the results of the phase 3 ALLY-1 trial in LTR treated with DCV/SOF/RBV reported overall SVR of 94% regardless of prior treatment experience[86]. Treatment with DCV/SOF/RBV has been included in the 2015 EASL (European Association for the Study of the Liver) recommendations for the treatment of HCV recurrence, including decompensated cirrhosis, in all genotypes[16].

A multicenter study including 34 LTR with mild genotype 1 HCV recurrence (F0-F2) treated with paritaprevir/ritonavir, ombitasvir, twice-daily dosed dasabuvir, and RBV for 24 wk showed overall SVR of 97%[103,117]. Dose adjustments were needed for cyclosporine and tacrolimus due to interactions between ritonavir and CNI. Only one discontinuation in a patient who achieved SVR was noted. Since the efficacy and tolerability in patients with more advanced HCV infection are not well known, this regimen is currently only recommended for LTR without cirrhosis. The association of SMV/SOF ± RBV is suggested as an alternative regimen in genotype 1 patients without liver decompensation and recurrent HCV disease post-LT. A retrospective analysis of a single center involving 61 patients with HCV genotype 1 infection who received a 12-wk combination regimen of SOF/SMV post-LT showed SVR12 of 93% compared with 67% in patients with advanced fibrosis[118]. No SAE occurred during treatment. Similar results were obtained in a large multicenter study encompassing 123 patients receiving SOF/SMV after a median time from LT of 32 mo. SVR12 was achieved in 90% of patients,

with rates around 70% in patients with advanced fibrosis[119]. While non-significant changes have been reported with tacrolimus use, up to 6-fold increases in SMV concentration have been noted in association with cyclosporine, due to inhibition of cytochrome P450 3A, ion-transporting polypeptide, and p-glycoprotein. Based on this data, SMV/SOF is preferred in patients receiving tacrolimus and represents a valid option in patients with impaired renal function or anemia who may not tolerate RBV. Additional data on SIM/SOF ± RBV came from a subgroup of 143 LTR from the TARGET cohort including 57% patients with cirrhosis. SVR4 rates were 94% among non-cirrhotic patients and 86% in patients with cirrhosis, showing a high level of concordance between cure rates obtained from clinical trials *vs* from real-life observational cohorts[120].

Treatment of LTR with Human immunodeficiency virus/ HCV coinfection

After the introduction of highly active antiretroviral therapy, ESLD has become the main cause of death among human immunodeficiency virus (HIV)/HCV-coinfected patients[121]. In patients that are not successfully treated for HCV, HIV infection accelerates the course of liver disease and increases the mortality rate[122]. LT is an effective treatment for HIV/HCV-coinfected patients with severe liver disease; LTR, however, display significantly lower survival rates (around 55% at 5 years) compared with HCV-monoinfected patients[123]. HIV infection alone has a minor impact on the outcome of organ transplantation; in fact, excellent results are reported among HIV monoinfected (or HIV/HBV-coinfected) patients undergoing LT, and better outcomes for HIV-positive compared to HCV-infected recipients of organ transplant have been recently demonstrated[124]. HIV/HCV coinfection, however, accelerates post-LT progression towards fibrosis and liver decompensation[125]. Furthermore, interactions between immunosuppressants and antiretrovirals *via* modulation of cytochrome P450 contribute to higher rates of acute graft rejections compared to non-HIV infected patients. Although new classes of antiretrovirals with limited interactions, such as integrase inhibitors and CCR5 receptor antagonist, are currently used in HIV/HCV-coinfected LTR, the presence of multiple and reciprocal drug-drug interactions or pathological conditions can still affect plasma drug concentrations[126,127]. Moreover, HIV/HCV-coinfected patients have historically shown high adverse effects and discontinuation rates following anti-HCV treatment[128,129]. Overall, poor survival along with limited effective therapeutic options still represent major barriers to LT in this cohort[130,131]. Data reporting the results of anti-HCV treatment in HIV/HCV-coinfected LTR is scarce. Responses to Peg-IFN/RBV were significantly lower in HCV/HIV-coinfected LTR compared to monoinfected transplant recipents (10% *vs* 33%, respectively), particularly among genotype 1 patients[129]. Nevertheless, HIV/HCV-coinfected patients achieving SVR showed survival rates up to 79%. The use of BOC and TVR in 7 HIV/HCV-coinfected LTR with severe HCV recurrence demonstrated 60% SVR and no response, respectively, along with high rates of SAE[132]. Preliminary results on SOF/RBV compassionate use, instead, showed SVR4 of 100% and good tolerability in 7 HIV/HCV-coinfected LTR[133].

Thanks to an improved efficacy, safety, and tolerability in HIV and transplant patients, the newly approved antiviral therapies have the potential to transform the treatment outcomes of HIV/HCV-coinfected patients with liver complications. Data from nontransplant patients suggests that HIV infection itself does not negatively impact SVR. Two trials involved a heterogeneous population of HIV/HCV-coinfected patients treated with SOF/RBV including different genotypes, patients with compensated cirrhosis, and treatment experienced patients. SVR12 were 90% in genotype 2 (irrespective of treatment duration) and above 80% among the other genotypes[129,134]. High relapse rates in genotype 1 patients, however, suggested that dual DAA combinations is preferred in this group; overall, lowest SVR were displayed in patients with genotype 3 treated for 12 wk and in patients with genotype 1 and cirrhosis. Therapy duration of 12 wk for genotype 2 and 24 wk for genotype 3 and 4 are recommended. Low rates of SAE and discontinuation (8% and 2.5%, respectively) were reported. Other key studies in this cohort included the combination of SOF/LDV administered for 12 wk to 50 GT1 coinfected patients with optimal baseline conditions (*e.g.,* absence of cirrhosis or previous treatment failures) showing SVR rates close to 100%[135]. The same combination showed SVR rates of 94% and 97% in cirrhotic and treatment-experienced patients, respectively, in a study encompassing 335 coinfected HIV-HCV patients[136].

In a trial including 20% of patients with cirrhosis, HIV/HCV-coinfected patients receiving paritaprevir/r/ombitasvir, dasabuvir and RBV had SVR rates above 90%, irrespective of treatment duration[137]. Combination of grazoprevir and elbasvir showed comparable results between monoinfected and coinfected subjects (SVR12 of 93% *vs* 97% with RBV and 98% *vs* 87% without RBV, respectively)[138]. Data on SMV use in coinfected patients is limited; its use in 12 HIV/HCV-positive patients showed SVR of 92%[139].

DCV/SOF regimens in HIV/HCV-coinfected patients showed SVR of 98% when administered for 12 wk in treatment-experienced patients. Shorter regimens (*e.g.,* 8 wk), however, were associated with high relapse rates especially in cirrhotic patients[140].

Although some trials were limited by a small number of patients or presented only interim results, anti-HCV treatment appeared to have similar efficacy among coinfected and monoinfected patients. Therefore, the new guidelines do not consider HIV/HCV coinfected

patients as a special population and recommend DAA-based treatments irrespective of HIV status. Among different anti-HCV regimens, paritaprevir/ritonavir/ombitasvir plus dasabuvir was the most susceptible to drug interactions with antiretrovirals. SMV can also cause drug interactions with PI, efavirenz, etravirine, and ciclosporin; conversely, minor or non-clinically significant interactions were seen with DCV, SOF, or LDV[141]. LDV/SOF, however, may increase tenofovir levels when associated with ritonavir-boosted HIV PI and its use is not recommended in patients with estimated CrCl < 60 mL/min.

Recently, recommendations for the treatment of HIV/HCV-coinfected LTR with recurrent HCV disease have been published by a group of experts[142]. Based on the efficacy and the low potential for drug interactions, SOF/RBV and SOF/daclatasvir ± RBV were identified as potentially preferred regimens in HIV/HCV-coinfected LTR[142].

Updated databases and publications detailing the interactions between anti-HCV regimens and antiretrovirals are available and should always be consulted for the management of coinfected patients[112,116].

CONCLUSION

Until recently, a well-tolerated and effective treatment protocol for the recurrence of HCV infection following LT has been an important unmet clinical need. The excellent response rates from new DAA combination therapies have opened new scenarios for patients with HCV-related advanced liver disease. Difficult-to-treat patients (including LT candidates and recipients), however, have been understudied in recent trials. Even if data is limited in these patient populations, overall cure rates in clinical practice compared to clinical trials remained high, suggesting that even in real-life patients the high SVR rates can be reproducible. The benefits provided by the new anti-HCV regimens apply to both pre-transplant and post-transplant periods. Good safety profiles, high SVR rates, and MELD score improvement among patients with CTP C cirrhosis on waiting list shown by SOF-based regimens may lead to a delay in organ allocation. This result was not reported with Peg-IFN/RBV and could be attributed to IFN-free regimes that lack the catabolic effects induced by IFN, hence allowing a significant clinical improvement over a short time frame. Among LTR, early antiviral treatment after transplant (*e.g.,* from 6 to 12 mo) may become standard and reduce the occurrence of advanced CPT scores that have been correlated to a limited response to anti-HCV treatment. IFN-free, DAA combinations may represent the future ideal option for patients on transplant waiting list and post-LT. Given that a high proportion of patients in recent trials still required concomitant erythropoietin or blood transfusions, the possibility to eliminate RVB appears very attractive. Nevertheless, drawbacks and open questions still apply to the scenario of new anti-HCV drugs. While compounds such as SOF, GS-5816, and daclatasvir have activity against various genotypes, most combinations are mainly active against genotype 1. Among patients with genotype 3 and cirrhosis, however, reduced SVR were reported. Furthermore, a growing number of patients who have failed under DAA-based therapy will need more potent treatment options in the near future. Specifically, cirrhotic genotype 1 patients with a history of previous HCV treatment failure represent a challenging population. Among patients with cirrhosis, including LTR, unanswered questions concern the need for RBV association to new therapies and the requirement to pursue longer treatment duration (12 wk *vs* 24 wk). Renal impairment, that often complicates ESLD, has not been fully addressed in the recent studies and necessitates further attention. Overall, a proportion of patients with advanced liver disease will progress towards ESLD despite the achievement of SVR, and the impact of new therapies is likely to be limited among patients with HCC. Finally, availability restrictions along with new treatments high cost still have a big impact on patient populations who necessitate prioritized treatment.

In conclusion, the availability of new options in the treatment of HCV infection is likely to have a major impact in liver transplant candidates and recipients. Further studies employing new DAA combinations in the treatment of patients with decompensated cirrhosis, HIV/HCV coinfection, and chronic kidney disease are awaited in order to improve the management of difficult-to-treat populations that often require urgent treatment.

REFERENCES

1 **Global Burden Of Hepatitis C Working Group**. Global burden of disease (GBD) for hepatitis C. *J Clin Pharmacol* 2004; **44**: 20-29 [PMID: 14681338 DOI: 10.1177/0091270003258669]

2 **Lavanchy D**. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81 [PMID: 19207969 DOI: 10.1111/j.1478-3231.2008.01934.x]

3 **Rodger AJ**, Roberts S, Lanigan A, Bowden S, Brown T, Crofts N. Assessment of long-term outcomes of community-acquired hepatitis C infection in a cohort with sera stored from 1971 to 1975. *Hepatology* 2000; **32**: 582-587 [PMID: 10960453 DOI: 10.1053/jhep.2000.9714]

4 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538 [PMID: 16879891 DOI: 10.1016/j.jhep.2006.05.013]

5 **Fattovich G**, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, Nevens F, Solinas A, Mura D, Brouwer JT, Thomas H, Njapoum C, Casarin C, Bonetti P, Fuschi P, Basho J, Tocco A, Bhalla A, Galassini R, Noventa F, Schalm SW, Realdi G. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997; **112**: 463-472 [PMID: 9024300 DOI: 10.1053/gast.1997.v112.pm9024300]

6 **Khan MH**, Farrell GC, Byth K, Lin R, Weltman M, George J, Samarasinghe D, Kench J, Kaba S, Crewe E, Liddle C. Which patients with hepatitis C develop liver complications? *Hepatology* 2000; **31**: 513-520 [PMID: 10655279 DOI: 10.1002/hep.510310236]

7 **Szabó E**, Lotz G, Páska C, Kiss A, Schaff Z. Viral hepatitis: new data on hepatitis C infection. *Pathol Oncol Res* 2003; **9**: 215-221 [PMID: 14688826 DOI: 10.1007/BF02893380]

8 **Biggins SW**, Bambha KM, Terrault NA, Inadomi J, Shiboski S, Dodge JL, Gralla J, Rosen HR, Roberts JP. Projected future increase in aging hepatitis C virus-infected liver transplant candidates: a potential effect of hepatocellular carcinoma. *Liver Transpl* 2012; **18**: 1471-1478 [PMID: 23008049 DOI: 10.1002/lt.23551]

9 **Adam R**, McMaster P, O'Grady JG, Castaing D, Klempnauer JL, Jamieson N, Neuhaus P, Lerut J, Salizzoni M, Pollard S, Muhlbacher F, Rogiers X, Garcia Valdecasas JC, Berenguer J, Jaeck D, Moreno Gonzalez E. Evolution of liver transplantation in Europe: report of the European Liver Transplant Registry. *Liver Transpl* 2003; **9**: 1231-1243 [PMID: 14625822 DOI: 10.1016/j.lts.2003.09.018]

10 **Terrault N**. Liver transplantation in the setting of chronic HCV. *Best Pract Res Clin Gastroenterol* 2012; **26**: 531-548 [PMID: 23199510 DOI: 10.1016/j.bpg.2012.09.010]

11 **Verna EC**, Saxena V, Burton JR, O'Leary JG, Dodge JL, Stravitz RT, Levitsky J, Trotter JF, Everson GT, Brown RS, Terrault NA. Telaprevir- and Boceprevir-based Triple Therapy for Hepatitis C in Liver Transplant Recipients With Advanced Recurrent Disease: A Multicenter Study. *Transplantation* 2015; **99**: 1644-1651 [PMID: 25715116 DOI: 10.1097/tp.0000000000000629]

12 AASLD and IDSA Guidelines. Recommendations for Testing, Managing, and Treating Hepatitis C. When and in whom to initiate HCV Therapy. Accessed April 2, 2015. Available from: URL: http://www.hcvguidelines.org/

13 **Manns MP**, Pockros PJ, Norkrans G, Smith CI, Morgan TR, Häussinger D, Shiffman ML, Hadziyannis SJ, Schmidt WN, Jacobson IM, Bárcena R, Schiff ER, Shaikh OS, Bacon B, Marcellin P, Deng W, Esteban-Mur R, Poynard T, Pedicone LD, Brass CA, Albrecht JK, Gordon SC. Long-term clearance of hepatitis C virus following interferon α-2b or peginterferon α-2b, alone or in combination with ribavirin. *J Viral Hepat* 2013; **20**: 524-529 [PMID: 23808990 DOI: 10.1111/jvh.12074]

14 **Backus LI**, Boothroyd DB, Phillips BR, Belperio P, Halloran J, Mole LA. A sustained virologic response reduces risk of all-cause mortality in patients with hepatitis C. *Clin Gastroenterol Hepatol* 2011; **9**: 509-516.e1 [PMID: 21397729 DOI: 10.1016/j.cgh.2011.03.004]

15 **Dienstag JL**, Ghany MG, Morgan TR, Di Bisceglie AM, Bonkovsky HL, Kim HY, Seeff LB, Szabo G, Wright EC, Sterling RK, Everson GT, Lindsay KL, Lee WM, Lok AS, Morishima C, Stoddard AM, Everhart JE. A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C. *Hepatology* 2011; **54**: 396-405 [PMID: 21520194 DOI: 10.1002/hep.24370]

16 **European Association for Study of Liver**. EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep.2015.03.025]

17 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]

18 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749 DOI: 10.1016/S0140-6736(01)06102-5]

19 **Hadziyannis SJ**, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355 [PMID: 14996676 DOI: 10.7326/0003-4819-140-5-200403020-00010]

20 **Venkatraman S**, Bogen SL, Arasappan A, Bennett F, Chen K, Jao E, Liu YT, Lovey R, Hendrata S, Huang Y, Pan W, Parekh T, Pinto P, Popov V, Pike R, Ruan S, Santhanam B, Vibulbhan B, Wu W, Yang W, Kong J, Liang X, Wong J, Liu R, Butkiewicz N, Chase R, Hart A, Agrawal S, Ingravallo P, Pichardo J, Kong R, Baroudy B, Malcolm B, Guo Z, Prongay A, Madison V, Broske L, Cui X, Cheng KC, Hsieh Y, Brisson JM, Prelusky D, Korfmacher W, White R, Bogdanowich-Knipp S, Pavlovsky A, Bradley P, Saksena AK, Ganguly A, Piwinski J, Girijavallabhan V, Njoroge FG. Discovery of (1R,5S)-N-[3-amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]-3-[2(S)-[[[(1,1-dimethylethyl)amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]- 6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(S)-carboxamide (SCH 503034), a selective, potent, orally bioavailable hepatitis C virus NS3 protease inhibitor: a potential therapeutic agent for the treatment of hepatitis C infection. *J Med Chem* 2006; **49**: 6074-6086 [PMID: 17004721 DOI: 10.1021/jm060325b]

21 **Lin C**, Kwong AD, Perni RB. Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect Disord Drug Targets* 2006; **6**: 3-16 [PMID: 16787300 DOI: 10.2174/187152606776056706]

22 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]

23 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]

24 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]

25 **Sherman KE**, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, Fried MW, Adler M, Reesink HW, Martin M, Sankoh AJ, Adda N, Kauffman RS, George S, Wright CI, Poordad F. Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med* 2011; **365**: 1014-1024 [PMID: 21916639 DOI: 10.1056/NEJMoa1014463]

26 **Ghany MG**, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 1433-1444 [PMID: 21898493 DOI: 10.1002/hep.24641]

27 Vertex Pharmaceuticals discontinues Incivek. Canadian Treatment Action Council website. Accessed February 19, 2015. Available from: URL: http://www.ctac.ca/multimedia-press/treatmentaccessnews/vertex-pharmaceuticals-discontinues-incivek

28 Clinical Pharmacology [database online]. Accessed March 12, 2015. Tampa, FL: Gold Standard, Inc., 2014. Available from: URL: http://clinicalpharmacology-ip.com/default.aspx

29 FDA approves Sovaldi for chronic hepatitis C. FDA news release US food and Drug administration. Accessed December 6, 2013. Available from: URL: http://www.Fda.gov/newsevents/newsroom/pressannouncements/ucm377888.htm

30 **Lam AM**, Espiritu C, Bansal S, Micolochick Steuer HM, Niu C, Zennou V, Keilman M, Zhu Y, Lan S, Otto MJ, Furman PA. Genotype and subtype profiling of PSI-7977 as a nucleotide inhibitor of hepatitis C virus. *Antimicrob Agents Chemother* 2012; **56**: 3359-3368 [PMID: 22430955 DOI: 10.1128/AAC.00054-12]

31 **Sofia MJ**, Bao D, Chang W, Du J, Nagarathnam D, Rachakonda S, Reddy PG, Ross BS, Wang P, Zhang HR, Bansal S, Espiritu C, Keilman M, Lam AM, Steuer HM, Niu C, Otto MJ, Furman PA.

Discovery of a β-d-2'-deoxy-2'-α-fluoro-2'-β-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J Med Chem* 2010; **53**: 7202-7218 [PMID: 20845908 DOI: 10.1021/jm100863x]

32 **Hsu CS**. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **369**: 678 [PMID: 23944317 DOI: 10.1056/NEJMoa1214853]

33 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214854]

34 **Mangia A**, Piazzolla V. Overall efficacy and safety results of sofosbuvir-based therapies in phase II and III studies. *Dig Liver Dis* 2014; **46** Suppl 5: S179-S185 [PMID: 25458780 DOI: 10.1016/j.dld.2014.09.026]

35 **Lawitz E**, Rodriguez-Torres M, Cornpropst M. The effect of hepatic impairment on the safety, pharmacokinetics, and antiviral activity of GS-7977 in hepatitis C infected patients treated for seven days (abstr). *J Hepatol* 2012; **56** (Suppl 1): S445-S446 [DOI: 10.1016/S0168-8278(12)61142-8]

36 **Lawitz E**, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, DeJesus E, Pearlman B, Rabinovitz M, Gitlin N, Lim JK, Pockros PJ, Scott JD, Fevery B, Lambrecht T, Ouwerkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay KL, Beumont M, Jacobson IM. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. *Lancet* 2014; **384**: 1756-1765 [PMID: 25078309 DOI: 10.1016/S0140-6736(14)61036-9]

37 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]

38 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]

39 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forns X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]

40 **Andreone P**, Colombo MG, Enejosa JV, Koksal I, Ferenci P, Maieron A, Müllhaupt B, Horsmans Y, Weiland O, Reesink HW, Rodrigues L, Hu YB, Podsadecki T, Bernstein B. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. *Gastroenterology* 2014; **147**: 359-365.e1 [PMID: 24818763 DOI: 10.1053/j.gastro.2014.04.045]

41 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]

42 **Lawitz E**, Poordad F, Brainard DM, Hyland RH, An D, Dvory-Sobol H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir with peginterferon-ribavirin for 12 weeks in previously treated patients with hepatitis C genotype 2 or 3 and cirrhosis. *Hepatology* 2015; **61**: 769-775 [PMID: 25322962 DOI: 10.1002/hep.27567]

43 **Bourlière M**, Bronowicki JP, de Ledinghen V, Hézode C, Zoulim F, Mathurin P, Tran A, Larrey DG, Ratziu V, Alric L, Hyland RH, Jiang D, Doehle B, Pang PS, Symonds WT, Subramanian GM, McHutchison JG, Marcellin P, Habersetzer F, Guyader D, Grangé JD, Loustaud-Ratti V, Serfaty L, Metivier S, Leroy V, Abergel A, Pol S. Ledipasvir-sofosbuvir with or without ribavirin to treat patients with HCV genotype 1 infection and cirrhosis non-responsive to previous protease-inhibitor therapy: a randomised, double-blind, phase 2 trial (SIRIUS). *Lancet Infect Dis* 2015; **15**: 397-404 [PMID: 25773757 DOI: 10.1016/S1473-3099(15)70050-2]

44 **Reddy KR**, Bourlière M, Sulkowski M, Omata M, Zeuzem S, Feld JJ, Lawitz E, Marcellin P, Welzel TM, Hyland R, Ding X, Yang J, Knox S, Pang P, Dvory-Sobol H, Subramanian GM, Symonds W, McHutchison JG, Mangia A, Gane E, Mizokami M, Pol S, Afdhal N. Ledipasvir and sofosbuvir in patients with genotype 1 hepatitis C virus infection and compensated cirrhosis: An integrated safety and efficacy analysis. *Hepatology* 2015; **62**: 79-86 [PMID: 25846144]

45 **Bourliere M**, Sulkowski MS, Omata M, Zeuzem S, Feld LL, Lawitz E, Marcellin P, Hyland RH, Ding X, Yang JC, Knox SJ, Pang PS, Subramanian M, Symonds WT, McHutchison JG, Mangia A, Gane EJ, Reddy KR, Mizokami M, Pol S, Afdhal NH. An Integrated Safety and Efficacy Analysis of >500 Patients with Compensated Cirrhosis Treated with Ledipasvir/Sofosbuvir with or without Ribavirin. 65th Annual Meeting of the American Association for the Study of Liver diseases, November 7-11, 2014; Boston, United States

46 **Dieterich D**, Bacon BR, Flamm SL, Kowdley KV, Milligan S, Tsai N, Yonoussi Z, Lawitz E. Evaluation of sofosbuvir and simeprevir-based regimens in the TRIO network: academic and community treatment of a real-world, heterogeneous population. 65th Annual Meeting of the American Association for the Study of Liver diseases, November 7-11, 2014; Boston, United States

47 **Lawitz E**, Gane E, Pearlman B, Tam E, Ghesquiere W, Guyader D, Alric L, Bronowicki JP, Lester L, Sievert W, Ghalib R, Balart L, Sund F, Lagging M, Dutko F, Shaughnessy M, Hwang P, Howe AY, Wahl J, Robertson M, Barr E, Haber B. Efficacy and safety of 12 weeks versus 18 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin for hepatitis C virus genotype 1 infection in previously untreated patients with cirrhosis and patients with previous null response with or without cirrhosis (C-WORTHY): a randomised, open-label phase 2 trial. *Lancet* 2015; **385**: 1075-1086 [PMID: 25467591 DOI: 10.1016/S0140-6736(14)61795-5]

48 ClinicalTrials.gov. Safety and Efficacy of Grazoprevir (MK-5172) Elbasvir (MK-8742) in Participants with Chronic Hepatitis C and Chronic Kidney Disease (MK-5172-052) (C-SURFER). Identifier: NCT02092350

49 **Muir AJ**, Poordad F, Lalezari J, Everson G, Dore GJ, Herring R, Sheikh A, Kwo P, Hézode C, Pockros PJ, Tran A, Yozviak J, Reau N, Ramji A, Stuart K, Thompson AJ, Vierling J, Freilich B, Cooper J, Ghesquiere W, Yang R, McPhee F, Hughes EA, Swenson ES, Yin PD. Daclatasvir in combination with asunaprevir and beclabuvir for hepatitis C virus genotype 1 infection with compensated cirrhosis. *JAMA* 2015; **313**: 1736-1744 [PMID: 25942724 DOI: 10.1001/jama.2015.3868]

50 **Wiesner RH**, Sorrell M, Villamil F. Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. *Liver Transpl* 2003; **9**: S1-S9 [PMID: 14586888 DOI: 10.1053/jlts.2003.50268]

51 **McCaughan GW**, Zekry A. Pathogenesis of hepatitis C virus recurrence in the liver allograft. *Liver Transpl* 2002; **8**: S7-S13

[PMID: 12362292 DOI: 10.1053/jlts.2002.35856]
52 **Ballardini G**, De Raffele E, Groff P, Bioulac-Sage P, Grassi A, Ghetti S, Susca M, Strazzabosco M, Bellusci R, Iemmolo RM, Grazi G, Zauli D, Cavallari A, Bianchi FB. Timing of reinfection and mechanisms of hepatocellular damage in transplanted hepatitis C virus-reinfected liver. *Liver Transpl* 2002; **8**: 10-20 [PMID: 11799480 DOI: 10.1053/jlts.2002.30141]
53 **Guerrero RB**, Batts KP, Burgart LJ, Barrett SL, Germer JJ, Poterucha JJ, Wiesner RH, Charlton MR, Persing DH. Early detection of hepatitis C allograft reinfection after orthotopic liver transplantation: a molecular and histologic study. *Mod Pathol* 2000; **13**: 229-237 [PMID: 10757333 DOI: 10.1038/modpathol.3880043]
54 **Garcia-Retortillo M**, Forns X, Feliu A, Moitinho E, Costa J, Navasa M, Rimola A, Rodes J. Hepatitis C virus kinetics during and immediately after liver transplantation. *Hepatology* 2002; **35**: 680-687 [PMID: 11870384 DOI: 10.1053/jhep.2002.31773]
55 **Rosen HR**, Hinrichs DJ, Gretch DR, Koziel MJ, Chou S, Houghton M, Rabkin J, Corless CL, Bouwer HG. Association of multispecific CD4(+) response to hepatitis C and severity of recurrence after liver transplantation. *Gastroenterology* 1999; **117**: 926-932 [PMID: 10500076 DOI: 10.1016/S0016-5085(99)70352-5]
56 **McCaughan GW**, Zekry A. Effects of immunosuppression and organ transplantation on the natural history and immunopathogenesis of hepatitis C virus infection. *Transpl Infect Dis* 2000; **2**: 166-185 [PMID: 11429029 DOI: 10.1034/j.1399-3062.2000.020403.x]
57 **Gane EJ**. The natural history of recurrent hepatitis C and what influences this. *Liver Transpl* 2008; **14** Suppl 2: S36-S44 [PMID: 18825724 DOI: 10.1002/lt.21646]
58 **Crespo G**, Mariño Z, Navasa M, Forns X. Viral hepatitis in liver transplantation. *Gastroenterology* 2012; **142**: 1373-1383.e1 [PMID: 22537446]
59 **Tong MJ**, el-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995; **332**: 1463-1466 [PMID: 7739682 DOI: 10.1056/NEJM199506013322202]
60 **Féray C**, Samuel D, Thiers V, Gigou M, Pichon F, Bismuth A, Reynes M, Maisonneuve P, Bismuth H, Bréchot C. Reinfection of liver graft by hepatitis C virus after liver transplantation. *J Clin Invest* 1992; **89**: 1361-1365 [PMID: 1313453 DOI: 10.1172/JCI115723]
61 **Reed A**, Howard RJ, Fujita S, Foley DP, Langham MR, Schold JD, Nelson D, Soldevila-Pico C, Firpi R, Abdelmalek M, Morrelli G, Hemming AW. Liver retransplantation: a single-center outcome and financial analysis. *Transplant Proc* 2005; **37**: 1161-1163 [PMID: 15848656 DOI: 10.1016/j.transproceed.2004.11.046]
62 **Veldt BJ**, Poterucha JJ, Watt KD, Wiesner RH, Hay JE, Rosen CB, Heimbach JK, Janssen HL, Charlton MR. Insulin resistance, serum adipokines and risk of fibrosis progression in patients transplanted for hepatitis C. *Am J Transplant* 2009; **9**: 1406-1413 [PMID: 19459812 DOI: 10.1111/j.1600-6143.2009.02642.x]
63 **Sabharwal S**, Delgado-Borrego A, Chung RT. Extrahepatic hepatitis C virus after transplantation: diabetes and renal dysfunction. *Liver Transpl* 2008; **14** Suppl 2: S51-S57 [PMID: 18825714 DOI: 10.1002/lt.21613]
64 **Hanouneh IA**, Feldstein AE, McCullough AJ, Miller C, Aucejo F, Yerian L, Lopez R, Zein NN. The significance of metabolic syndrome in the setting of recurrent hepatitis C after liver transplantation. *Liver Transpl* 2008; **14**: 1287-1293 [PMID: 18756451 DOI: 10.1002/lt.21524]
65 **Forman LM**, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology* 2002; **122**: 889-896 [PMID: 11910340 DOI: 10.1053/gast.2002.32418]
66 **Charlton MR**, Thompson A, Veldt BJ, Watt K, Tillmann H, Poterucha JJ, Heimbach JK, Goldstein D, McHutchison J. Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. *Hepatology* 2011; **53**: 317-324 [PMID: 21254179 DOI: 10.1002/hep.24074]
67 **Firpi RJ**, Clark V, Soldevila-Pico C, Morelli G, Cabrera R, Levy C, Machicao VI, Chaoru C, Nelson DR. The natural history of hepatitis C cirrhosis after liver transplantation. *Liver Transpl* 2009; **15**: 1063-1071 [PMID: 19718647 DOI: 10.1002/lt.21784]
68 **Gallegos-Orozco JF**, Yosephy A, Noble B, Aqel BA, Byrne TJ, Carey EJ, Douglas DD, Mulligan D, Moss A, de Petris G, Williams JW, Rakela J, Vargas HE. Natural history of post-liver transplantation hepatitis C: A review of factors that may influence its course. *Liver Transpl* 2009; **15**: 1872-1881 [PMID: 19938138 DOI: 10.1002/lt.21954]
69 **Rosen HR**, Gretch DR, Oehlke M, Flora KD, Benner KG, Rabkin JM, Corless CL. Timing and severity of initial hepatitis C recurrence as predictors of long-term liver allograft injury. *Transplantation* 1998; **65**: 1178-1182 [PMID: 9603164 DOI: 10.1097/00007890-199805150-00006]
70 **Charlton M**, Seaberg E, Wiesner R, Everhart J, Zetterman R, Lake J, Detre K, Hoofnagle J. Predictors of patient and graft survival following liver transplantation for hepatitis C. *Hepatology* 1998; **28**: 823-830 [PMID: 9731579 DOI: 10.1002/hep.510280333]
71 **Bruno S**, Shiffman ML, Roberts SK, Gane EJ, Messinger D, Hadziyannis SJ, Marcellin P. Efficacy and safety of peginterferon alfa-2a (40KD) plus ribavirin in hepatitis C patients with advanced fibrosis and cirrhosis. *Hepatology* 2010; **51**: 388-397 [PMID: 19918980 DOI: 10.1002/hep.23340]
72 **Everson GT**, Hoefs JC, Seeff LB, Bonkovsky HL, Naishadham D, Shiffman ML, Kahn JA, Lok AS, Di Bisceglie AM, Lee WM, Dienstag JL, Ghany MG, Morishima C. Impact of disease severity on outcome of antiviral therapy for chronic hepatitis C: Lessons from the HALT-C trial. *Hepatology* 2006; **44**: 1675-1684 [PMID: 17133499 DOI: 10.1002/hep.21440]
73 **Van Thiel DH**, Faruki H, Friedlander L, Fagiuoli S, Caraceni P, Molloy PJ, Kania RJ, Wright HI. Combination treatment of advanced HCV associated liver disease with interferon and G-CSF. *Hepatogastroenterology* 1995; **42**: 907-912 [PMID: 8847044]
74 **Everson GT**, Trotter J, Forman L, Kugelmas M, Halprin A, Fey B, Ray C. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. *Hepatology* 2005; **42**: 255-262 [PMID: 16025497 DOI: 10.1002/hep.20793]
75 **Crippin JS**, McCashland T, Terrault N, Sheiner P, Charlton MR. A pilot study of the tolerability and efficacy of antiviral therapy in hepatitis C virus-infected patients awaiting liver transplantation. *Liver Transpl* 2002; **8**: 350-355 [PMID: 11965579 DOI: 10.1053/jlts.2002.31748]
76 **Thomas RM**, Brems JJ, Guzman-Hartman G, Yong S, Cavaliere P, Van Thiel DH. Infection with chronic hepatitis C virus and liver transplantation: a role for interferon therapy before transplantation. *Liver Transpl* 2003; **9**: 905-915 [PMID: 12942451 DOI: 10.1053/jlts.2003.50166]
77 **Carrión JA**, Navasa M, García-Retortillo M, García-Pagan JC, Crespo G, Bruguera M, Bosch J, Forns X. Efficacy of antiviral therapy on hepatitis C recurrence after liver transplantation: a randomized controlled study. *Gastroenterology* 2007; **132**: 1746-1756 [PMID: 17484872 DOI: 10.1053/j.gastro.2007.03.041]
78 **Carrión JA**, Martínez-Bauer E, Crespo G, Ramírez S, Pérez-del-Pulgar S, García-Valdecasas JC, Navasa M, Forns X. Antiviral therapy increases the risk of bacterial infections in HCV-infected cirrhotic patients awaiting liver transplantation: A retrospective study. *J Hepatol* 2009; **50**: 719-728 [PMID: 19217183 DOI: 10.1016/j.jhep.2008.11.015]
79 **Everson GT**, Terrault NA, Lok AS, Rodrigo del R, Brown RS, Saab S, Shiffman ML, Al-Osaimi AM, Kulik LM, Gillespie BW, Everhart JE. A randomized controlled trial of pretransplant antiviral therapy to prevent recurrence of hepatitis C after liver transplantation. *Hepatology* 2013; **57**: 1752-1762 [PMID: 22821361 DOI: 10.1002/hep.25976]
80 **Osinusi A**, Meissner EG, Lee YJ, Bon D, Heytens L, Nelson A, Sneller M, Kohli A, Barrett L, Proschan M, Herrmann E, Shivakumar B, Gu W, Kwan R, Teferi G, Talwani R, Silk R, Kotb C, Wroblewski S, Fishbein D, Dewar R, Highbarger H, Zhang X, Kleiner D, Wood BJ, Chavez J, Symonds WT, Subramanian M, McHutchison J, Polis MA, Fauci AS, Masur H, Kottilil S. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients

with unfavorable treatment characteristics: a randomized clinical trial. *JAMA* 2013; **310**: 804-811 [PMID: 23982366 DOI: 10.1001/jama.2013.109309]

81 **Curry MP**, Forns X, Chung RT, Terrault NA, Brown R, Fenkel JM, Gordon F, O'Leary J, Kuo A, Schiano T, Everson G, Schiff E, Befeler A, Gane E, Saab S, McHutchison JG, Subramanian GM, Symonds WT, Denning J, McNair L, Arterburn S, Svarovskaia E, Moonka D, Afdhal N. Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology* 2015; **148**: 100-107.e1 [PMID: 25261839]

82 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N. Ledipasvir and Sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659 [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]

83 **Casselman JW**, Peene PT, Coppens F, Vanneste F. Pneumatocele in a traumatic ruptured lacrimal sac mucocele. *Rofo* 1989; **150**: 106-107 [PMID: 2536488 DOI: 10.7326/M14-1211]

84 **Wyles D**, Pockros P, Morelli G, Younes Z, Svarovskaia E, Yang JC, Pang PS, Zhu Y, McHutchison JG, Flamm S, Lawitz E. Ledipasvir-sofosbuvir plus ribavirin for patients with genotype 1 hepatitis C virus previously treated in clinical trials of sofosbuvir regimens. *Hepatology* 2015; **61**: 1793-1797 [PMID: 25846014 DOI: 10.1002/hep.27814]

85 **Poordad F**, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, McPhee F, Hughes E, Noviello S, Swenson ES. Daclatasvir, sofosbuvir, and ribavirin combination for HCV patients with advanced cirhhosis or posttransplant recurrence: phase 3 ALLY-1 study. 50th International Liver Congress. April 22-26 2015; Vienna, Austria

86 AASLD and IDSA Guidelines. Recommendations for Testing, Managing, and Treating Hepatitis C. Unique patient populations: patients with decompensated cirrhosis. Accessed June 30, 2015. Available from: URL: http://www.hcvguidelines.org/

87 **Lens S**, Mariño Z, Forns X. Efficacy of new direct acting antivirals in transplant recipients and patients with advanced disease. *Dig Liver Dis* 2014; **46** Suppl 5: S197-S205 [PMID: 25458782 DOI: 10.1016/j.dld.2014.10.002]

88 **Terrault NA**. Prophylactic and preemptive therapies for hepatitis C virus-infected patients undergoing liver transplantation. *Liver Transpl* 2003; **9**: S95-S100 [PMID: 14586903 DOI: 10.1053/jlts.2003.50255]

89 **Powers KA**, Ribeiro RM, Patel K, Pianko S, Nyberg L, Pockros P, Conrad AJ, McHutchison J, Perelson AS. Kinetics of hepatitis C virus reinfection after liver transplantation. *Liver Transpl* 2006; **12**: 207-216 [PMID: 16447184 DOI: 10.1002/lt.20572]

90 **Tamura S**, Sugawara Y, Yamashiki N, Kaneko J, Kokudo N, Makuuchi M. Pre-emptive antiviral therapy in living donor liver transplantation for hepatitis C: observation based on a single-center experience. *Transpl Int* 2010; **23**: 580-588 [PMID: 20028490 DOI: 10.1111/j.1432-2277.2009.01023.x]

91 **Shergill AK**, Khalili M, Straley S, Bollinger K, Roberts JP, Ascher NA, Terrault NA. Applicability, tolerability and efficacy of preemptive antiviral therapy in hepatitis C-infected patients undergoing liver transplantation. *Am J Transplant* 2005; **5**: 118-124 [PMID: 15636619 DOI: 10.1111/j.1600-6143.2004.00648.x]

92 **Berenguer M**, Palau A, Aguilera V, Rayón JM, Juan FS, Prieto M. Clinical benefits of antiviral therapy in patients with recurrent hepatitis C following liver transplantation. *Am J Transplant* 2008; **8**: 679-687 [PMID: 18294165 DOI: 10.1111/j.1600-6143.2007.02126.x]

93 **Hanouneh IA**, Miller C, Aucejo F, Lopez R, Quinn MK, Zein NN. Recurrent hepatitis C after liver transplantation: on-treatment prediction of response to peginterferon/ribavirin therapy. *Liver Transpl* 2008; **14**: 53-58 [PMID: 18161839 DOI: 10.1002/lt.21312]

94 **Biselli M**, Andreone P, Gramenzi A, Lorenzini S, Loggi E, Bonvicini F, Cursaro C, Bernardi M. Pegylated interferon plus ribavirin for recurrent Hepatitis C infection after liver transplantation in naïve and non-responder patients on a stable immunosuppressive regimen. *Dig Liver Dis* 2006; **38**: 27-32 [PMID: 16311084 DOI: 10.1016/j.dld.2005.08.009]

95 **Fernández I**, Meneu JC, Colina F, García I, Muñoz R, Castellano G, Fuertes A, Abradelo M, Lumbreras C, Moreno E, Solís-Herruzo JA. Clinical and histological efficacy of pegylated interferon and ribavirin therapy of recurrent hepatitis C after liver transplantation. *Liver Transpl* 2006; **12**: 1805-1812 [PMID: 17133585 DOI: 10.1002/lt.20883]

96 **Oton E**, Barcena R, Garcia-Garzon S, Moreno-Zamora A, Moreno A, Garcia-Gonzalez M, Blesa C, Foruny JR, Ruiz P. Pegylated interferon and ribavirin for the recurrence of chronic hepatitis C genotype 1 in transplant patients. *Transplant Proc* 2005; **37**: 3963-3964 [PMID: 16386597 DOI: 10.1016/j.transproceed.2005.10.060]

97 **Oton E**, Barcena R, Moreno-Planas JM, Cuervas-Mons V, Moreno-Zamora A, Barrios C, Garcia-Garzon S, Moreno A, Boullosa-Graña E, Rubio-Gonzalez EE, Garcia-Gonzalez M, Blesa C, Mateos ML. Hepatitis C recurrence after liver transplantation: Viral and histologic response to full-dose PEG-interferon and ribavirin. *Am J Transplant* 2006; **6**: 2348-2355 [PMID: 16869810 DOI: 10.1111/j.1600-6143.2006.01470.x]

98 **Castells L**, Vargas V, Allende H, Bilbao I, Luis Lázaro J, Margarit C, Esteban R, Guardia J. Combined treatment with pegylated interferon (alpha-2b) and ribavirin in the acute phase of hepatitis C virus recurrence after liver transplantation. *J Hepatol* 2005; **43**: 53-59 [PMID: 15876467 DOI: 10.1016/j.jhep.2005.02.015]

99 **Rodriguez-Luna H**, Khatib A, Sharma P, De Petris G, Williams JW, Ortiz J, Hansen K, Mulligan D, Moss A, Douglas DD, Balan V, Rakela J, Vargas HE. Treatment of recurrent hepatitis C infection after liver transplantation with combination of pegylated interferon alpha2b and ribavirin: an open-label series. *Transplantation* 2004; **77**: 190-194 [PMID: 14742979 DOI: 10.1097/01.TP.0000100481.14514.BB]

100 **Dumortier J**, Scoazec JY, Chevallier P, Boillot O. Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. *J Hepatol* 2004; **40**: 669-674 [PMID: 15030984 DOI: 10.1016/j.jhep.2003.12.015]

101 **Mukherjee S**, Rogge J, Weaver L, Schafer DF. Pilot study of pegylated interferon alfa-2b and ribavirin for recurrent hepatitis C after liver transplantation. *Transplant Proc* 2003; **35**: 3042-3044 [PMID: 14697974 DOI: 10.1016/j.transproceed.2003.10.083]

102 **Wang CS**, Ko HH, Yoshida EM, Marra CA, Richardson K. Interferon-based combination anti-viral therapy for hepatitis C virus after liver transplantation: a review and quantitative analysis. *Am J Transplant* 2006; **6**: 1586-1599 [PMID: 16827859 DOI: 10.1111/j.1600-6143.2006.01362.x]

103 **Kwo PY**, Mantry PS, Coakley E, Te HS, Vargas HE, Brown R, Gordon F, Levitsky J, Terrault NA, Burton JR, Xie W, Setze C, Badri P, Pilot-Matias T, Vilchez RA, Forns X. An interferon-free antiviral regimen for HCV after liver transplantation. *N Engl J Med* 2014; **371**: 2375-2382 [PMID: 25386767 DOI: 10.1056/NEJMoa1408921]

104 **Werner CR**, Egetemeyr DP, Lauer UM, Nadalin S, Königsrainer A, Malek NP, Berg CP. Feasibility of telaprevir-based triple therapy in liver transplant patients with hepatitis C virus: SVR 24 results. *PLoS One* 2013; **8**: e80528 [PMID: 24265827 DOI: 10.1371/journal.pone.0080528]

105 **Kwo P**, Ghabril M, Lacerda M, Vinayek R, Tector AJ, Fridell J, Vianna R. Use of telaprevir plus peginterferon/ribavirin for null responders postOL with advanced fibrosis/cholestatic hepatitis C. *J Hepatol* 2012; **56** Suppl 2: S86 [DOI: 10.1016/S0168-8278(12)60215-3]

106 **Burton JR**, Everson GT. Initial experience with telaprevir for treating hepatitis C virus in liver recipients: virologic response, safety, and tolerability. *Am J Transplant* 2012; **12** Suppl 3: 188

107 **Coilly A**, Roche B, Dumortier J, Leroy V, Botta-Fridlund D, Radenne S, Pageaux GP, Si-Ahmed SN, Guillaud O, Antonini

TM, Haïm-Boukobza S, Roque-Afonso AM, Samuel D, Duclos-Vallée JC. Safety and efficacy of protease inhibitors to treat hepatitis C after liver transplantation: a multicenter experience. *J Hepatol* 2014; **60**: 78-86 [PMID: 23994384 DOI: 10.1016/j.jhep.2013.08.018]

108 **Pungpapong S**, Aqel BA, Koning L, Murphy JL, Henry TM, Ryland KL, Yataco ML, Satyanarayana R, Rosser BG, Vargas HE, Charlton MR, Keaveny AP. Multicenter experience using telaprevir or boceprevir with peginterferon and ribavirin to treat hepatitis C genotype 1 after liver transplantation. *Liver Transpl* 2013; **19**: 690-700 [PMID: 23696372 DOI: 10.1002/lt.23669]

109 **Verna EC**, Shetty K, Lukose T, Terry N, Mentore K, Olsen SK, Fox AN, Dove LM, Brown RS. High post-transplant virological response in hepatitis C virus infected patients treated with pretransplant protease inhibitor-based triple therapy. *Liver Int* 2015; **35**: 510-517 [PMID: 24905624 DOI: 10.1111/liv.12616]

110 **Charlton M**, Gane E, Manns MP, Brown RS, Curry MP, Kwo PY, Fontana RJ, Gilroy R, Teperman L, Muir AJ, McHutchison JG, Symonds WT, Brainard D, Kirby B, Dvory-Sobol H, Denning J, Arterburn S, Samuel D, Forns X, Terrault NA. Sofosbuvir and ribavirin for treatment of compensated recurrent hepatitis C virus infection after liver transplantation. *Gastroenterology* 2015; **148**: 108-117 [PMID: 25304641 DOI: 10.1053/j.gastro.2014.10.001]

111 **Forns X**, Charlton M, Denning J, McHutchison JG, Symonds WT, Brainard D, Brandt-Sarif T, Chang P, Kivett V, Castells L, Prieto M, Fontana RJ, Baumert TF, Coilly A, Londoño MC, Habersetzer F. Sofosbuvir compassionate use program for patients with severe recurrent hepatitis C after liver transplantation. *Hepatology* 2015; **61**: 1485-1494 [PMID: 25557906 DOI: 10.1002/hep.27681]

112 AASLD and IDSA Guidelines. Recommendations for Testing, Managing, and Treating Hepatitis C. Summary of Recommendations for Patients Who Develop Recurrent HCV Infection Post-Liver Transplantation. Accessed April 3, 2015. Available from: URL: http://www.hcvguidelines.org/

113 **Reddy KR**, Everson GT, Flamm SL, Denning JM, Arterburn S, Brandt-Sarif T, Pang PS, Dvory-Sobol H, McHutchison GH, Curry MP, Charlton M. Ledipasvir/Sofosbuvir With Ribavirin for the Treatment of HCV in Patients With Post-Transplant Recurrence: Preliminary Results of a Prospective, Multicenter Study. 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), November 7-11, 2014; Boston, United States

114 **Gane EJ**, Hyland RH, An D, Svarovskaia E, Pang PS, Brainard D, Stedman CA. Efficacy of Ledipasvir and Sofosbuvir, With or Without Ribavirin, for 12 Weeks in Patients With HCV Genotype 3 or 6 Infection. *Gastroenterology* 2015; Epub ahead of print [PMID: 26261007 DOI: 10.1053/j.gastro.2015.07.063]

115 **Pellicelli AM**, Montalbano M, Lionetti R, Durand C, Ferenci P, D'Offizi G, Knop V, Telese A, Lenci I, Andreoli A, Zeuzem S, Angelico M. Sofosbuvir plus daclatasvir for post-transplant recurrent hepatitis C: potent antiviral activity but no clinical benefit if treatment is given late. *Dig Liver Dis* 2014; **46**: 923-927 [PMID: 24997638 DOI: 10.1016/j.dld.2014.06.004]

116 **Fontana R**. High efficacy and favorable safety profile of Daclatasvir based all oral antiviral therapy in liver Transplant recipients with severe recurrent HCV. 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), November 7-11, 2014; Boston, United States

117 **Mantry P**, Kwo PY, Coakley E, Te HS, Vargas HE, Brown RS, Gordon FD, Levitsky J, Terrault N, Burton JR, Xie W, Setze C, Badri P, Vilchez RA, Forns X. High Sustained Virologic Response Rates in Liver Transplant Recipients With Recurrent HCV Genotype 1 Infection Receiving ABT-450/r/Ombitasvir Dasabuvir Plus Ribavirin. 65th Annual Meeting of the American Association for the Study of Liver diseases, November 7-11, 2014, Boston, United States

118 **Gutierrez JA**, Carrion AF, Avalos D, O'Brien C, Martin P, Bhamidimarri KR, Peyton A. Sofosbuvir and simeprevir for treatment of hepatitis C virus infection in liver transplant recipients. *Liver Transpl* 2015; **21**: 823-830 [PMID: 25825070 DOI: 10.1002/lt.24126]

119 **Pungpapong S**, Aqel B, Leise M, Werner KT, Murphy JL, Henry TM, Ryland K, Chervenak AE, Watt KD, Vargas HE, Keaveny AP. Multicenter experience using simeprevir and sofosbuvir with or without ribavirin to treat hepatitis C genotype 1 after liver transplant. *Hepatology* 2015; **61**: 1880-1886 [PMID: 25722203 DOI: 10.1002/hep.27770]

120 **Brown RS**, Reddy KRJ, O'Leary JG, Kuo A, Morelli G, Stravitz RT. Safety and efficacy of new DAA-based therapy for hepatitis C post-transplant: interval results from the HCV-TARGET longitudinal, observational study. *Hepatology* 2014; **60**: 1269A

121 **Weber R**, Sabin CA, Friis-Møller N, Reiss P, El-Sadr WM, Kirk O, Dabis F, Law MG, Pradier C, De Wit S, Akerlund B, Calvo G, Monforte Ad, Rickenbach M, Ledergerber B, Phillips AN, Lundgren JD. Liver-related deaths in persons infected with the human immunodeficiency virus: the D: A: D study. *Arch Intern Med* 2006; **166**: 1632-1641 [PMID: 16908797 DOI: 10.1001/archinte.166.15.1632]

122 **Marcellin P**, Pequignot F, Delarocque-Astagneau E, Zarski JP, Ganne N, Hillon P, Antona D, Bovet M, Mechain M, Asselah T, Desenclos JC, Jougla E. Mortality related to chronic hepatitis B and chronic hepatitis C in France: evidence for the role of HIV coinfection and alcohol consumption. *J Hepatol* 2008; **48**: 200-207 [PMID: 18086507 DOI: 10.1016/j.jhep.2007.09.010]

123 **Moreno A**, Cervera C, Fortún J, Blanes M, Montejo E, Abradelo M, Len O, Rafecas A, Martín-Davila P, Torre-Cisneros J, Salcedo M, Cordero E, Lozano R, Pérez I, Rimola A, Miró JM. Epidemiology and outcome of infections in human immunodeficiency virus/hepatitis C virus-coinfected liver transplant recipients: a FIPSE/GESIDA prospective cohort study. *Liver Transpl* 2012; **18**: 70-81 [PMID: 21898772 DOI: 10.1002/lt.22431]

124 **Sawinski D**, Forde KA, Eddinger K, Troxel AB, Blumberg E, Tebas P, Abt PL, Bloom RD. Superior outcomes in HIV-positive kidney transplant patients compared with HCV-infected or HIV/HCV-coinfected recipients. *Kidney Int* 2015; **88**: 341-349 [PMID: 25807035 DOI: 10.1038/ki.2015.74]

125 **Norris S**, Taylor C, Muiesan P, Portmann BC, Knisely AS, Bowles M, Rela M, Heaton N, O'Grady JG. Outcomes of liver transplantation in HIV-infected individuals: the impact of HCV and HBV infection. *Liver Transpl* 2004; **10**: 1271-1278 [PMID: 15376307 DOI: 10.1002/lt.20233]

126 **Righi E**, Londero A, Pea F, Bonora S, Nasta P, Della Siega P, Delle Foglie P, Villa G, Giglio O, Dal Zoppo S, Baccarani U, Bassetti M. Antiretroviral blood levels in HIV/HCV-coinfected patients with cirrhosis after liver transplant: a report of three cases. *Transpl Infect Dis* 2015; **17**: 147-153 [PMID: 25620392 DOI: 10.1111/tid.12339]

127 **Wyles DL**, Gerber JG. Antiretroviral drug pharmacokinetics in hepatitis with hepatic dysfunction. *Clin Infect Dis* 2005; **40**: 174-181 [PMID: 15614709 DOI: 10.1086/426021]

128 **Righi E**, Beltrame A, Bassetti M, Lindstrom V, Mazzarello G, Dentone C, Di Biagio A, Ratto S, Viscoli C. Therapeutical aspects and outcome of HIV/HCV coinfected patients treated with pegylated interferon plus ribavirin in an Italian cohort. *Infection* 2008; **36**: 358-361 [PMID: 18642111 DOI: 10.1007/s15010-008-7319-5]

129 **Castells L**, Rimola A, Manzardo C, Valdivieso A, Montero JL, Barcena R, Abradelo M, Xiol X, Aguilera V, Salcedo M, Rodriguez M, Bernal C, Suarez F, Antela A, Olivares S, Del Campo S, Laguno M, Fernandez JR, de la Rosa G, Agüero F, Perez I, González-García J, Esteban-Mur JI, Miro JM. Pegylated interferon plus ribavirin in HIV-infected patients with recurrent hepatitis C after liver transplantation: a prospective cohort study. *J Hepatol* 2015; **62**: 92-100 [PMID: 25127748 DOI: 10.1016/j.jhep.2014.07.034]

130 **Baccarani U**, Righi E, Adani GL, Lorenzin D, Pasqualucci A, Bassetti M, Risaliti A. Pros and cons of liver transplantation in human immunodeficiency virus infected recipients. *World J Gastroenterol* 2014; **20**: 5353-5362 [PMID: 24833865 DOI: 10.3748/wjg.v20.i18.5353]

131 **Duclos-Vallée JC**, Féray C, Sebagh M, Teicher E, Roque-Afonso

AM, Roche B, Azoulay D, Adam R, Bismuth H, Castaing D, Vittecoq D, Samuel D. Survival and recurrence of hepatitis C after liver transplantation in patients coinfected with human immunodeficiency virus and hepatitis C virus. *Hepatology* 2008; **47**: 407-417 [PMID: 18098295 DOI: 10.1002/hep.21990]

132 **Antonini TM**, Furlan V, Teicher E, Haim-Boukobza S, Sebagh M, Coilly A, Bonhomme-Faivre L, Roque-Afonso AM, Vittecoq D, Samuel D, Taburet AM, Duclos-Vallée JC. Therapy with boceprevir or telaprevir in HIV/hepatitis C virus co-infected patients to treat recurrence of hepatitis C virus infection after liver transplantation. *AIDS* 2015; **29**: 53-58 [PMID: 25387314 DOI: 10.1097/QAD.0000000000000516]

133 **Moreno A**, Perez-Elias MJ, Barcena R, Quereda C, Casado JL, Dronda F, Mateos ML, Diaz A, Moreno S. Safety and Efficacy of IFN-free, Sofosbuvir/RBV Therapy in HIV/HCV Liver Transplanted Patients. 21st Conference on Retroviruses and Opportunistic Infections (CROI) March 3-6, 2014, Boston, United States

134 **Rockstroh JK**, Puoti M, Rodriguez-Torres M, Dieterich D, Gaggar A, Ni L, Massetto B, Svarovskaia ES, Brainard DM, Subramanian M, McHutchison JG, Naggie S, Orkin C, Molina JM, Sulkowski MS. Sofosbuvir and Ribavirin therapy for the Treatment of HIV/HCV coinfected patients with HCV GT1-4 Infection: The PHOTON-1 and -2 Trials. Presented at: 65th Annual Meeting of the American Association for the Study of Liver diseases, November 7-11, 2014, Boston, United States

135 **Osinusi A**, Townsend K, Kohli A, Nelson A, Seamon C, Meissner EG, Bon D, Silk R, Gross C, Price A, Sajadi M, Sidharthan S, Sims Z, Herrmann E, Hogan J, Teferi G, Talwani R, Proschan M, Jenkins V, Kleiner DE, Wood BJ, Subramanian GM, Pang PS, McHutchison JG, Polis MA, Fauci AS, Masur H, Kottilil S. Virologic response following combined ledipasvir and sofosbuvir administration in patients with HCV genotype 1 and HIV co-infection. *JAMA* 2015; **313**: 1232-1239 [PMID: 25706232 DOI: 10.1001/jama.2015.1373]

136 **Naggie S**, Cooper C, Saag M, Yang J. Ledipasvir/sofosbuvir for 12 wk in patients coinfected with HCV and HIV-1. Presented at: 22nd Conference on Retroviruses and Opportunistic Infections (CROI) 2015. February 23-26, 2015; Seattle, United States

137 **Sulkowski MS**, Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, Slim J, Bhatti L, Gathe J, Ruane PJ, Elion R, Bredeek F, Brennan R, Blick G, Khatri A, Gibbons K, Hu YB, Fredrick L, Schnell G, Pilot-Matias T, Tripathi R, Da Silva-Tillmann B, McGovern B, Campbell AL, Podsadecki T. Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. *JAMA* 2015; **313**: 1223-1231 [PMID: 25706092 DOI: 10.1001/jama.2015.1328]

138 **Sulkowski M**, Hezode C, Gerstoft J, Vierling JM, Mallolas J, Pol S, Kugelmas M, Murillo A, Weis N, Nahass R, Shibolet O, Serfaty L, Bourliere M, DeJesus E, Zuckerman E, Dutko F, Shaughnessy M, Hwang P, Howe AY, Wahl J, Robertson M, Barr E, Haber B. Efficacy and safety of 8 weeks versus 12 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin in patients with hepatitis C virus genotype 1 mono-infection and HIV/hepatitis C virus co-infection (C-WORTHY): a randomised, open-label phase 2 trial. *Lancet* 2015; **385**: 1087-1097 [PMID: 25467560 DOI: 10.1016/S0140-6736(14)61793-1]

139 **Del Bello DP**, Bichoupan K, Yalamanchili R. Real-world data on HIV positive patients with HCV genotype 1,2 and 3 on sofosbuvir- and simeprevir containing regimens. Presented at: 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). November 7-1, 2014, Boston, United States

140 **Wyles D**, Ruane P, Sulkowski, Dieterich D. Daclatasvir in combination with sofosbuvir for HIV/HCV coinfection: ALLY-2 study. 22nd Conference on Retroviruses and Opportunistic Infections (CROI), February 23-26, 2015; Seattle, United States

141 **Burgess S**, Partovi N, Yoshida EM, Erb SR, Azalgara VM, Hussaini T. Drug Interactions With Direct-Acting Antivirals for Hepatitis C: Implications for HIV and Transplant Patients. *Ann Pharmacother* 2015; **49**: 674-687 [PMID: 25770114 DOI: 10.1177/1060028015576180]

142 **Miro JM**, Stock P, Teicher E, Duclos-Vallée JC, Terrault N, Rimola A. Outcome and management of HCV/HIV coinfection pre- and post-liver transplantation. A 2015 update. *J Hepatol* 2015; **62**: 701-711 [PMID: 25450714 DOI: 10.1016/j.jhep.2014.10.032]

143 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]

144 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]

145 **Forns X**, García-Retortillo M, Serrano T, Feliu A, Suarez F, de la Mata M, García-Valdecasas JC, Navasa M, Rimola A, Rodés J. Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation. *J Hepatol* 2003; **39**: 389-396 [PMID: 12927925 DOI: 10.1016/S0168-8278(03)00310-6]

146 **Ueda Y**, Takada Y, Marusawa H, Egawa H, Uemoto S, Chiba T. Individualized extension of pegylated interferon plus ribavirin therapy for recurrent hepatitis C genotype 1b after living-donor liver transplantation. *Transplantation* 2010; **90**: 661-665 [PMID: 20110853 DOI: 10.1097/TP.0b013e3181d2bfca]

P- Reviewer: Morioka D **S- Editor**: Ma YJ **L- Editor**: A
E- Editor: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10776

World J Gastroenterol 2015 October 14; 21(38): 10776-10782
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

Modulation of host lipid metabolism by hepatitis C virus: Role of new therapies

José A Del Campo, Manuel Romero-Gómez

José A Del Campo, Manuel Romero-Gómez, Digestive Diseases and CIBERehd, Valme University Hospital, 41014 Seville, Spain

Author contributions: Del Campo JA and Romero-Gómez M contributed equally to this work, by analyzing published papers and writing the manuscript.

Conflict-of-interest statement: The authors have no conflict of interest to report.

Correspondence to: José A Del Campo, PhD, Digestive Diseases and CIBERehd, Valme University Hospital, University of Seville, Avda. Bellavista s/n, 41014 Seville, Spain. jantonio.delcampo@ciberehd.org
Telephone: +34-95-5015485
Fax: +34-95-5015799

Received: April 21, 2015
Peer-review started: April 22, 2015
First decision: June 23, 2015
Revised: July 7, 2015
Accepted: September 14, 2015
Article in press: September 14, 2015
Published online: October 14, 2015

Abstract

It is well established that hepatitis C virus (HCV) infection and replication relies on host lipid metabolism. HCV proteins interact and associate with lipid droplets to facilitate virion assembly and production. Besides, circulating infective particles are associated with very low-density lipoprotein. On the other hand, higher serum lipid levels have been associated with sustained viral response to pegylated interferon and ribavirin therapy in chronic HCV infection, suggesting a relevant role in viral clearance for host proteins. Host and viral genetic factors play an essential role in chronic infection. Lipid metabolism is hijacked by viral infection and could determine the success of viral replication. Recently development of direct acting antiviral agents has shown a very high efficacy (> 90%) in sustained viral response rates even for cirrhotic patients and most of the viral genotypes. HCV RNA clearance induced by Sofosbuvir has been associated with an increased concentration and size of the low-density lipoprotein particles. In this review, host genetic factors, viral factors and the interaction between them will be depicted to clarify the major issues involved in viral infection and lipid metabolism.

Key words: Hepatitis C virus; Lipid metabolism; Direct acting antiviral agents; Genetic interaction; Sofosbuvir

Core tip: Hepatitis C virus (HCV) is known to be closely related and associated with host lipid metabolism. Recently development of direct acting antiviral agents has shown a very high efficacy (> 90%) in sustained viral response rates even for cirrhotic patients and most of the viral genotypes. HCV RNA clearance induced by Sofosbuvir has been associated with an increased concentration and size of the low-density lipoprotein particles. Host and viral genetic factors play an essential role in chronic infection. Lipid metabolism is hijacked by viral infection and could determine the success of viral replication.

Del Campo JA, Romero-Gómez M. Modulation of host lipid metabolism by hepatitis C virus: Role of new therapies. *World J*

Gastroenterol 2015; 21(38): 10776-10782 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10776.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10776

INTRODUCTION

Hepatitis C virus (HCV) infection is a relevant public health problem, infecting approximately 170 million people worldwide[1]. About 70% of infected patients will develop chronic HCV infection. One third of them have a significant increased risk of advanced liver fibrosis, cirrhosis development and finally, hepatocellular carcinoma. With the recent emergence of first generation direct acting antivirals (DAAs), and the development of a second generation DAAs, They have been a near-final step towards the eradication of HCV infection[2-5].

HCV is known to be closely related and associated with host lipid metabolism. HCV proteins interact and associate with lipid droplets to facilitate virion assembly and production[6]. Besides, circulating infective particles are associated with very low-density lipoprotein (VLDL)-like particles, referred as lipoviral particles (LVP)[7]. A proposed mechanism to facilitate HCV entry has been postulated based on the incorporation of host apolipoproteins into the LVP[7-9]. It has been shown that several apolipoproteins are necessary for viral assembly and the production of infective particles[10-12]. Moreover, elevated serum lipid levels have been associated with the rate of sustained viral response to pegylated interferon and ribavirin (Peg-IFN + RBV) therapy for chronic HCV infected patients, suggesting a key role for host proteins in the eradication of viral infection[13,14]. In this review, host genetic factors, viral factors and the interaction between them will be depicted to clarify the major issues involved in viral infection and lipid metabolism.

HOST GENETIC FACTORS

All viruses, as obligate intracellular parasites, are implicitly dependent on host cell functions for their survival and propagation. There is an emerging understanding of the possible role played by lipid droplets (LDs) in the life cycle of a growing number of viruses, including HCV[15,16]. In the establishment of HCV infection, LDs occupy a central role in the generation of infectious virions and are specifically targeted by viral proteins for this purpose[17]. Diacylglycerol acyltransferase-1 (DGAT1) catalyses the final stage in triglyceride synthesis, and also plays a central role in formation of LDs. It has been shown that DGAT1 interacts with both core and NS5A to facilitate their recruitment to LDs[18]. DGAT1 also appears to facilitate interaction between core and NS5A, thereby functioning as a molecular bridge between the two proteins to ensure that they are targeted to the same LD[19].

The close relationship between serum LDL-cholesterol (LDL-C) concentration and the chance of achieving sustained viral response has been reported largely in patients under Peg-IFN + RBV therapy[20] as well as with direct-acting antiviral-based triple therapy[21]. Lipid-conforming LVPs are released after HCV eradication, thus increasing concentration can be found in plasma and their concentrations increase in plasma. As previously pointed out, the higher the baseline LDL-C serum level, the greater the chance of curing hepatitis C. This finding is especially relevant in patients in patients bearing non-favourable IL28B genotype, together with previous non-responders patients to Peg-IFN + RBV when treated with triple therapy using telaprevir[22]. Some works have analyzed several genes implicated in lipid transport, such as *APOB*, *APOC-Ⅲ*, *APO-L3*, and lipid-signaling leptin receptor, MTTP together with liver X receptor/retinoid X receptor pathways. Several changes in these genes have corroborated the link between HCV infection and lipid metabolism and could also identify these genes as therapeutic targets for HCV infection, like FASN inhibition or DGAT activity blockage for inhibition of viral particles production, together with the prevention of the viral entry in the cell[23,24] (Figure 1).

The liver is the main organ for lipid homeostasis in the entire body, through production and uptake of lipoproteins. Lipid homeostasis is a complex mechanism that involves a large amount of genes. Several genetic analysis, including Genome-Wide analysis have been performed to shed some light on this process. This type of analysis has identified a strong association between single nucleotide polymorphisms (SNPs) near the *IL28B* locus and the chance of achieving sustained virologic response (SVR) to Peg-IFN + RBV therapy in HCV patients, as well as spontaneous viral clearance[25,26]. Moreover, higher plasma levels of ApoB have been associated with sustained virological response in HCV patients bearing the rs8099917 responder genotype (located proximal to rs12979860) in the *IL28B* gene[27]. Besides, Duggal *et al*[28] described the association of SNP rs4273729 related to the HLA class Ⅱ genes on Chromosome 6 with spontaneous HCV clearance independently of *IL28B* genotype. Nowadays, the role of the *IL28B* genotype on SVR is attenuated - non significant - in the setting new therapies with NS3 protease, NS5A or NS5B polymerase inhibitors.

Adiponutrin or patatin-like phospholipase domain containing 3 (*PNPLA3*) is a member of the patatin-like phospholipase family. It is expressed in several human tissues with highest expression in the liver[29]. *PNPLA3* acts as a transacylase, which synthesises intracellular triglycerides by transferring acyl groups from monoglycerides to mono- and diglycerides[30]. A study by Trépo *et al*[31] found, in Caucasian chronic hepatitis C (CHC) patients, a strong and independent association between *PNPLA3* and liver damage. Patients with

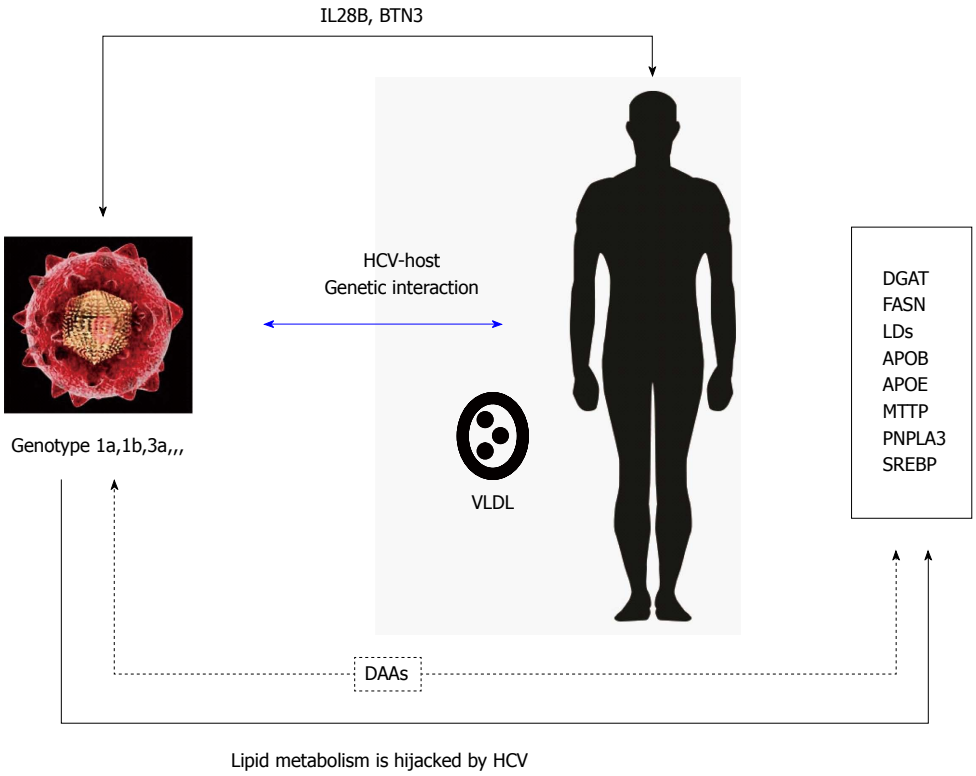


Figure 1 Schematic representation of hepatitis C virus and host interplay during hepatitis C virus infection. Viral infection has a direct effect on lipid metabolism through two main mechanisms: first, by deregulating gene expression (*FASN, DGAT, MTTP, SREBP*). This effect can be modulated by certain SNPs in PNPLA3, among others. Secondly, VLDL synthesis is affected, since HCV replication takes place on lipid droplets. SNPs: Single nucleotide polymorphisms; VLDL: Very low-density lipoprotein; HCV: Hepatitis C virus; DGAT: Diacylglycerol acyltransferase; LD: Lipid droplet; PNPLA3: patatin-like phospholipase domain containing 3.

homozygosity of the risk allele had a 2.5-fold higher risk for hepatic steatosis and an over three-fold higher risk for fibrosis as well as for fibrosis progression.

HCV interacts with several proteins of the VLDL secretion pathway for the production of infectious particles. Circulating LVP in an infected patient indicate that HCV virions are associated with hepatically derived triglyceride-rich lipoproteins (TRL) containing apoB-100. These lipo-viro-particles are also associated with gut related lipoproteins containing apoB[8,32]. HCV infection also leads to TRL accumulation through transcriptional activation of lipogenic genes, thus stimulating synthesis of lipids in patients[33]. Besides, several studies on HCV patients have indicated that the virus induced lipogenic genes over-expression. This process may exert a strong influence on inflammation and fibrosis progression in HCV patients, rather than causing the lipid accumulation observed in hepatic steatosis[34].

ApoE plays a relevant role in the assembly and production of viral particles during HCV infection. ApoE depletion has a significant effect in HCV particles production compared to apoB or apoA1 in the same model. This effect may be related to the role of apoE in HCV assembly and interaction with the viral protease NS5A, as previously described[11,12,35]. The interplay NS5A-apoE is a key factor for the building of the viral assembly machinery.

VIRAL FACTORS

A previous work performed by our group demonstrated a relationship between *IL28B* polymorphism and lipid profile in patients with hepatitis C genotype 1[20]. This association was not present in patients with hepatitis C genotype 3 or 4 and in the non-infected control group. LDL and total cholesterol levels were higher in patients infected with *HCV* genotypes 1 and 4 harbouring the favourable (CC) genotype for *IL28B* gene. HCV directly causes the appearance of large lipid droplets in hepatocytes. Remarkably, HCV replication rates are higher in patients infected with genotype 3, concomitant with more frequent and severe hepatic steatosis[36]. In addition, HCV-induced steatosis related to genotype 3 infections is abolished when antiviral therapy is achieved. Moreover, studies performed *in vitro*, where cells are transfected with HCV core protein from different genotypes show that core protein is sufficient for lipid droplets induction in the hepatocytes, which is especially relevant - more efficient - in the case of genotype 3a core protein[37]. Lack of understanding for these mechanisms still hamper the characterizarion of these processes, including the appearance of very large lipid droplets in genotype 3. The reasons to explain why genotype 3 is more efficient in steatosis development are still unknown, since very limited studies have been performed using different genotypes

in the same model[38].

HCV (including genotype 3a) has been reported to activate *in vitro* the sterol regulatory element binding proteins 1c and 2, two transcription factors involved in the control of neolipogenesis[39]. However, the evidence obtained in patients with different viral genotypes is inconclusive[34,40] and thus it is unclear whether steatosis in genotype 3 is favoured by an increased fatty acid and/or cholesterol synthesis.

HOST-VIRAL INTERACTIONS AND LIPIDS

HCV belongs to the *Flaviviridae* family. These viruses use the secretory pathway of the cell for their way out. Lipoprotein metabolism is tightly associated to the secretory pathway. For this reason, it has been suggested that in HCV infection, the virus uses for its own benefit the VLDL synthesis mechanism of the host cell. Based on an extensive siRNA analysis, it has been shown that most of the host proteins involved in HCV secretion belongs to the classical trafficking pathway, including microtubules, Golgi recycling endosomes, VAMP1 secretory vesicles and the lipoprotein apoE, which is linked to the core protein in the trafficking pathway[41].

High frequency of chronic infection reflects the fact that HCV has evolved several mechanisms to evade and suppress innate immunity, resulting in HCV progression to chronicity[42]. The viral NS3/4A protease is a central component of the HCV innate immune evasion strategy. The multifunctional NS3/4A protease is required for HCV replication, during which it processes the HCV polyprotein at several sites to liberate the viral NS proteins[43]. NS3/4A also targets and cleaves mitochondrial antiviral signaling protein (MAVS) from intracellular membranes to prevent signal transduction[44,45] thus, MAVS cleavage by the HCV NS3/4A protease disrupts RIG-I signaling of innate antiviral immunity and attenuates IFN production[46].

The interaction host-virus resulted on clone selection, immune response modulation and induction/inhibition of proteins involved in the viral entry into the hepatocyte. Recent insights into how HCV regulates innate immune signaling within the liver reveal a complex interaction of patient genetic background with viral and host factors of innate immune triggering and control that imparts the outcome of HCV infection and immunity[47]. Host immune responses, both innate[48] and adaptive[49] together with factors regulating HCV entry into the cell and viral quasispecies, have been explored[50]. In a previous analysis, we identified BTN3A2 (rs9104) to be associated with the selection of viral genotype[51]. Our group is currently exploring HCV susceptibility and to determine the influence of butyrophilin (BTN) family on the selection of HCV genotype. An association between BTN3A2 SNP rs9104 and HCV infection by genotype 1 has been recently described, where genetic variants play a relevant role in selecting a HCV genotype and influencing disease progression[52].

ROLE OF NEW HCV THERAPIES IN LIPID METABOLISM

Sofosbuvir is one of the most relevant drugs for hepatitis C therapy. It is a nucleotide analogue inhibitor of the NS5B polymerase which has been recently approved by the Food and Drug Administration and European Medicines Agency for HCV treatment and is currently used in combination with other antivirals like daclatasvir and ledipasvir (NS5A inhibitors). Other combinations include a protease inhibitor such as simeprevir or even with the formerly defined as Standard of Care for hepatitis C (peg-IFN + RBV). Sofosbuvir has demonstrated a consistently potent antiviral activity across several HCV genotypes, and has been found to be safe and well tolerated, showing a very high efficacy (> 90%) in sustained viral response rates even for cirrhotic patients. HCV RNA clearance induced by Sofosbuvir has been associated with an increased concentration and size of the LDL particles. Recently, Meissner *et al*[53] have demonstrated rapid changes in serum lipoprotein particle concentration during treatment of chronic HCV, genotype 1-infected patients with an IFN-free regimen of SOF and RBV. This likely reflects an altered balance of lipogenesis subsequent to removal of host lipid metabolism perturbation induced by HCV. This fact could be due to differential regulation of genes associated with lipid transport (*APOC3* and *APOL3*) and lipid assembly and signaling (*LEPR* and *MTTP*) that has been observed in patients with paired liver biopsies available for analysis[54,55].

Several studies have suggested that statins [3-hydroxy-3-methylglutaryl CoA reductase (HMG Co-A) inhibitors] that inhibit *de novo* cholesterol synthesis, can block HCV replication[56]. Statins appear to inhibit HCV replication *via* inhibition of geranylgeranylation of a host protein FBL2 which is required for HCV replication[57]. Rao *et al*[58] have demonstrated that statin use was associated with an improved SVR among both diabetic patients and non-diabetic patients receiving combination antiviral therapy. Hence, poor diabetes control leads to a lower SVR rate.

CONCLUSION

Host and viral genetic factors play an essential role in chronic infection. Lipid metabolism is hijacked by viral infection and could determine the success of viral replication. Mechanisms of treatment relapse with DAA therapy are nuclear and differential regulation of host lipid metabolic pathways may be associated with treatment relapse and support further investigation of lipid metabolites as predictors of treatment response

to DAA-therapy.

REFERENCES

1 **Lavanchy D**. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]

2 **Feld JJ**, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]

3 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]

4 **Kowdley KV**, Lawitz E, Poordad F, Cohen DE, Nelson DR, Zeuzem S, Everson GT, Kwo P, Foster GR, Sulkowski MS, Xie W, Pilot-Matias T, Liossis G, Larsen L, Khatri A, Podsadecki T, Bernstein B. Phase 2b trial of interferon-free therapy for hepatitis C virus genotype 1. *N Engl J Med* 2014; **370**: 222-232 [PMID: 24428468 DOI: 10.1056/NEJMoa1306227]

5 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forns X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]

6 **Popescu CI**, Dubuisson J. Role of lipid metabolism in hepatitis C virus assembly and entry. *Biol Cell* 2010; **102**: 63-74 [PMID: 19857204 DOI: 10.1042/BC20090125]

7 **André P**, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, Pol S, Bréchot C, Paranhos-Baccalà G, Lotteau V. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. *J Virol* 2002; **76**: 6919-6928 [PMID: 12072493 DOI: 10.1128/JVI.76.14.6919-6928.2002]

8 **Diaz O**, Delers F, Maynard M, Demignot S, Zoulim F, Chambaz J, Trépo C, Lotteau V, André P. Preferential association of Hepatitis C virus with apolipoprotein B48-containing lipoproteins. *J Gen Virol* 2006; **87**: 2983-2991 [PMID: 16963757 DOI: 10.1099/vir.0.82033-0]

9 **Meunier JC**, Russell RS, Engle RE, Faulk KN, Purcell RH, Emerson SU. Apolipoprotein c1 association with hepatitis C virus. *J Virol* 2008; **82**: 9647-9656 [PMID: 18667498 DOI: 10.1128/JVI.00914-08]

10 **Chang KS**, Jiang J, Cai Z, Luo G. Human apolipoprotein e is required for infectivity and production of hepatitis C virus in cell culture. *J Virol* 2007; **81**: 13783-13793 [PMID: 17913825 DOI: 10.1128/JVI.01091-07]

11 **Jiang J**, Luo G. Apolipoprotein E but not B is required for the formation of infectious hepatitis C virus particles. *J Virol* 2009; **83**: 12680-12691 [DOI: 10.1128/JVI.01476-09]

12 **Benga WJ**, Krieger SE, Dimitrova M, Zeisel MB, Parnot M, Lupberger J, Hildt E, Luo G, McLauchlan J, Baumert TF, Schuster C. Apolipoprotein E interacts with hepatitis C virus nonstructural protein 5A and determines assembly of infectious particles. *Hepatology* 2010; **51**: 43-53 [PMID: 20014138 DOI: 10.1002/hep.23278]

13 **Ramcharran D**, Wahed AS, Conjeevaram HS, Evans RW, Wang T, Belle SH, Yee LJ; Virahep-C Study Group. Associations between serum lipids and hepatitis C antiviral treatment efficacy. *Hepatology* 2010; **52**: 854-863 [PMID: 20690192 DOI: 10.1002/hep.23796]

14 **Ramcharran D**, Wahed AS, Conjeevaram HS, Evans RW, Wang T, Belle SH, Yee LJ. Serum lipids and their associations with viral levels and liver disease severity in a treatment-naïve chronic hepatitis C type 1-infected cohort. *J Viral Hepat* 2011; **18**: e144-e152 [PMID: 21070504 DOI: 10.1111/j.1365-2893.2010.01394.x]

15 **Seo JY**, Cresswell P. Viperin regulates cellular lipid metabolism during human cytomegalovirus infection. *PLoS Pathog* 2013; **9**: e1003497 [PMID: 23935494 DOI: 10.1371/journal.ppat.1003497]

16 **Boulant S**, Montserret R, Hope RG, Ratinier M, Targett-Adams P, Lavergne JP, Penin F, McLauchlan J. Structural determinants that target the hepatitis C virus core protein to lipid droplets. *J Biol Chem* 2006; **281**: 22236-22247 [PMID: 16704979 DOI: 10.1074/jbc.M601031200]

17 **Filipe A**, McLauchlan J. Hepatitis C virus and lipid droplets: finding a niche. *Trends Mol Med* 2015; **21**: 34-42 [PMID: 25496657 DOI: 10.1016/j.molmed.2014.11.003]

18 **Herker E**, Harris C, Hernandez C, Carpentier A, Kaehlcke K, Rosenberg AR, Farese RV, Ott M. Efficient hepatitis C virus particle formation requires diacylglycerol acyltransferase-1. *Nat Med* 2010; **16**: 1295-1298 [PMID: 20935628 DOI: 10.1038/nm.2238]

19 **Camus G**, Herker E, Modi AA, Haas JT, Ramage HR, Farese RV, Ott M. Diacylglycerol acyltransferase-1 localizes hepatitis C virus NS5A protein to lipid droplets and enhances NS5A interaction with the viral capsid core. *J Biol Chem* 2013; **288**: 9915-9923 [PMID: 23420847 DOI: 10.1074/jbc.M112.434910]

20 **Rojas Á**, del Campo JA, Maraver M, Aparcero R, García-Valdecasas M, Diago M, Carmona I, Andrade RJ, Solà R, Romero-Gómez M. Hepatitis C virus infection alters lipid metabolism depending on IL28B polymorphism and viral genotype and modulates gene expression in vivo and in vitro. *J Viral Hepat* 2014; **21**: 19-24 [PMID: 24188401 DOI: 10.1111/jvh.12209]

21 **Serfaty L**, Forns X, Goeser T, Ferenci P, Nevens F, Carosi G, Drenth JP, Lonjon-Domanec I, DeMasi R, Picchio G, Beumont M, Marcellin P. Insulin resistance and response to telaprevir plus peginterferon α and ribavirin in treatment-naive patients infected with HCV genotype 1. *Gut* 2012; **61**: 1473-1480 [PMID: 22387529 DOI: 10.1136/gutjnl-2011-300749]

22 **Ogawa E**, Furusyo N, Kajiwara E, Nomura H, Dohmen K, Takahashi K, Nakamuta M, Satoh T, Azuma K, Kawano A, Tanabe Y, Kotoh K, Shimoda S, Hayashi J. Influence of low-density lipoprotein cholesterol on virological response to telaprevir-based triple therapy for chronic HCV genotype 1b infection. *Antiviral Res* 2014; **104**: 102-109 [PMID: 24462955 DOI: 10.1016/j.antiviral.2014.01.004]

23 **Yang W**, Hood BL, Chadwick SL, Liu S, Watkins SC, Luo G, Conrads TP, Wang T. Fatty acid synthase is up-regulated during hepatitis C virus infection and regulates hepatitis C virus entry and production. *Hepatology* 2008; **48**: 1396-1403 [PMID: 18830996 DOI: 10.1002/hep.22508]

24 **Sung PS**, Murayama A, Kang W, Kim MS, Yoon SK, Fukasawa M, Kondoh M, Kim JS, Kim H, Kato T, Shin EC. Hepatitis C virus entry is impaired by claudin-1 downregulation in diacylglycerol acyltransferase-1-deficient cells. *J Virol* 2014; **88**: 9233-9244 [PMID: 24899196 DOI: 10.1128/JVI.01428-14]

25 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]

26 **Balagopal A**, Thomas DL, Thio CL. IL28B and the control of hepatitis C virus infection. *Gastroenterology* 2010; **139**: 1865-1876 [PMID: 20950615 DOI: 10.1053/j.gastro.2010.10.004]

27 **Yoshizawa K**, Abe H, Aida Y, Ishiguro H, Ika M, Shimada N, Tsubota A, Aizawa Y. Serum apolipoprotein B-100 concentration predicts the virological response to pegylated interferon plus ribavirin combination therapy in patients infected with chronic hepatitis C virus genotype 1b. *J Med Virol* 2013; **85**: 1180-1190 [PMID: 23918536 DOI: 10.1002/jmv.23597]

28 **Duggal P**, Thio CL, Wojcik GL, Goedert JJ, Mangia A, Latanich R, Kim AY, Lauer GM, Chung RT, Peters MG, Kirk GD, Mehta SH, Cox AL, Khakoo SI, Alric L, Cramp ME, Donfield SM, Edlin BR,

Tobler LH, Busch MP, Alexander G, Rosen HR, Gao X, Abdel-Hamid M, Apps R, Carrington M, Thomas DL. Genome-wide association study of spontaneous resolution of hepatitis C virus infection: data from multiple cohorts. *Ann Intern Med* 2013; **158**: 235-245 [PMID: 23420232 DOI: 10.7326/0003-4819-158-4-201302190-00003]

29 **Huang Y**, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, Hobbs HH. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci USA* 2010; **107**: 7892-7897 [PMID: 20385813 DOI: 10.1073/pnas.1003585107]

30 **Jenkins CM**, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J Biol Chem* 2004; **279**: 48968-48975 [PMID: 15364929 DOI: 10.1074/jbc.M407841200]

31 **Trépo E**, Gustot T, Degré D, Lemmers A, Verset L, Demetter P, Ouziel R, Quertinmont E, Vercruysse V, Amininejad L, Deltenre P, Le Moine O, Devière J, Franchimont D, Moreno C. Common polymorphism in the PNPLA3/adiponutrin gene confers higher risk of cirrhosis and liver damage in alcoholic liver disease. *J Hepatol* 2011; **55**: 906-912 [PMID: 21334404 DOI: 10.1016/j.jhep.2011.01.028]

32 **Felmlee DJ**, Sheridan DA, Bridge SH, Nielsen SU, Milne RW, Packard CJ, Caslake MJ, McLauchlan J, Toms GL, Neely RD, Bassendine MF. Intravascular transfer contributes to postprandial increase in numbers of very-low-density hepatitis C virus particles. *Gastroenterology* 2010; **139**: 1774-1783, 1783.e1-6 [PMID: 20682323 DOI: 10.1053/j.gastro.2010.07.047]

33 **Fujino T**, Nakamuta M, Yada R, Aoyagi Y, Yasutake K, Kohjima M, Fukuizumi K, Yoshimoto T, Harada N, Yada M, Kato M, Kotoh K, Taketomi A, Maehara Y, Nakashima M, Enjoji M. Expression profile of lipid metabolism-associated genes in hepatitis C virus-infected human liver. *Hepatol Res* 2010; **40**: 923-929 [PMID: 20887597 DOI: 10.1111/j.1872-034X.2010.00700.x]

34 **McPherson S**, Jonsson JR, Barrie HD, O'Rourke P, Clouston AD, Powell EE. Investigation of the role of SREBP-1c in the pathogenesis of HCV-related steatosis. *J Hepatol* 2008; **49**: 1046-1054 [PMID: 18752865 DOI: 10.1016/j.jhep.2008.06.022]

35 **Cun W**, Jiang J, Luo G. The C-terminal alpha-helix domain of apolipoprotein E is required for interaction with nonstructural protein 5A and assembly of hepatitis C virus. *J Virol* 2010; **84**: 11532-11541 [PMID: 20719944 DOI: 10.1128/JVI.01021-10]

36 **Rubbia-Brandt L**, Leandro G, Spahr L, Giostra E, Quadri R, Malé PJ, Negro F. Liver steatosis in chronic hepatitis C: a morphological sign suggesting infection with HCV genotype 3. *Histopathology* 2001; **39**: 119-124 [PMID: 11493327 DOI: 10.1016/S0168-8278(00)80166-X]

37 **Abid K**, Pazienza V, de Gottardi A, Rubbia-Brandt L, Conne B, Pugnale P, Rossi C, Mangia A, Negro F. An in vitro model of hepatitis C virus genotype 3a-associated triglycerides accumulation. *J Hepatol* 2005; **42**: 744-751 [PMID: 15826725 DOI: 10.1016/j.jhep.2004.12.034]

38 **Goossens N**, Negro F. Is genotype 3 of the hepatitis C virus the new villain? *Hepatology* 2014; **59**: 2403-2412 [PMID: 24155107 DOI: 10.1002/hep.26905]

39 **Waris G**, Felmlee DJ, Negro F, Siddiqui A. Hepatitis C virus induces proteolytic cleavage of sterol regulatory element binding proteins and stimulates their phosphorylation via oxidative stress. *J Virol* 2007; **81**: 8122-8130 [PMID: 17507484 DOI: 10.1128/JVI.00125-07]

40 **Nakamuta M**, Yada R, Fujino T, Yada M, Higuchi N, Tanaka M, Miyazaki M, Kohjima M, Kato M, Yoshimoto T, Harada N, Taketomi A, Maehara Y, Koga M, Nishinakagawa T, Nakashima M, Kotoh K, Enjoji M. Changes in the expression of cholesterol metabolism-associated genes in HCV-infected liver: a novel target for therapy? *Int J Mol Med* 2009; **24**: 825-828 [PMID: 19885625]

41 **Coller KE**, Heaton NS, Berger KL, Cooper JD, Saunders JL, Randall G. Molecular determinants and dynamics of hepatitis C virus secretion. *PLoS Pathog* 2012; **8**: e1002466 [PMID: 22241992 DOI: 10.1371/journal.ppat.1002466]

42 **Horner SM**, Gale M. Intracellular innate immune cascades and interferon defenses that control hepatitis C virus. *J Interferon Cytokine Res* 2009; **29**: 489-498 [PMID: 19708811 DOI: 10.1089/jir.2009.0063]

43 **Morikawa K**, Lange CM, Gouttenoire J, Meylan E, Brass V, Penin F, Moradpour D. Nonstructural protein 3-4A: the Swiss army knife of hepatitis C virus. *J Viral Hepat* 2011; **18**: 305-315 [PMID: 21470343 DOI: 10.1111/j.1365-2893.2011.01451.x]

44 **Baril M**, Racine ME, Penin F, Lamarre D. MAVS dimer is a crucial signaling component of innate immunity and the target of hepatitis C virus NS3/4A protease. *J Virol* 2009; **83**: 1299-1311 [PMID: 19036819 DOI: 10.1128/JVI.01659-08]

45 **Li XD**, Sun L, Seth RB, Pineda G, Chen ZJ. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proc Natl Acad Sci USA* 2005; **102**: 17717-17722 [PMID: 16301520 DOI: 10.1073/pnas.0508531102]

46 **Bellecave P**, Sarasin-Filipowicz M, Donzé O, Kennel A, Gouttenoire J, Meylan E, Terracciano L, Tschopp J, Sarrazin C, Berg T, Moradpour D, Heim MH. Cleavage of mitochondrial antiviral signaling protein in the liver of patients with chronic hepatitis C correlates with a reduced activation of the endogenous interferon system. *Hepatology* 2010; **51**: 1127-1136 [PMID: 20044805 DOI: 10.1002/hep.23426]

47 **Horner SM**, Gale M. Regulation of hepatic innate immunity by hepatitis C virus. *Nat Med* 2013; **19**: 879-888 [PMID: 23836238 DOI: 10.1038/nm.3253]

48 **Romero V**, Azocar J, Zúñiga J, Clavijo OP, Terreros D, Gu X, Husain Z, Chung RT, Amos C, Yunis EJ. Interaction of NK inhibitory receptor genes with HLA-C and MHC class II alleles in Hepatitis C virus infection outcome. *Mol Immunol* 2008; **45**: 2429-2436 [PMID: 18289678 DOI: 10.1016/j.molimm.2008.01.002]

49 **Montes-Cano MA**, Caro-Oleas JL, Romero-Gómez M, Diago M, Andrade R, Carmona I, Aguilar Reina J, Núñez-Roldán A, González-Escribano MF. HLA-C and KIR genes in hepatitis C virus infection. *Hum Immunol* 2005; **66**: 1106-1109 [PMID: 16571411 DOI: 10.1016/j.humimm.2006.02.001]

50 **Cubero M**, Gregori J, Esteban JI, García-Cehic D, Bes M, Perales C, Domingo E, Rodríguez-Frías F, Sauleda S, Casillas R, Sanchez A, Ortega I, Esteban R, Guardia J, Quer J. Identification of host and viral factors involved in a dissimilar resolution of a hepatitis C virus infection. *Liver Int* 2014; **34**: 896-906 [PMID: 24134179 DOI: 10.1111/liv.12362]

51 **Rojas L**, Ampuero J, Del Campo JA, Garcia-Lozano RJ, Solá R, Forns X, Romero-Gómez M. Fine mapping of the butyrophilin genomics region: Role in hepatitis C virus infection (HCV). *J Hepatol* 2014; **60** Suppl: S139 [DOI: 10.1016/S0168-8278(14)60381-0]

52 **Ampuero J**, Del Campo JA, Rojas L, García-Lozano RJ, Buti M, Solá R, Forns X, Moreno-Otero R, Andrade R, Diago M, Salmerón J, Rodrigo L, Pons JA, Navarro JM, Calleja JL, García-Samaniego J, García-Valdecasas M, Rojas Á, Millán R, González-Escribano MF, Romero-Gómez M. Fine-mapping butyrophilin family genes revealed several polymorphisms influencing viral genotype selection in hepatitis C infection. *Genes Immun* 2015; **16**: 297-300 [PMID: 25928882 DOI: 10.1038/gene.2015.14]

53 **Meissner EG**, Lee YJ, Osinusi A, Sims Z, Qin J, Sturdevant D, McHutchison J, Subramanian M, Sampson M, Naggie S, Patel K, Remaley AT, Masur H, Kottilil S. Effect of sofosbuvir and ribavirin treatment on peripheral and hepatic lipid metabolism in chronic hepatitis C virus, genotype 1-infected patients. *Hepatology* 2015; **61**: 790-801 [PMID: 25203718 DOI: 10.1002/hep.27424]

54 **Huang H**, Sun F, Owen DM, Li W, Chen Y, Gale M, Ye J. Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. *Proc Natl Acad Sci USA* 2007; **104**: 5848-5853 [PMID: 17376867 DOI: 10.1073/pnas.0700760104]

55 **Sun HY**, Lin CC, Lee JC, Wang SW, Cheng PN, Wu IC, Chang TT, Lai MD, Shieh DB, Young KC. Very low-density lipoprotein/lipo-viro particles reverse lipoprotein lipase-mediated inhibition of

hepatitis C virus infection via apolipoprotein C-III. *Gut* 2013; **62**: 1193-1203 [PMID: 22689516 DOI: 10.1136/gutjnl-2011-301798]

56 **Ikeda M**, Abe K, Yamada M, Dansako H, Naka K, Kato N. Different anti-HCV profiles of statins and their potential for combination therapy with interferon. *Hepatology* 2006; **44**: 117-125 [PMID: 16799963 DOI: 10.1002/hep.21232]

57 **Kapadia SB**, Chisari FV. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. *Proc Natl Acad Sci USA* 2005; **102**: 2561-2566 [PMID: 15699349 DOI: 10.1073/pnas.0409834102]

58 **Rao GA**, Pandya PK. Statin therapy improves sustained virologic response among diabetic patients with chronic hepatitis C. *Gastroenterology* 2011; **140**: 144-152 [PMID: 20833169 DOI: 10.1053/j.gastro.2010.08.055]

P- Reviewer: Fanning LJ, Ho SB, Nakajima H, Panduro A, Shimizu Y
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10783

World J Gastroenterol 2015 October 14; 21(38): 10783-10789
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

What's new in hepatitis C virus infections in children?

Malgorzata Pawlowska, Krzysztof Domagalski, Anna Pniewska, Beata Smok, Waldemar Halota, Andrzej Tretyn

Malgorzata Pawlowska, Anna Pniewska, Beata Smok, Waldemar Halota, Department of Paediatric Infectious Diseases and Haepatology, Faculty of Medicine, Collegium Medicum Bydgoszcz, Nicolaus Copernicus University, 85-030 Bydgoszcz, Poland

Krzysztof Domagalski, Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, 87-100 Toruń, Poland

Andrzej Tretyn, Department of Plant Physiology and Biotechnology, Nicolaus Copernicus University, 87-100 Toruń, Poland

Author contributions: Pawlowska M, Domagalski K, Pniewska A, Smok B, Halota W and Tretyn A contributed in writing and reviewing this article; all authors approved the final version.

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

Correspondence to: Malgorzata Pawlowska, MD, PhD, Professor, Department of Paediatric Infectious Diseases and Haepatology, Faculty of Medicine, Collegium Medicum Bydgoszcz, Nicolaus Copernicus University, Floriana 12, 85-030 Bydgoszcz, Poland. mpawlowska@cm.umk.pl
Telephone: +48-52-3255605
Fax: +48-52-3255650

Received: April 3, 2015
Peer-review started: April 4, 2015
First decision: June 2, 2015
Revised: June 17, 2015
Accepted: September 2, 2015
Article in press: September 2, 2015
Published online: October 14, 2015

Abstract

The number of hepatitis C virus (HCV) infection cases is relatively low in children. This low number may be connected with the lack of screening tests and the asymptomatic course of infection. Currently, mother-to-infant transmission is the most common cause of HCV infection amongst children in developed countries. It is important to introduce routine screening tests for HCV in pregnant women. The risk of vertical transmission of HCV is estimated at approximately 5% (3%-10%). Currently, we do not have HCV transmission prevention methods. Some factors could potentially be eliminated by elective caesarean section. Currently, the method of prevention of perinatal HCV infection is the early identification and effective treatment of infections in young women in the preconception period. We describe genetic tests (IL-28B single nucleotide polymorphisms) to identify children with an increased chance of spontaneous clearance or sustained virologic response achievement and vitamin D level as a potential predictor of treatment response in children. It is also important to develop non-invasive tests that can predict liver fibrosis. The existence of differences in the mechanisms leading to liver injury between children and adults creates new perspectives of action to reduce liver disease progression in children in the early years of life.

Key words: Hepatitis C virus; Infection in children; Single nucleotide polymorphisms; Epidemiology; Biomarkers of liver injury; Vertical infection

Core tip: Vertical transmission (VT) is the most common cause of hepatitis C virus (HCV) infection in children. It is important to introduce routine HCV screening tests in pregnant women. Some hopes for VTC prophylaxis are associated with directly acting antiviral agents. IL-28B single nucleotide polymorphisms may help to identify children with spontaneous clearance and with good

treatment prognosis. Developing non-invasive tests that can predict liver fibrosis in children is important. New biomarkers of liver injury (ITIH4, C4a, arginase 1) have been shown to reflect liver fibrosis and steatosis. The differences in liver injury between children and adults create new perspectives of action to reduce liver disease progression in children.

Pawlowska M, Domagalski K, Pniewska A, Smok B, Halota W, Tretyn A. What's new in hepatitis C virus infections in children? *World J Gastroenterol* 2015; 21(38): 10783-10789 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10783.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10783

INTRODUCTION AND EPIDEMIOLOGY

Hepatitis C virus (HCV) infection is a major health problem affecting approximately 150-180 million people worldwide, with its estimated 19 million persons infected in Europe[1]. Moreover, 3-4 million people worldwide are newly infected every year, and 350000 patients die every year due to HCV-related disorders[1,2]. According to a report from eighty-seven countries, the total global anti-HCV prevalence was estimated to be 1.6% (1.3%-2.1%)[3], whereas a recent study by the World Health Organization estimated that the prevalence of HCV varies between 2.4% in Western and Central Europe and 2.9% in Eastern Europe[1].

There are a few current global studies evaluating the prevalence of HCV infection in children, and accessible data are mainly estimated and local; there are no worldwide studies. The reason for this lack of data could be the lack of screening tests and the asymptomatic course of HCV infection. According to NASPGHAN recommendations, HCV screening should be performed in all children born to HCV-infected mothers (with assessment of anti-HCV antibodies in children after 18 mo of age and HCV RNA at a younger age), in children with chronically elevated transaminases and in children from regions with a high prevalence of HCV infection by assessing anti-HCV antibody levels. All positive anti-HCV antibody tests should be followed by a HCV RNA test to determine whether the infection is active[4].

The prevalence of HCV infection in children varies from 0.05%-0.36% in the United States and Europe and up to 1.8%-5.8% in some developing countries[5]. In studies conducted in Rio de Janeiro State in 1999-2012, Villar *et al*[6] collected data from approximately 1217 children aged 0 to 18 years. 20 individuals (1.7%) had anti-HCV antibodies in serum samples, but only 3 individuals (0.2% of all patients) were HCV RNA-positive. Recently, an epidemiological survey of HCV infection was conducted amongst children aged 10 to 19 years living in Brazil, and it reported an overall seroprevalence rate of 0.75%[7]. According to Abd-Elgawad *et al*[8], the global prevalence of HCV infection is relatively low in children, with an anti-HCV prevalence rate of 0.2%-0.4% in the Western world, with the exception of Egypt, which has the highest prevalence of adult HCV infection in the world, averaging 15%-25% in rural communities. In two Egyptian studies, anti-HCV antibodies were detectable in 3%-9% of children, depending on the region. Similar to global studies, the most common type of HCV infection in children is genotype 1. The following findings were confirmed in British research: 63% children were infected with genotype 1, 33% with genotype 3 and only 3% with genotype 2[9]. According to data from the National Institute of Public Health in Poland, the number of HCV infections in 2013 was 2641 (6.86 per 100000 people), and only 45 cases were registered in children from 0 to 18 years of age. There was also a significant decrease in cases amongst children and adolescents younger than 19 years of age[10].

There are a few most common routes of HCV transmission in children: blood transfusions, trans-plantations, unsafe injection procedures and vertical transmission. In studies conducted in Birmingham, Great Britain in 1991-2008, 133 children infected with HCV were analysed. The route of transmission was vertically acquired in 49%, transfusion acquired in 47% and transplantation acquired in 2%. Moreover, Abdel-Hady *et al*[9] showed that transfusion-associated hepatitis C was the main cause of HCV infection in children between the years 1991 and 1995. However, this route was superseded by vertically acquired HCV infection from 1995 onwards. These data correspond with other global studies. Prior to the 1990s, the principal routes of HCV infection were *via* blood transfusion and the unsafe use and reuse of injection equipment in hospitals. Unfortunately, in developing countries, these causes of HCV transmission are still reported amongst children[5].

Currently, mother-to-infant transmission of HCV is the most common cause of HCV infection amongst children in developed countries[1,5,9,11]. The incidence of HCV infection in pregnant women ranges from 1%-2% in the United States and Northern Europe and up to 8% in developing countries. Sood *et al*[12] estimated a HCV RNA prevalence rate of 1.43% in pregnant women in northern India, although this rate is comparable to those reported in other parts of the world (0.1%-2.4%)[9]. In a meta-analysis conducted by Benova *et al*[11], the risk of vertical HCV infection in children of HCV antibody-positive and RNA-positive women was 5.8% for children of human immunodeficiency virus (HIV)-negative women and 10.8% for children of HIV-positive women. These results correspond with data from Yeung *et al*[13], which showed that the risk was 1.7% amongst children born to all HCV antibody-positive women and 4.3% amongst children of HCV RNA-positive women. In Europe, the estimated rate of vertical HCV transmission also ranges

from 2% to 5%[1,9]. Therefore, routine screening tests for HCV infection need to be introduced in pregnant women.

PREVENTION OF VERTICAL HCV INFECTION

Mother-to-child transmission (MTCT) is currently responsible for 60%-90% cases of chronic hepatitis C in paediatric patients in developed countries[14]. For many years, many studies have been conducted to identify the risk factors of vertical transmission of HCV and potential ways to prevent this infection. Most of the published works come from developing countries.

High maternal serum viral load (HVL - $\geqslant 10^6$ copies/mL) during the perinatal period, the inflammatory activity in the maternal liver, coexisting HIV infection, and female sex of the baby are considered to be factors that potentially increase the risk of MTCT. Additionally, potential factors associated with higher risk of transmission are prolonged labour, premature rupture of the membranes, a long period from rupture of membranes to childbirth, newborn massive exposure to maternal blood and HCV-contaminated fluids, maternal intravenous drug abuse, HCV infection in the mother's sexual partner, maternal liver inflammation activity, the use of invasive foetal testing and assisted vaginal delivery (forceps and vacuum extractor)[1,9,11,12]. The length of labour and the time of rupture of membranes are associated with newborn exposure to the maternal blood and fluids, which are potential sources of infection. Maternal intravenous drug abuse is associated with a higher risk of the presence of HCV RNA in maternal peripheral blood mononuclear cells and thus with a higher risk of perinatal transmission[15]. Breastfeeding in the context of the risk of HCV transmission has also been the subject of many studies.

High maternal viral load in the perinatal period and coexisting HIV infection seem to be the most important factors that increase the risk of MTCT[16,17]. According to Murakami *et al*[17], the risk was higher in children of mothers with a high viral load in the perinatal period, prolonged exposure to the maternal blood and fluids in the genital tract and premature rupture of membranes. Cottrell *et al*[18] performed an extensive meta-analysis of published studies on the risk factors of vertical transmission of HCV, including invasive foetal testing and prolonged rupture of membranes. They found divergent data. Most of the available studies excluded the impact of breastfeeding on the risk of transmission.

Currently, we do not have HCV transmission prevention methods that could be used in newborns, in contrast to HIV and HBV. A vaccine against HCV or a specific immunoglobulin has not yet been developed, and recommended chemoprophylaxis is not available. The identification of risk factors for MTCT is therefore the basis for developing recommendations of procedures to prevent or at least reduce the likelihood of transmission. The most important risk factor seems to be a high viral load in the mother during the perinatal period. Its reduction can only be achieved by the use of antiviral treatment. There is currently no recommended chemoprophylaxis of perinatal HCV infection.

The use of pegylated interferon (PegIFN), especially during the first trimester of pregnancy, may be associated with an increased risk of miscarriage and low birth weight, and ribavirin (RBV) is classified by the FDA as category X because of its teratogenic effects. Accordingly, prevention using standard therapy cannot be applied during pregnancy, and women who have begun treatment before pregnancy should discontinue the therapy immediately after the confirmation of pregnancy. Some hopes are associated with direct acting antiviral agents, which appear to have a greater safety profile in pregnancy and show no teratogenic effects; they are applicable and effective without PegIFN and RBV[19]. Another proven risk factor for MTCT is concomitant HIV infection. The risk of transmission of HCV infection in children of HCV- and HIV-positive mothers without antiretroviral treatment is estimated to be 15%, which is 3 times higher than in children of HIV-negative mothers. To some extent, we have the opportunity to reduce this risk through the use of highly active antiretroviral therapy. The effectiveness of such a procedure in reducing HIV viral load is indicated by the significant reduction in the risk of HCV transmission, probably by reducing the HCV viral load[20].

Another group of potential risk factors for MTCT is related to the duration of labour or rupture of membranes time (more than 6 h), as well as the use of invasive foetal testing and assisted vaginal delivery. These factors could potentially be eliminated by performing an elective caesarean section. The effectiveness of this approach is the subject of a number of prospective and retrospective studies, the results of which are divergent; currently, there is no evidence to recommend elective caesarean section to reduce the risk of perinatal transmission of HCV. The results of the analysed studies were inconsistent; some showed a reduction in the risk of infection by using elective caesarean section compared with vaginal delivery or emergency caesarean section, but the differences in most studies were not statistically significant, and subsequent studies have not confirmed these observations. The conclusion was that it is not currently possible to indicate any particular intervention that would involve a reduction in the risk of infection[18].

A meta-analysis conducted by Ghamar Chehreh *et al*[20] showed that caesarean section does not reduce the risk of perinatal transmission of hepatitis C virus from HCV-RNA (+)/HIV (-) mothers to their infants. However, Murakami *et al*[17] conducted a prospective study, which, inter alia, assessed the risks associated with various modes of delivery in patients with high viral load in the perinatal period. According to the obtained data, elective caesarean section in patients

with HVL was associated with a significant reduction in the risk of transmission of infection; MTCT was found in 41% of infants born vaginally and none of those born by elective caesarean section. The authors therefore suggested that elective caesarean section could be an effective method to prevent MTCT in women with HVL. Furthermore, in a retrospective study, it was found that the effectiveness of elective caesarean section reduced the risk of transmission of HCV infection in patients co-infected with HIV[21].

Currently, there are no recommendations regarding chemoprophylaxis of perinatal HCV infection. There is also no obvious evidence that the mode of delivery affects the risk. Therefore, despite the significant progress that has been made in recent years in the treatment of chronic HCV infection, the only unequivocally recommended method of prevention of perinatal HCV infection is the early identification and effective treatment of infections in young women in the preconception period, but the treatment should be completed at least 6 mo before a planned pregnancy due to the potential teratogenicity of currently used drugs.

NEW TESTING DIRECTIONS

The rest of the work describes only the latest aspects and directions of the research recently conducted in paediatric patients with chronic hepatitis C (CHC), which may have a potential impact on the development of diagnostic tests for monitoring patients, on the prediction of adverse consequences in the course of the disease and on treatment results.

NEW TESTING DIRECTIONS FOR THE PREDICTION OF PERSISTENT INFECTION

The estimated rates of spontaneous clearance of the HCV RNA in vertically infected children vary considerably, and in the European population, the rates do not exceed 30%[22-24]. Spontaneous clearance of HCV in vertically infected children has been associated with HCV genotype 3 infection and with transaminase flare in the first year of life. Recently, Garazzino *et al*[22] confirmed the results of previous studies by showing that the resolution of infection is higher in patients infected with HCV genotype 3 and in patients with higher ALT levels in the first two years of life. Currently, we are additionally able to identify a group of children with an increased chance of spontaneous clearance by performing a genetic test determining the single nucleotide polymorphisms (SNPs) in the *IL-28B* gene. In 2011, a preliminary study showed the independent association of the rs12979860 polymorphism with the spontaneous clearance of HCV genotype 1 in infants infected by perinatal transmission[25]. This connection was confirmed by multicentre collaborative studies[26,27]. One of these studies enrolled 177 Italian children, of which 30 (16.9%) had spontaneous clearance and 147 had a persistent HCV infection[27]. This study demonstrated that the favourable CC *IL-28B* genotype increases the chances of spontaneous elimination of the HCV more than twice compared to the CT and TT genotypes combined (OR = 2.7; 90%CI: 1.3-5.8). Additionally, an ethnically matched control group with unknown hepatitis C status obtained from the 1000 Genome Project data was used for the analysis. It was demonstrated that in children with spontaneous viral clearance, the prevalence rate of the favourable genotype CC is significantly higher compared to that of ethnically matched individuals (56.7% and 34.7%, respectively, $P = 0.03$). However, the predictive potential of IL-28B variation is diversified, which is associated with variations in geographical distributions of HCV genotypes and differences in frequency of *IL-28B* SNPs by race. The adult study showed that the global pattern of *IL-28B* SNPs distribution may partly explain the observed discrepancy in the frequency of viral clearance across various ethnic groups[28]. In a recent study conducted in 130 Chinese paediatric patients with spontaneous clearance, rs12979860 and rs8099917 SNPs independently predicted spontaneous clearance[29]. The odds ratio was 7.39 (95%CI: 1.07-50.41) and 14.27 (95%CI: 3.07-108.50) for rs12979860 and rs8099917, respectively. In this study group, the frequency of spontaneous clearance was 47%, which is related to a high frequency (> 85% for both) of favourable genotype CC of rs12979860 and genotype TT rs8099917.

NEW TESTING DIRECTIONS FOR THE PREDICTION OF TREATMENT RESPONSE

Currently, in the case of a confirmed HCV infection, to comprehensively qualify a patient for treatment, the HCV RNA levels, HCV genotype and SNPs of the *IL-28B* gene should be determined. These are well-known predictors of response to interferon-based therapy in adults. The connection between high baseline HCV viral load and the unfavourable HCV genotypes 1 and 4 with a higher likelihood of failed interferon and RBV combination therapy was also confirmed in children[30-32]. The importance of the favourable genotypes CC rs12979860 and TT rs8099917 in the *IL-28B* gene associated with higher sustained virologic response (SVR) rates in PegIFN-based treatment for HCV infection in children has been demonstrated in several studies[33-36]. Thus, the determination of the *IL-28B* SNPs may be useful in clinical practice in enhancing the correct prediction of SVR achievement in children. In contrast, no associations were found between the rs8099917 marker and the final treatment outcome in Japanese children who were treated with response-guided PegIFN or a PegIFN plus RBV combination[37]. The results of these studies suggests that, similar to adults, the SNPs of IL-28B appear to have limited

potential for predicting treatment response, and *IL-28B* genotype testing cannot be used alone to predict the final outcome. Despite limited prognostic potential, *IL-28B* SNPs - as one of the strongest pretreatment predictors of SVR - are greatly needed for standard PegIFN-based therapy in CHC children. Although new specifically targeted antiviral agents are being introduced in adults, currently, these types of drugs are not allowed to be used in children because the safety of this therapy in children has still not been determined. Therefore, further paediatric studies are needed to evaluate the potential role of *IL-28B* genotype testing together with other known prognostic factors in new treatment strategies targeting children who poorly tolerate IFN-based regimens.

Recently, vitamin D levels have also been identified as potential predictors of response to HCV therapy in children. A study of Egyptian HCV children showed a high frequency of vitamin D deficiency and significant decreases in bone density compared with healthy children control groups matched by age and sex[38]. It was demonstrated that children treated with vitamin D showed higher early and sustained virological responses. Therefore, the authors suggest that the assessment of vitamin D levels before the start of PegIFN/RBV therapy and correction of any detected deficiency during the course of therapy may be needed to improve viral response.

NEW DIRECTIONS FOR BIOMARKERS OF LIVER INJURY

Whereas chronic hepatitis C is usually asymptomatic during childhood, long-term infection can lead to severe and decompensating liver disease in later childhood or adulthood[22,39]. The results of several paediatric studies reveal that the degree of liver injury generally correlates with age and duration of infection[8,40-42], although progression seems to be slower than observed in those infected later in life. In contrast to previous studies[43,44] that suggest that co-infection with HBV and HCV is associated with more severe liver disease and frequent progression to cirrhosis, in a recent study conducted in Polish children, HBV/HCV co-infection did not enhance fibrosis compared with HCV or HBV mono-infection groups[45]. However, in this study group, HBV/HCV co-infection was associated with moderate to severe necro-inflammation, irrespective of age of biopsy or duration of infection, and led to significantly higher necro-inflammatory activity than HCV mono-infection.

Liver biopsy still represents the gold standard for evaluating the current status of liver injury, including inflammatory and fibrosis scores in CHC. However, in children, this may result in a higher risk of complications; therefore, it is less accepted in paediatric patients than in adults[46]. Thus, developing non-invasive tests that can predict liver fibrosis, especially in paediatric populations, is attractive. Several years ago, Fibrotest and ActiTest were found to be potential non-invasive assays for the assessment of hepatic fibrosis and necro-inflammatory activity in CHC paediatric patients in comparison with liver biopsy[47-49]. In fact, they have limited prognostic potential. In the Hermeziu *et al*[48] study, it was shown that the global concordance between FibroTest-ActiTest and METAVIR scores was found in 48% of paediatric cases. A recent study that used proteomic analysis of serum from adult patients with CHC revealed that Complement C4a and inter-alpha-trypsin inhibitor heavy chain 4 (ITIH4) were potential factors to predict liver fibrosis[50]. A study including 30 Egyptian CHC children showed that C4a was not associated with histological scores, but it could predict significant fibrosis (presence of bridging fibrosis) with acceptable clinical performance[51]. The paediatric experience with serum level of ITIH4 showed notable correlation of this marker with later stages of fibrosis[52]. ITIH4 serum levels were substantially higher in patients with significant fibrosis than in those at lower fibrosis stages. Based on the available data, non-invasive tests designed to predict the degree of liver injury currently have too many limitations to constitute an alternative to liver biopsy; however, they may be useful to detect significant fibrosis. Non-invasive detection of significant fibrosis is very important for treatment decisions. Patients with significant fibrosis progression are commonly prone to cirrhosis, so antiviral treatment should be strongly considered in this group. In another preliminary study conducted in children, the aberrant expression of arginase 1 in liver tissue correlated with liver steatosis in HCV infection[53]. Immunochemistry and western blot analysis showed that there was higher expression of arginase 1 in HCV patients with steatosis than in those without it. These findings open new horizons for diagnostic markers for steatosis, but the tools need to be confirmed by larger studies.

There is relatively little information on the histopathology of chronic hepatitis C in children. It is currently accepted that both immune system-mediated reactions and viral cytopathic effects are involved in CHC pathogenesis; however, the effects of each component on the final result in children and adults have not been previously studied. In a recent preliminary study, Valva *et al*[54] evaluated an intrahepatic viral infection by comparing apoptosis and portal and periportal infiltrates in paediatric and adult patients. The results of this comparative study provided the first suggestions that liver injury in paediatric CHC may be substantially associated with viral cytopathic effects mediated by apoptosis, whereas in adults, it could be mainly associated with an exacerbated immune response. Knowing the existence of differences in the mechanisms leading to liver injury between children and adults creates new perspectives of action to reduce liver disease progression in children in the early years of life.

REFERENCES

1 **Negro F**. Epidemiology of hepatitis C in Europe. *Dig Liver Dis* 2014; **46** Suppl 5: S158-S164 [PMID: 25453870 DOI: 10.1016/j.dld.2014.09.023]

2 **Webster DP**, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; **385**: 1124-1135 [PMID: 25687730 DOI: 10.1016/S0140-6736(14)62401-6]

3 **Gower E**, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol* 2014; **61**: S45-S57 [PMID: 25086286 DOI: 10.1016/j.jhep.2014.07.027]

4 **Mack CL**, Gonzalez-Peralta RP, Gupta N, Leung D, Narkewicz MR, Roberts EA, Rosenthal P, Schwarz KB. NASPGHAN practice guidelines: Diagnosis and management of hepatitis C infection in infants, children, and adolescents. *J Pediatr Gastroenterol Nutr* 2012; **54**: 838-855 [PMID: 22487950 DOI: 10.1097/MPG.0b013e318258328d]

5 **El-Shabrawi MH**, Kamal NM. Burden of pediatric hepatitis C. *World J Gastroenterol* 2013; **19**: 7880-7888 [PMID: 24307782 DOI: 10.3748/wjg.v19.i44.7880]

6 **Villar LM**, Amado LA, de Almeida AJ, de Paula VS, Lewis-Ximenez LL, Lampe E. Low prevalence of hepatitis B and C virus markers among children and adolescents. *Biomed Res Int* 2014; **2014**: 324638 [PMID: 25093164 DOI: 10.1155/2014/324638]

7 **Pereira LM**, Martelli CM, Moreira RC, Merchan-Hamman E, Stein AT, Cardoso MR, Figueiredo GM, Montarroyos UR, Braga C, Turchi MD, Coral G, Crespo D, Lima ML, Alencar LC, Costa M, dos Santos AA, Ximenes RA. Prevalence and risk factors of Hepatitis C virus infection in Brazil, 2005 through 2009: a cross-sectional study. *BMC Infect Dis* 2013; **13**: 60 [PMID: 23374914 DOI: 10.1186/1471-2334-13-60]

8 **Abd-Elgawad MM**, Baddour NM, Salem MAE. Chronic hepatitis C in children: Clinical spectrum and histopathological study. *Alexandria Med J* 2013; **49**: 363-368 [DOI: 10.1016/j.ajme.2013.03.008]

9 **Abdel-Hady M**, Bunn SK, Sira J, Brown RM, Brundler MA, Davies P, Kelly DA. Chronic hepatitis C in children--review of natural history at a National Centre. *J Viral Hepat* 2011; **18**: e535-e540 [PMID: 21914074 DOI: 10.1111/j.1365-2893.2011.01456.x]

10 **National Institute of Public Health**. Infectious diseases and poisonings in Poland in 2004-2013. Accessed March 29, 2015. Available from: URL: http://wwwold.pzh.gov.pl/oldpage/epimeld/index_p.html#04

11 **Benova L**, Mohamoud YA, Calvert C, Abu-Raddad LJ. Vertical transmission of hepatitis C virus: systematic review and meta-analysis. *Clin Infect Dis* 2014; **59**: 765-773 [PMID: 24928290 DOI: 10.1093/cid/ciu447]

12 **Sood A**, Midha V, Bansal M, Sood N, Puri S, Thara A. Perinatal transmission of hepatitis C virus in northern India. *Indian J Gastroenterol* 2012; **31**: 27-29 [PMID: 22362316 DOI: 10.1007/s12664-012-0163-7]

13 **Yeung LT**, King SM, Roberts EA. Mother-to-infant transmission of hepatitis C virus. *Hepatology* 2001; **34**: 223-229 [PMID: 11481604 DOI: 10.1053/jhep.2001.25885]

14 **Méndez-Sánchez N**, Ridruejo E, Alves de Mattos A, Chávez-Tapia NC, Zapata R, Paraná R, Mastai R, Strauss E, Guevara-Casallas LG, Daruich J, Gadano A, Parise ER, Uribe M, Aguilar-Olivos NE, Dagher L, Ferraz-Neto BH, Valdés-Sánchez M, Sánchez-Avila JF. Latin American Association for the Study of the Liver (LAASL) clinical practice guidelines: management of hepatocellular carcinoma. *Ann Hepatol* 2014; **13** Suppl 1: S4-40 [PMID: 24998696]

15 **Indolfi G**, Azzari C, Resti M. Perinatal transmission of hepatitis C virus. *J Pediatr* 2013; **163**: 1549-1552.e1 [PMID: 23919905 DOI: 10.1016/j.jpeds.2013.06.077]

16 **Mast EE**, Hwang LY, Seto DS, Nolte FS, Nainan OV, Wurtzel H, Alter MJ. Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. *J Infect Dis* 2005; **192**: 1880-1889 [PMID: 16267758 DOI: 10.1086/497701]

17 **Murakami J**, Nagata I, Iitsuka T, Okamoto M, Kaji S, Hoshika T, Matsuda R, Kanzaki S, Shiraki K, Suyama A, Hino S. Risk factors for mother-to-child transmission of hepatitis C virus: Maternal high viral load and fetal exposure in the birth canal. *Hepatol Res* 2012; **42**: 648-657 [PMID: 22404371 DOI: 10.1111/j.1872-034X.2012.00968.x]

18 **Cottrell EB**, Chou R, Wasson N, Rahman B, Guise JM. Reducing risk for mother-to-infant transmission of hepatitis C virus: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2013; **158**: 109-113 [PMID: 23437438 DOI: 10.7326/0003-4819-158-2-201301150-00575]

19 **Hagan LM**, Schinazi RF. Best strategies for global HCV eradication. *Liver Int* 2013; **33** Suppl 1: 68-79 [PMID: 23286849 DOI: 10.1111/liv.12063]

20 **Ghamar Chehreh ME**, Tabatabaei SV, Khazanehdari S, Alavian SM. Effect of cesarean section on the risk of perinatal transmission of hepatitis C virus from HCV-RNA+/HIV- mothers: a meta-analysis. *Arch Gynecol Obstet* 2011; **283**: 255-260 [PMID: 20652289 DOI: 10.1007/s00404-010-1588-9]

21 **European Paediatric Hepatitis C Virus Network**. Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. European Paediatric Hepatitis C Virus Network. *BJOG* 2001; **108**: 371-377 [PMID: 11305543 DOI: 10.1111/j.1471-0528.2001.00088.x]

22 **Garazzino S**, Calitri C, Versace A, Alfarano A, Scolfaro C, Bertaina C, Vatrano S, Mignone F, Licciardi F, Gabiano C, Tovo PA. Natural history of vertically acquired HCV infection and associated autoimmune phenomena. *Eur J Pediatr* 2014; **173**: 1025-1031 [PMID: 24585099 DOI: 10.1007/s00431-014-2286-6]

23 **Bortolotti F**, Verucchi G, Cammà C, Cabibbo G, Zancan L, Indolfi G, Giacchino R, Marcellini M, Marazzi MG, Barbera C, Maggiore G, Vajro P, Bartolacci S, Balli F, Maccabruni A, Guido M. Long-term course of chronic hepatitis C in children: from viral clearance to end-stage liver disease. *Gastroenterology* 2008; **134**: 1900-1907 [PMID: 18439604 DOI: 10.1053/j.gastro.2008.02.082]

24 **Pembrey L**, Newell ML, Tovo PA. The management of HCV infected pregnant women and their children European paediatric HCV network. *J Hepatol* 2005; **43**: 515-525 [PMID: 16144064 DOI: 10.1016/j.jhep.2005.06.002]

25 **Ruiz-Extremera A**, Muñoz-Gámez JA, Salmerón-Ruiz MA, de Rueda PM, Quiles-Pérez R, Gila-Medina A, Casado J, Belén Martín A, Sanjuan-Nuñez L, Carazo A, Pavón EJ, Ocete-Hita E, León J, Salmerón J. Genetic variation in interleukin 28B with respect to vertical transmission of hepatitis C virus and spontaneous clearance in HCV-infected children. *Hepatology* 2011; **53**: 1830-1838 [PMID: 21413051 DOI: 10.1002/hep.24298]

26 **Indolfi G**, Mangone G, Calvo PL, Bartolini E, Regoli M, Serranti D, Calitri C, Tovo PA, de Martino M, Azzari C, Resti M. Interleukin 28B rs12979860 single-nucleotide polymorphism predicts spontaneous clearance of hepatitis C virus in children. *J Pediatr Gastroenterol Nutr* 2014; **58**: 666-668 [PMID: 24792632 DOI: 10.1097/MPG.0000000000000275]

27 **Indolfi G**, Mangone G, Bartolini E, Nebbia G, Calvo PL, Moriondo M, Tovo PA, de Martino M, Azzari C, Resti M. Comparative analysis of rs12979860 SNP of the IFNL3 gene in children with hepatitis C and ethnic matched controls using 1000 Genomes Project data. *PLoS One* 2014; **9**: e85899 [PMID: 24465773 DOI: 10.1371/journal.pone.0085899]

28 **Thomas DL**, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798-801 [PMID: 19759533 DOI: 10.1038/nature08463]

29 **Wu RR**, Liu FQ, Zhu SS, Han J. Association of Hepatitis C Virus Infection and Interleukin-28B Gene Polymorphism in Chinese Children. *Pak J Med Sci* 2014; **30**: 519-524 [PMID: 24948971 DOI: 10.12669/pjms.303.4267]

30 **Druyts E**, Thorlund K, Wu P, Kanters S, Yaya S, Cooper CL, Mills EJ. Efficacy and safety of pegylated interferon alfa-2a or

alfa-2b plus ribavirin for the treatment of chronic hepatitis C in children and adolescents: a systematic review and meta-analysis. *Clin Infect Dis* 2013; **56**: 961-967 [PMID: 23243171 DOI: 10.1093/cid/cis1031]

31 **Wirth S**, Ribes-Koninckx C, Calzado MA, Bortolotti F, Zancan L, Jara P, Shelton M, Kerkar N, Galoppo M, Pedreira A, Rodriguez-Baez N, Ciocca M, Lachaux A, Lacaille F, Lang T, Kullmer U, Huber WD, Gonzalez T, Pollack H, Alonso E, Broue P, Ramakrishna J, Neigut D, Valle-Segarra AD, Hunter B, Goodman Z, Xu CR, Zheng H, Noviello S, Sniukiene V, Brass C, Albrecht JK. High sustained virologic response rates in children with chronic hepatitis C receiving peginterferon alfa-2b plus ribavirin. *J Hepatol* 2010; **52**: 501-507 [PMID: 20189674 DOI: 10.1016/j.jhep.2010.01.016]

32 **Pawłowska M**, Pilarczyk M, Halota W. Virologic response to treatment with Pegylated Interferon alfa-2b and Ribavirin for chronic hepatitis C in children. *Med Sci Monit* 2010; **16**: CR616-CR621 [PMID: 21119580]

33 **Domagalski K**, Pawłowska M, Tretyn A, Halota W, Pilarczyk M, Smukalska E, Linkowska K, Grzybowski T. Impact of IL-28B polymorphisms on pegylated interferon plus ribavirin treatment response in children and adolescents infected with HCV genotypes 1 and 4. *Eur J Clin Microbiol Infect Dis* 2013; **32**: 745-754 [PMID: 23314745 DOI: 10.1007/s10096-012-1799-z]

34 **Shaker OG**, Nassar YH, Nour ZA, El Raziky M. Single-nucleotide polymorphisms of IL-10 and IL-28B as predictors of the response of IFN therapy in HCV genotype 4-infected children. *J Pediatr Gastroenterol Nutr* 2013; **57**: 155-160 [PMID: 23880623 DOI: 10.1097/MPG.0b013e31828febf0]

35 **Tajiri H**, Tanaka Y, Takano T, Suzuki M, Abukawa D, Miyoshi Y, Shimizu T, Brooks S. Association of IL28B polymorphisms with virological response to peginterferon and ribavirin therapy in children and adolescents with chronic hepatitis C. *Hepatol Res* 2014; **44**: E38-E44 [PMID: 23841718 DOI: 10.1111/hepr.12206]

36 **Hierro L**, Alvarez L, Andueza S, Gordo-Giralt R, Lledin D, Camarena C, de la Vega A, Munoz-Bartolo G, Frauca E, Diaz C, Jara P. Influence of IL28B gene polymorphisms on sustained response to peginterferon plus ribavirin in children with chronic hepatitis C. *J Hepatol* 2011; **54** Suppl 1: S524-525 [DOI: 10.1016/s0168-8278(11)61331-7]

37 **Komatsu H**, Inui A, Tsunoda T, Sogo T, Fujisawa T. Association between an IL-28B genetic polymorphism and the efficacy of the response-guided pegylated interferon therapy in children with chronic hepatic C infection. *Hepatol Res* 2013; **43**: 327-338 [PMID: 22970660 DOI: 10.1111/j.1872-034X.2012.01087.x]

38 **Eltayeb AA**, Abdou MA, Abdel-aal AM, Othman MH. Vitamin D status and viral response to therapy in hepatitis C infected children. *World J Gastroenterol* 2015; **21**: 1284-1291 [PMID: 25632203 DOI: 10.3748/wjg.v21.i4.1284]

39 **Mohan P**, Barton BA, Narkewicz MR, Molleston JP, Gonzalez-Peralta RP, Rosenthal P, Murray KF, Haber B, Schwarz KB, Goodman ZD. Evaluating progression of liver disease from repeat liver biopsies in children with chronic hepatitis C: a retrospective study. *Hepatology* 2013; **58**: 1580-1586 [PMID: 23703847 DOI: 10.1002/hep.26519]

40 **Guido M**, Bortolotti F, Leandro G, Jara P, Hierro L, Larrauri J, Barbera C, Giacchino R, Zancan L, Balli F, Crivellaro C, Cristina E, Pucci A, Rugge M. Fibrosis in chronic hepatitis C acquired in infancy: is it only a matter of time? *Am J Gastroenterol* 2003; **98**: 660-663 [PMID: 12650803 DOI: 10.1111/j.1572-0241.2003.07293.x]

41 **Goodman ZD**, Makhlouf HR, Liu L, Balistreri W, Gonzalez-Peralta RP, Haber B, Jonas MM, Mohan P, Molleston JP, Murray KF, Narkewicz MR, Rosenthal P, Smith LJ, Robuck PR, Schwarz KB. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C Trial. *Hepatology* 2008; **47**: 836-843 [PMID: 18167062 DOI: 10.1002/hep.22094]

42 **Cesaro S**, Bortolotti F, Petris MG, Brugiolo A, Guido M, Carli M. An updated follow-up of chronic hepatitis C after three decades of observation in pediatric patients cured of malignancy. *Pediatr Blood Cancer* 2010; **55**: 108-112 [PMID: 20127849 DOI: 10.1002/pbc.22438]

43 **Sagnelli E**, Pasquale G, Coppola N, Scarano F, Marrocco C, Scolastico C, Santantonio T, Gentile A, Piccinino F. Influence of chronic coinfection with hepatitis B and C virus on liver histology. *Infection* 2004; **32**: 144-148 [PMID: 15188073 DOI: 10.1007/s15010-004-3080-6]

44 **Fiel MI**. Pathology of chronic hepatitis B and chronic hepatitis C. *Clin Liver Dis* 2010; **14**: 555-575 [PMID: 21055682 DOI: 10.1016/j.cld.2010.07.001]

45 **Pokorska-Śpiewak M**, Kowalik-Mikołajewska B, Aniszewska M, Walewska-Zielecka B, Marczyńska M. The influence of hepatitis B and C virus coinfection on liver histopathology in children. *Eur J Pediatr* 2015; **174**: 345-353 [PMID: 25172445 DOI: 10.1007/s00431-014-2402-7]

46 **Martínez SM**, Crespo G, Navasa M, Forns X. Noninvasive assessment of liver fibrosis. *Hepatology* 2011; **53**: 325-335 [PMID: 21254180 DOI: 10.1002/hep.24013]

47 **de Lédinghen V**, Le Bail B, Rebouissoux L, Fournier C, Foucher J, Miette V, Castéra L, Sandrin L, Merrouche W, Lavrand F, Lamireau T. Liver stiffness measurement in children using FibroScan: feasibility study and comparison with Fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy. *J Pediatr Gastroenterol Nutr* 2007; **45**: 443-450 [PMID: 18030211 DOI: 10.1097/MPG.0b013e31812e56ff]

48 **Hermeziu B**, Messous D, Fabre M, Munteanu M, Baussan C, Bernard O, Poynard T, Jacquemin E. Evaluation of FibroTest-ActiTest in children with chronic hepatitis C virus infection. *Gastroenterol Clin Biol* 2010; **34**: 16-22 [PMID: 19726147 DOI: 10.1016/j.gcb.2009.06.007]

49 **El-Shabrawi MH**, Mohsen NA, Sherif MM, El-Karaksy HM, Abou-Yosef H, El-Sayed HM, Riad H, Bahaa N, Isa M, El-Hennawy A. Noninvasive assessment of hepatic fibrosis and necroinflammatory activity in Egyptian children with chronic hepatitis C virus infection using FibroTest and ActiTest. *Eur J Gastroenterol Hepatol* 2010; **22**: 946-951 [PMID: 20110820 DOI: 10.1097/MEG.0b013e328336ec84]

50 **Yang L**, Rudser KD, Higgins L, Rosen HR, Zaman A, Corless CL, David L, Gourley GR. Novel biomarker candidates to predict hepatic fibrosis in hepatitis C identified by serum proteomics. *Dig Dis Sci* 2011; **56**: 3305-3315 [PMID: 21590334 DOI: 10.1007/s10620-011-1745-4]

51 **Behairy BE**, El-Mashad GM, Abd-Elghany RS, Ghoneim EM, Sira MM. Serum complement C4a and its relation to liver fibrosis in children with chronic hepatitis C. *World J Hepatol* 2013; **5**: 445-451 [PMID: 24023984 DOI: 10.4254/wjh.v5.i8.445]

52 **Sira MM**, Behairy BE, Abd-Elaziz AM, Abd Elnaby SA, Eltahan EE. Serum Inter-Alpha-Trypsin Inhibitor Heavy Chain 4 (ITIH4) in Children with Chronic Hepatitis C: Relation to Liver Fibrosis and Viremia. *Hepat Res Treat* 2014; **2014**: 307942 [PMID: 25295185 DOI: 10.1155/2014/307942]

53 **Alisi A**, Comparcola D, De Stefanis C, Nobili V. Arginase 1: a potential marker of a common pattern of liver steatosis in HCV and NAFLD children. *J Hepatol* 2015; **62**: 1207-1208 [PMID: 25678387 DOI: 10.1016/j.jhep.2014.12.036]

54 **Valva P**, Gismondi MI, Casciato PC, Galoppo M, Lezama C, Galdame O, Gadano A, Galoppo MC, Mullen E, De Matteo EN, Preciado MV. Distinctive intrahepatic characteristics of paediatric and adult pathogenesis of chronic hepatitis C infection. *Clin Microbiol Infect* 2014; **20**: O998-1009 [PMID: 24942073 DOI: 10.1111/1469-0691.12728]

P- Reviewer: Chmiela M **S- Editor**: Ma YJ **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10790

World J Gastroenterol 2015 October 14; 21(38): 10790-10810
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

Epidemiology of hepatitis C virus in Iran

Reza Taherkhani, Fatemeh Farshadpour

Reza Taherkhani, Fatemeh Farshadpour, Department of Microbiology and Parasitology, School of Medicine, Bushehr University of Medical Sciences, Bushehr 7514633341, Iran

Reza Taherkhani, Persian Gulf Biomedical Research Center, Bushehr University of Medical Sciences, Bushehr 7514633341, Iran

Fatemeh Farshadpour, Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr 7514633341, Iran

Author contributions: Taherkhani R and Farshadpour F solely contributed to this paper.

Conflict-of-interest statement: The authors declare there are no conflicts of interest in the content of this review.

Correspondence to: Fatemeh Farshadpour, PhD, Department of Microbiology and Parasitology, School of Medicine, Bushehr University of Medical Sciences, Moallem Street, PO Box 3631, Bushehr 7514633341, Iran. f.farshadpour@yahoo.com
Telephone: +98-9171712653
Fax: +98-7714550235

Received: March 27, 2015
Peer-review started: March 28, 2015
First decision: April 24, 2015
Revised: May 20, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 14, 2015

Abstract

In Iran, the prevalence of hepatitis C virus (HCV) infection is relatively low according to the population-based epidemiological studies. However, the epidemiology of HCV is changing and the rate of HCV infection is increasing due to the growth in the number of injecting drug users in the society. In addition, a shift has occurred in the distribution pattern of HCV genotypes among HCV-infected patients in Iran. Genotype 1a is the most prevalent genotype in Iran, but in recent years, an increase in the frequency of 3a and a decrease in 1a and 1b have been reported. These variations in the epidemiology of HCV reflect differences in the routes of transmission, status of public health, lifestyles, and risk factors in different groups and geographic regions of Iran. Health policy makers should consider these differences to establish better strategies for control and prevention of HCV infection. Therefore, this review was conducted to present a clear view regarding the current epidemiology of HCV infection in Iran.

Key words: Hepatitis C virus; Blood donors; Injecting drug users; Hemodialysis; Hemophilia; Thalassemia; Genotypes; Occult hepatitis C virus; Epidemiology; Iran

Core tip: The distribution patterns of hepatitis C virus (HCV) infection are related to different status of public health and the presence of risk factors in the society. In Iran, the predominance of risk factors for transmission of HCV has changed from blood transfusion to intravenous drug use; and due to the growth in the number of injecting drug users, the prevalence of HCV infection is rising in the country. Even the recent changes in the distribution pattern of HCV genotypes confirm this issue. Overall, the epidemiology of HCV is

changing in Iran. Therefore, this review was conducted to present a clear view about current epidemiology of HCV in Iran.

Taherkhani R, Farshadpour F. Epidemiology of hepatitis C virus in Iran. *World J Gastroenterol* 2015; 21(38): 10790-10810 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10790.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10790

INTRODUCTION

Hepatitis C virus (HCV) is a small, enveloped positive-stranded RNA virus, belonging to the family *Flaviviridae* and the genus *Hepacivirus*[1,2]. Based on genomic heterogeneity, HCV has been classified into seven genotypes and over 70 different subtypes[3,4]. HCV is transmitted through exposure to infected blood and blood products. Blood transfusion, injecting drug use, sexual intercourse, surgery, and tattooing are some possible ways to spread HCV infection[5,6]. Among these, HCV transmission by sexual intercourse is less common and includes those that lead to mucosal exposure to infectious blood or blood-derived body fluids and is related to the presence of mucosal tears and genital ulcerative disease[7,8].

HCV is the major cause of chronic liver disease, and can lead to cirrhosis and hepatocellular carcinoma (HCC)[3,9]. Although the infection is preliminary acute with a wide spectrum of clinical manifestations from asymptomatic to mild or even severe clinical illness[10], about 75% to 85% of acute HCV infections slowly progress to chronic infection[11]. Approximately 10%-20% of those chronically infected are at risk of developing liver cirrhosis within 20 to 30 years, and of those with cirrhosis, 1%-5% per year will develop HCC[12].

HCV infection is defined as the presence of HCV-RNA and anti-HCV antibodies in serum or plasma. A positive HCV antibody test [enzyme linked immunosorbent assay (ELISA) and immunoblot assay] indicates exposure to HCV, however, it cannot distinguish between current or past infection. In general, anti-HCV antibody positive samples can be defined as current HCV infection if the HCV RNA test [reverse transcriptase polymerase chain reaction (RT-PCR)] is positive[8,13].

According to the World Health Organization reports, about 130-150 million of the world population have chronic HCV infection[14]. In addition, 3-4 million new cases of HCV infection emerge globally each year[15,16]. The chronic infection might result in cirrhosis, hepatic failure, or HCC, which are responsible for approximately 350000 to 500000 deaths per year[5,14,17,18]. Therefore, HCV is a life threatening global health problem, and its prevention is the main objective.

HCV has a high rate of genetic heterogeneity, therefore, no vaccine or immunoglobulin exist to prevent this infection[18]. Recent advances in HCV therapy have led to the development of new antiviral drugs for treatment of HCV infection, including the protease inhibitors telaprevir, simeprevir, boceprevir, and paritaprevir; NS5A inhibitors ledipasvir, daclatasvir, and ombitasvir; the nucleotide analog NS5B polymerase inhibitor sofosbuvir; and the non-nucleotide polymerase inhibitor dasabuvir[8,19,20]. These new therapies are well-tolerated and safer and much more effective than the previous therapies pegylated interferon (IFN)/ribavirin[20]. Despite these advantages, pegylated IFN-α in combination with ribavirin is recommended as the standard treatment for HCV infection in Iran[21-24]. The reasons for this are the high cost and restricted availability of the new medications in low- and middle-income countries[25].

Iran is a vast country with various ethnicities in different provinces. This country, with an area of about 1700000 km^2, is located in the Middle East between Arab peninsula, Indian subcontinent, Europe, and Middle Asia[26,27]. There are variations in the prevalence and epidemiology of HCV in different groups and regions throughout the country. To achieve better strategies for the prevention and management of HCV infection, the current knowledge regarding the epidemiology of HCV infection merits reviewing. Therefore, we present here a clear review about the current epidemiology of HCV in Iran.

HCV IN BLOOD DONORS

In Iran, the prevalence of HCV infection among blood donors in different studies varies considerably, depending on the study population, sample sizes, study periods, the geographic regions, risk factors, and the methods and type of kits used to determine HCV[15,28]. According to the results of a meta-analysis study, the prevalence of anti-HCV among 10739221 blood donors was 0.5% during 1996 to 2011[28]. In another study, the rate of anti-HCV seropositivity among 6499851 blood donors was 0.13% during 2004 to 2007[29]. The highest anti-HCV prevalence of 1.39% was declared in 2005, followed by a significant decreasing rate from 0.13% in 2007 to 0.03% in 2009[4,28]. The reasons for this decline were the implementation of more restrictive rules in physical examination prior to donation and the application of more sensitive HCV test kits for screening the blood by Iran Blood Transfusion Centers[27,28]. In addition, the public has become more aware of the routes of transmission of HCV infection in recent years[29].

Iran has the lowest anti-HCV prevalence among blood donors compared to corresponding figures in the Middle East countries, such as 0.6% in Lebanon, 0.8% in Kuwait, 0.9% in Oman, 2.7% in Yemen, and 5%-25% in Egypt[4,27,28,30,31]. Globally, however, the lowest HCV prevalence of 0.01%-0.1% has been reported in the

Table 1 Prevalence of hepatitis C virus among blood donors in Iran

Author	Year of study	City or province	Location	No. of participants	No. of positive samples	Prevalence	Test	Ref.
Taheri Azbarmi	2003-2005	Rasht, Gilan province	North	49820	91	0.18%	ELISA and RIBA	[36]
Mansour-Ghanaei	1998-2003	Gilan	North	221508	3603	1.62%	ELISA	[37]
					709	0.32%	RIBA	
Bani Aghil	2006-2008	Golestan	North-East	128198	161	0.12%	ELISA and immunoblot	[38]
Khedmat	2003-2005	Tehran	North-Center	1004889	21390	2.10%	ELISA	[39]
					1005	0.10%	RT-PCR	
Attarchi	2003-2004	Tehran	North-Center	26645	42	0.20%	ELISA and RIBA	[40]
Khedmat	2005-2006	Tehran	North-Center	318029	323	0.09%	ELISA, immunoblot and RT-PCR	[35]
Bozorgi	2002-2004	Qazvin	West-Center	48116	73	0.15%	ELISA and RIBA	[41]
Mahdaviani	2004	Arak	West-Center	11615	81	0.70%	ELISA	[42]
					33	0.20%	RIBA	
Bozorgi	2009	Qazvin	West-Center	20591	328	1.59%	ELISA	[43]
					35	0.17%	HCV confirmatory tests (ND)	
Afzali	1996-2001	Kashan	Center	43731	477	1.10%	ELISA	[44]
Moniri	2001-2002	Kashan	Center	600	3	0.50%	ELISA	[45]
Karimi	2004-2006	Shahr-e Kord	Central	35124	70	0.20%	ELISA and immunoblot	[46]
Masaeli	2002-2003	Isfahan	Center	29458	24	0.27%	ELISA and RIBA	[47]
Esmaieli	2006-2007	Bushehr	South	20294	42	0.20%	ELISA and immunoblot	[48]
Ghavanini	1998	Shiraz	South	7897	47	0.59%	ELISA and immunoblot	[49]
Emamghorashi	2001-2003	Jahrom	South	3000	9	0.30%	ELISA and immunoblot	[50]
Kasraian	2002-2005	Shiraz	South	507531	710	0.14%	ELISA	[51]
Kasraian	2007-2008	Shiraz	South	93987	203	0.21%	ELISA and RIBA	[52]
Delavari	2003	Kerman	South-East	15252	60	0.39%	ELISA	[53]
Tajbakhsh	2004	Shahr-e kord	West	11472	69	0.60%	ELISA	[54]
Doosti	2003-2004	Shahrekord	West	11200	76	0.67%	ELISA	[55]
						0.59%	immunoblot	
						0.41%	RT-PCR	
Ghafouri	2006-2009	South Khorasan	East	42652	31	0.07%	ELISA	[56]
					13	0.03%	RIBA	

ND: Not defined; ELISA: Enzyme linked immunosorbent assay; RT-PCR: Reverse transcriptase polymerase chain reaction; RIBA: Recombinant immunoblot assay.

United Kingdom and Scandinavia[5,18,32-34].

At present, the ELISA and confirmatory recombinant immunoblot assay (RIBA) are used routinely for screening of the blood donors by the Iranian blood bank transfusion centers. It seems screening of blood is an important factor in controlling and reducing the rate of HCV infection in the general population. However, the presence of asymptomatic or occult HCV infected donors with no detectable HCV Ab or low copy number of HCV genomes in their blood is a potential source of HCV transmission. Thus, the risk of HCV transmission through blood transfusion is considered an important public health concern[28,35] (Table 1[35-56]).

HCV IN GENERAL POPULATION

With an overall anti-HCV prevalence of less than 1% in the general population, Iran is considered a country with low frequency HCV infection[27]. However, it seems the prevalence of HCV is slightly rising in the country[57,58]. The prevalence of HCV infection in the general population varies considerably in different regions of Iran (Table 2[58-68]). These variations in the prevalence of HCV might be due to the differences in the quality of public health services, lifestyles, habits, and rates of high-risk behaviors in different geographic regions[15,28].

In Iran, the prevalence of HCV infection in the general population is lower than those of the neighboring countries such as Afghanistan (1.1%), Turkey (1%-2.1%), Pakistan (4.7%), Iraq (7.1%), and Qatar (6.3%)[4,30]. Globally, the highest HCV prevalence of 17.5% (13%-22%) has been reported in Egypt[59].

The general population-based prevalence of HCV infection is used to describe and compare the local and global epidemiology of HCV infection[10,16]. The surveys on prevalence of HCV in the blood donor population fail to assess the true prevalence in an entire community. Since a large number of HCV positive cases are excluded from donating blood, the donor population is representative of a population at low risk of HCV infection. A recent study reported a HCV prevalence of 9.2% in the excluded individuals[27]. Therefore, the prevalence of HCV in the general population is higher than that in the donor population[27,28].

HCV IN HIGH-RISK GROUPS

HCV in intravenous drug users

Presently, injecting drug use is the main route of HCV

Table 2 Prevalence of hepatitis C virus among general population in Iran

Author	Year of study	City or province	Location	No. of participants	No. of positive samples	Prevalence	Test	Ref.
Zamani	2008-2011	Amol, Mazandaran	North	6145	12	0.20%	ELISA	[60]
					5	0.08%	RIBA	
					3	0.05%	RT-PCR	
Mansour-Ghanaei	2003	Gilan	North	383	9	2.30%	ELISA	[61]
					5	1.30%	RT-PCR	
Shakeri	2010-2011	Mashhad	North-East	3870	8	0.20%	ELISA	[62]
					5	0.13%	RT-PCR	
Ghadir	2006	Golestan	North-East	2123	56	2.60%	ELISA	[63]
					22	1.00%	RIBA	
Merat	2006	Golestan	North-East	1895	18	1.00%	ELISA and RIBA	[58]
Merat	2006	Tehran	North-Center	2326	8	0.30%	ELISA and RIBA	[58]
Merat	2006	Hormozgan	South	1463	24	1.60%	ELISA and RIBA	[58]
Motlagh	2001	Ahvaz	South-West	80	5	6.25%	ELISA	[64]
					0	0.00%	Immunoblot	
Nikbakht	2007-2008	Ahvaz	South-West	712	9	0.63%	ELISA	[65]
Moradi	2001-2002	Saravan, Sistan and Baluchestan	South-East	365	3	0.80%	ELISA	[66]
Sayad	2006	Kermanshah	West	1721	15	0.87%	ELISA, immunoblot and RT-PCR	[67]
Mohebbi	2007-2008	Lorestan	West	827	2	0.20%	ELISA	[68]

ELISA: Enzyme linked immunosorbent assay; RT-PCR: Reverse transcriptase polymerase chain reaction; RIBA: Recombinant immunoblot assay.

Table 3 Prevalence of hepatitis C virus among injecting drug users in Iran

Author	Year of study	City or province	location	No. of participants	No. of positive samples	Prevalence	Test	Ref.
Mohtasham Amiri	2003	Gilan	North	81	72	88.9%	ELISA	[72]
Rahimi-Movaghar	2006-2007	Tehran	North-Center	895	309	34.5%	ELISA	[73]
Hosseini	2006	Tehran	North-Center	417	334	80.0%	ELISA	[74]
Zali	1995	Tehran	North-Center	402 (Male imprisoned IDUs)	182	45.3%	ELISA, RIBA	[75]
Zamani	2004	Tehran	North-Center	202	105	52.0%	Particle Agglutination (PA) assay	[76]
Hajinasrollah	2005	Tehran	North-Center	65	11	17.0%	ELISA	[77]
Amin-Esmaeili	2006-2007	Tehran	North-Center	895	309	34.5%	ELISA	[78]
Nokhodian	2008-2009	Isfahan	Center	531	250	47.1%	ELISA	[79]
Zamani	2008	Isfahan	Center	117	71	60.7%	EIA	[80]
Kassaian	2009	Isfahan	Center	943	392	41.6%	ELISA	[81]
Fadaei Nobari	2011	Isfahan	Center	1747	595	34.0%	ELISA	[82]
Sofian	2009	Arak, Markazi	West-Center	153 (Male IDUs)	91	59.5%	ELISA	[83]
Ramezani	2012	Arak	West-Center	100 (Male IDUs)	56	56.0%	ELISA	[84]
Honarvar	2012-2013	Shiraz	South	569 (High risk groups)	109	19.1%	ELISA and immunoblot	[70]
				233 (IDUs)	94	40.3%		
				336 (non-IDUs)	15	4.4%		
Davoodian	2002	Bandar Abbas, Hormozgan	South	249	163	64.8%	ELISA	[85]
Sarkari	2009-2010	Kohgiloyeh and Boyerahmad	South-West	158	67	42.4%	ELISA	[86]
Imani	2004	Shahr-e Kord	Sout-West	133	15	11.3%	ELISA	[87]
Alavi	2002-2006	Ahvaz	South-West	333	103	30.9%	ND	[88]
Mohammad Alizadeh	2002	Hamadan	West	149 (IDUs Prisoners)	47	31.5%	ELISA, immunoblot	[89]
Keramat	2005-2007	Hamadan	West	379 (High risk groups)	135	35.6%	ELISA, immunoblot	[90]
				199 (IDUs)	126	63.3%		

IDUs: Injecting drug users; ND: Not defined; ELISA: Enzyme linked immunosorbent assay; RIBA: Recombinant immunoblot assay.

transmission[6,9,69]. Iran has one of the highest numbers of drug addicts in the world[9,70]. It has been reported that 2.8% of Iranian adults aged 15-64 years are drug abusers and about 180000 (12.2%) of this population are injecting drug users (IDUs)[9]. Estimates from Iran show a HCV prevalence of 50%-75% among IDUs[6].

Table 4 Prevalence of hepatitis C virus among hemodialysis in Iran

Author	Year of study	City or province	Location	No. of participants	No. of positive samples	Prevalence	Test	Ref.
Makhlough	2006	Mazandaran	North	186	39	21.0%	ELISA	[99]
					21	11.3%	RT-PCR	
Amiri	2001	Gilan	North	298	80	26.8%	ELISA	[100]
					74	24.8%	Immunoblot	
Joukar	2008	Gilan	North	514	61	11.9%	ELISA	[101]
					32	6.2%	RT-PCR	
Samimi-rad	2005	Markazi	West-Center	204	11	5.4%	ELISA, RIBA and RT-PCR	[102]
Bozorghi	2006	Qazvin	West-Center	89	9	10.3%	ELISA	[103]
					6	6.4%	RIBA	
Somi	2012	Tabriz	North-West	455	37	8.1%	ELISA	[104]
Zahedi	2010	Kerman	South-East	228	16	7.0%	ELISA	[105]
					7	3.0%	PCR	
Kalantari	2010-2011	Isfahan	Center	499	26	5.2%	ELISA	[106]
Zamani	1998–2005	Amol, Tonekabon, Rasht and Ramsar Mazandaran and Gilan provinces	North	334	67	20.0%	ELISA, RT-PCR	[107]
Assarehzadegan	2005-2006	Khuzestan	South-West	214	34	7.9%	ELISA, RT-PCR	[108]
Nemati	1990-2006	Tehran	Center	112	6	5.3%	ELISA, RT-PCR	[109]
Sotoudehjahromi	2006	Jahrom	South	34	3	8.8%	ELISA	[110]
					2	5.9%	RIBA	
Alavian	2003	Tehran	North-Center	838	176	21.0%	ELISA	[111]
					111	13.2%	RIBA	
Broumand	2002	Tehran	North-Center	548	105	19.6%	ELISA	[112]
					51	9.33%	RT-PCR	
Nasiri-Toosi	2007	Tehran	North-Center	130	11	8.5%	ND	[113]
Mohammad-Alizadeh	2002	Hamedan	West	96	9	11.4%	ELISA	[114]
Saboor	1999-2000	Kermanshah	West	140	37	26.4%	ELISA	[115]
Jabbari	2008	Golestan	North-East	93	23	24.7%	ELISA, RIBA	[116]
Ansari	2005-2006	Urmia	North-West	50	19	38.0%	EIA	[117]
					12	24.0%	RT-PCR	
Hassanshahi	2006-2007	Kerman	South-East	203	64	31.5%	ELISA, RT-PCR	[118]
Ansar	1997-1998	Gilan	North	93	52	55.9%	ELISA	[119]

ND: Not defined; ELISA: Enzyme linked immunosorbent assay; RT-PCR: Reverse transcriptase polymerase chain reaction; RIBA: Recombinant immunoblot assay.

However, the prevalence of anti-HCV among IDUs varies considerably in different regions of Iran (Table 3[70-90]). The outcomes revealed that Gilan, Hamedan, Tehran, and Hormozgan provinces have the highest rate of HCV infection, while Shahre Kord had the lowest rate of infection (Table 3). As a result, IDUs are the main source of HCV infection in Iran and account for the large proportion of current HCV transmission in the society[6,9,27]. In addition, the prevalence of HCV infection in prisons of Iran is extremely high, where 38% to 90% of imprisoned IDUs have been infected with HCV[9]. Interestingly, tattooing more effectively transmits HCV infection than injecting drug use among Iranian prisoners[6].

The global prevalence of HCV infection among IDUs varies considerably from 9.8% to 97.4%[71]. Approximately 10 million IDUs with a global midpoint prevalence of 67% are positive for anti-HCV. The highest rate of HCV infection among the IDUs has been reported in China (67%, 1.6 million), the United States (73.4%, 1.5 million), and Russia (72.5%, 1.3 million)[71].

HCV in hemodialysis patients

Distribution of HCV infection among hemodialysis patients has a vast geographic variation in different regions of Iran (Table 4[91-119]). According to a recent meta-analysis study in Iran, prevalence of HCV infection among this group of patients was reported to be 13.6%, 12.2%, and 7.6% by ELISA, RIBA, and PCR, respectively, which is lower than those of Saudi Arabia (50.5%), Kuwait (43.4%), Jordan (32.5%), and Pakistan (23.7%)[91-94] but higher than those of Australia (2.3%), United Kingdom (2.7%), Germany (3.9%), and Bahrain (7.4%)[95-97]. The risk of HCV infection is extremely high among hemodialysis patients[11]. Recent surveys show that the prevalence of HCV infection among hemodialysis patients is not related to history of blood transfusion. Considering the fact that the length of time on dialysis is significantly associated with HCV seropositivity, the nosocomial transmission is the main route of HCV transmission

Table 5 Prevalence of hepatitis C virus among hemophilia patients in Iran

Author	Year of study	City or province	Location	No. of participants	No. of positive samples	Prevalence	Test	Ref.
Mansour-Ghanaei	1999	Gilan	North	101	72	71.30%	RIBA	[120]
Torabi	2004	East Azarbaijan	North-West	130	72	56.00%	ELISA, RIBA	[121]
Valizadeh	2010	West Azarbaijan	North-West	35	3	8.57%	ELISA, RIBA and RT-PCR	[122]
Mousavian	2003-2005	Tehran	North-Center	1095	802	72.30%	ELISA and RT-PCR	[123]
Kalantari	2008-2010	Isfahan	Center	615	495	80.50%	ELISA	[124]
					347	56.40%	RT-PCR	
Mobini	2006	Yazd	Center	77	41	53.20%	ELISA	[125]
					38	49.40%	RT-PCR	
Yazdani	1996-2010	Isfahan	Center	350	231	66.00%	ELISA	[126]
Javadzadeh Shahshahani	2003	Yazd	Center	74	36	48.60%	ELISA and RIBA	[127]
Samimi-Rad	2004	Markazi	West-Center	76	34	44.70%	ELISA	[128]
					33	43.40%	RIBA	
					23	30.26%	RT-PCR	
Mahdaviani	2004	Markazi	West-Center	68	26	38.20%	ELISA	[129]
					25	36.70%	RIBA	
Karimi	1999-2000	Shiraz	South	281	44	15.65%	ELISA and immunoblot	[130]
Assarehzadegan	2008-2009	Ahvaz	South-West	87	47	54.00%	ELISA	[131]
					42	48.30%	RT-PCR	
Zahedi	2002	Kerman	South-East	97	43	44.30%	ELISA and RIBA	[132]
Sharifi-Mood	2003-2006	Zahedan, Sistan and Baluchistan	South-East	81	24	29.60%	ELISA and immunoblot	[133]
Esfahani	2012	Hamadan	West	89	44	49.40%	ELISA	[134]
					15	16.70%	RT-PCR	

ELISA: Enzyme linked immunosorbent assay; RT-PCR: Reverse transcriptase polymerase chain reaction; RIBA: Recombinant immunoblot assay.

among Iranian hemodialysis patients[11,98].

HCV in hemophilia patients

Hemophilia patients may acquire HCV infection *via* contaminated blood products[98]. In Iran, the prevalence of HCV among hemophilia patients is very high, with an overall prevalence of 40.8%[98] and has a wide geographic variation (Table 5[120-134]). Most of the HCV infections among hemophilia patients are asymptomatic and may lead to liver failure. Therefore, routine screening for HCV infection in hemophilia patients is required to prevent the serious consequences of HCV infection[27].

HCV in thalassemia patients

HCV is a major cause of mortality in thalassemia patients due to post-transfusion HCV infection, which dramatically progresses to liver failure or even HCC[27,135]. Therefore, HCV infection is currently considered the main health problem in thalassemia patients, and much more attention to HCV screening in the blood transfusion process may improve survival of thalassemia patients[136]. Even though the current policies of blood banks have considerably decreased the incidence of HCV infection in thalassemia patients, blood transfusion remains the main risk factor for HCV infection among this group of patients because of transfusion of HCV-infected seronegative blood donated during the window period[27,136,137]. Therefore, the rate of HCV infection is high among thalassemia patients[137].

The geographical distribution of HCV infection among thalassemia patients varies widely in different regions of Iran (Table 6[86,118,119,127-129,137-151]), but a recent meta-analysis study reported the overall HCV prevalence is 18% among thalassemia patients in Iran[136]. Iran has the lowest rate of HCV infection among thalassemia patients in comparison with Eastern Mediterranean countries[136]. High prevalence of HCV infection has been reported among thalassemia in Egypt (69%), Saudi Arabia (63%), and Pakistan (45%)[136].

HCV in health care workers

Health care workers are at the risk of acquiring HCV infection due to occupational exposures to blood and blood-derived body fluids[152]. There are few reports on the prevalence of HCV infection among health care workers in Iran. In Shoaei *et al*[153], HCV infection status was negative in 203 health care workers in Isfahan city in 2012. Similarly, all 191 health care workers were tested negative for HCV antibodies in Shahrud province in 2010[154]. Hadadi *et al*[155] reported a HCV prevalence of 6.6% (31/467) among health care workers in Tehran in 2004-2005, and Sarkari *et al*[86] reported a HCV seroprevalence of 4.2% among 212 health care workers in Kohgiloyeh and

Table 6 Prevalence of hepatitis C virus among thalassemia patients in Iran

Author	Year of study	City or province	Location	No. of participants	No. of positive samples	Prevalence	Test	Ref.
Mirmomen	2002	Tehran	North-Center	410	80	19.6%	ELISA, RIBA	[138]
		Kerman	South-East	100	18	18.8%		
		Qazvin	West-Center	95	23	25.3%		
		Semnan	East-Center	81	19	24.4%		
		Zanjan	West	46	1	2.2%		
		Total		732	141	19.6%		
Ansar	1997-1998	Rasht	North	105	67	63.8%	ELISA	[119]
Ghane	2010-2011	Gilan and Mazandaran	North	245	46	18.8%	ELISA	[139]
					28	11.4%	Nested-PCR	
Tamaddoni	2005	Babol	North	113	12	10.6%	ELISA	[140]
Mansouritorghabeh	2007	Mashhad	North-East	360	30	8.33%	ELISA	[137]
Alavi	ND	Tehran	North-Center	90 (pediatric patients)	12	13.3%	ELISA, RT-PCR	[141]
Alavian	2002	Qazvin	West-Center	96	23	24.2%	ELISA, RIBA	[142]
Samimi-Rad	2004	Markazi	West-Center	98	7	7.1%	ELISA	[128]
					5	5.1%	RIBA	
					2	2.04%	RT-PCR	
Bozorgi	2005	Qazvin	West-Central	207	54	26.1%	ELISA	[143]
					50	24.01%	RIBA	
Azarkeivan	1996-2009	Tehran	North-Center	395	109	27.5%	EIA, RIBA	[144]
Mahdaviani	2004	Markazi	West-Center	97	9	9.2%	ELISA	[129]
					7	7.2%	RIBA	
Nakhaie	1999-2000	Tehran	North-Center	507	122	24.0%	ELISA	[145]
					41	8.1%	RT-PCR	
Kalantari	2008-2010	Isfahan	Center	545	50	9.1%	ELISA	[124]
					31	5.6%	RT-PCR	
Ataei	1996-2011	Isfahan	Center	466	37	8.0%	ND	[146]
Javadzadeh Shahshahani	2003	Yazd	Center	85	8	9.4%	ELISA, RIBA	[127]
Karimi	1999-2000	Shiraz	South	466 (pediatric patients)	73	15.7%	ELISA and immunoblot	[147]
Kashef	2006	Shiraz	South	131	24	18.3%	ELISA and immunoblot	[148]
					7	5.3%	RT-PCR	
Kadivar	1999	Shiraz	South	147	40	27.2%	ELISA	[149]
Shahraki	2005-2007	Zahedan	South-East	560 (pediatric patients)	30	5.3%	ELISA	[150]
					20	3.5%	PCR	
Hassanshahi	2006-2007	Kerman	South-East	181	81	44.7%	ELISA, RT-PCR	[118]
Ghafourian	2005-2006	Ahvaz	South-West	206	58	28.2%	ELISA	[151]
Boroujerdnia					46	22.3%	RT-PCR	
Sarkari	2009-2010	Kohgiloyeh and Boyerahmad	South-West	49	3	6.1%	ELISA	[86]

ND: Not defined; ELISA: Enzyme linked immunosorbent assay; RT-PCR: Reverse transcriptase polymerase chain reaction; RIBA: Recombinant immunoblot assay.

Boyerahmad province in 2009-2010.

The global prevalence of HCV infection among health care workers is 1%-6%[156]. After HBV, HCV is the most common blood-borne infection found among health care workers. Needle-stick or sharp injuries and mucosal exposure following blood splash are the most common risk factors for HCV infection among health care workers[152,153]. Therefore, prevention strategies and training programs are needed for health care workers to reduce the incidence of HCV infection in this group.

HCV in homeless people

Homeless people are one of the main high-risk groups for acquiring HCV infection because of high-risk behaviors, lifestyle, low levels of education, poverty, and poor hygiene[157,158]. There are over 100 million homeless people worldwide, and the prevalence of HCV infection among this group varies from 3.9% to 36.2% in different parts of the world[159]. Currently, there are no data on the number of homeless people in Iran, and only a few studies are available on the prevalence of HCV infection among homeless people in Tehran, the capital of Iran. Amiri *et al*[157] reported a HCV prevalence of 23.3% among 593 homeless individuals in Tehran in 2012. In another study by Vahdani *et al*[158], the prevalence of HCV infection was found to be 42.8% among 202 homeless men in Tehran city in 2007. According to the available data in Iran, the prevalence of HCV infection is considerably high among

Table 7 Prevalence of hepatitis C virus among HIV-positive patients in Iran

Author	Year of study	City or province	Location	No. of participants	No. of positive samples	Prevalence	Test	Ref.
Babamahmoodi	2008-2010	Mazandaran	North	80	27	33.8%	ELISA	[173]
Ramezani	1999-2004	Tehran	North-Center	95	65	68.0%	ELISA	[167]
SeyedAlinaghi	2004- 2005	Tehran	North-Center	201	135	67.2%	ELISA	[170]
Ataei	1998-2007	Isfahan	Center	130	100	77.0%	ELISA and RIBA	[168]
Davarpanah	2006-2007	Shiraz	South	226	200	88.5%	ELISA	[166]
					196	86.7%	RIBA	
					59	26.1%	RT-PCR	
Khosravi		Fars	South	101	87	86.1%	ELISA	[172]
Alipour	2011	Shiraz	South	1444	1132	78.4%	ELISA	[169]
Davoodian	2002	Bandar Abbas and Roodan	South	38	35	94.0%	ELISA	[85]
Zahedi	2011	Kerman	South-East	165	122	73.9%	ELISA	[165]
Sharifi-Mood	2000-2005	Zahedan	South-East	52	6	11.5%	ND	[162]
Alavi	2001-2003	Ahvaz	South-West	104	77	74.04%	ELISA	[171]
Saleh	2013	Khorramabad, Lorestan	West	103	23	22.3%	ELISA	[174]
Mohammadi	2007-2008	Lorestan	West	391	282	72.0%	ELISA	[163]

ND: Not defined; ELISA: Enzyme linked immunosorbent assay; RT-PCR: Reverse transcriptase polymerase chain reaction; RIBA: Recombinant immunoblot assay.

the older homeless population and homeless IDUs, especially those with a history of imprisonment[157,158]. The seroprevalence of HCV was reported to be 3.5% among the street children in Tehran city in 2008[160], while it was 1.0% in Isfahan city in 2005-2007[161].

The prevalence of HCV infection among homeless populations is higher than the other blood-borne infections, therefore, HCV infection is the main health problem among homeless population of Iran and implementation of HCV-controlling and educational programs are required to reduce HCV infection among this population[157,158,161].

HCV in human immunodeficiency virus-positive patients

Prevalence of HCV coinfection among human immunodeficiency virus (HIV) positive patients ranges from 11.5% to 94.0% in different regions of Iran[85,162] (Table 7[85,162-174]). This geographic variation in HCV/HIV coinfection reflects diversity of the risk factors, the types of exposure, and the epidemiology of these viruses in different regions of the country[163,164]. However, in all of these studies, intravenous drug use and a history of imprisonment were the most prevalent risk factors for HCV/HIV co-infection in Iran[164-169].

The prevalence of HCV coinfection is noticeably high among HIV-positive patients in Iran. The shared modes of transmission and the lack of an effective vaccine for HCV could explain this high prevalence[163-165,169,170]. In Iran, HCV and HIV are predominantly transmitted by injecting drug use[165,170,171]. Moreover, the rate of IDUs is increasing in Iran, which may boost the rate of HCV/HIV coinfection in the country[163].

HCV coinfection adversely affects HIV disease outcomes and leads to severe liver disorders, progression to cirrhosis and HCC, and subsequently lower survival of HIV infected patients[163-165]. HIV infection leads to higher rates of HCV persistence, increased risk of hepatotoxicity due to the extensive use of anti-retroviral drugs, and subsequently accelerated end stage liver disease[164,169-171]. Overall, one third of mortalities in HIV infected patients are related to liver diseases[163,164,170]. Therefore, HCV coinfection is considered a potential threat to HIV positive patients, and routine screening for HCV infection, as well as HCV treatment, seem to be necessary in all HIV-positive patients[164,165,169,172].

HCV IN IMMUNOLOGICAL DISORDERS

HCV in patients with mixed cryoglobulinemia

Mixed cryoglobulinemia is the most common immunological disorder reported in patients with chronic HCV infection[175-177]. The prevalence of HCV infection in patients with mixed cryoglobulinemia ranges from 40% to 90% worldwide[178]. Several studies have reported HCV infection as the etiological agent of mixed cryoglobulinemia[176,179,180]. Gharagozloo *et al*[181] reported an anti-HCV prevalence of 69% among patients with mixed cryoglobulinemia in Iran. In Owlia *et al*[182], 16% of patients (8/50) with HCV infection had cryoglobulins in central regions of Iran. However, this rate was relatively low in comparison with the high incidence of mixed cryoglobulinemia (19%-> 50%) among patients with chronic HCV infection.

HCV in patients with diabetes mellitus

Diabetes mellitus is one of the most prevalent metabolic disorders, and it affects 4.6%-10.0% of the Iranian population[183]. In 1994, a possible association between HCV infection and diabetes mellitus was first introduced[184]. Since then, many studies have demonstrated that HCV infection has a role in the activation of host innate immune responses and, *via* the TNF-α pathway, induces the destruction of insulin

signaling pathways and subsequently the development of insulin resistance[185]. In addition, immune-mediated pathogenesis or direct cytotoxic effects of HCV on pancreatic islet cells results in dysfunction of β cells and declines the insulin production[183,185-188]. Although HCV infects the pancreas, autoimmunity is not involved in the occurrence of diabetes[186].

Several studies have shown that the prevalence of HCV among diabetic patients is significantly higher than that in non-diabetic patients[187,189,190]. Interestingly, male gender, age over 40 years, and abnormal liver enzymes are associated with high prevalence of HCV infection among patients with diabetes mellitus[191]. Although there are several reports on the prevalence of HCV infection among patients with diabetes mellitus in Iran, the results show great heterogeneity. Aghamohammadzadeh *et al*[192] reported HCV seropositivity in 2.5% (10/400) of Iranian patients with diabetes mellitus in Tabriz. In addition, Alavian *et al*[193] showed an increased risk of diabetes mellitus among Iranian patients with chronic HCV infection in Tehran. While, Janbakhsh *et al*[194] reported no association between HCV infection and the occurrence of diabetes in Kermanshah. Metanat *et al*[195] found no association between HCV and diabetes in Zahedan, and Bahar *et al*[196] reported similar findings in Tehran.

According to the epidemiological data, patients with chronic HCV infection are at an increased risk for developing diabetes[191,197,198]. HCV infection is a risk factor for occurrence of diabetes, and diabetes will enhance the risk of liver fibrosis, cirrhosis, and finally progression to HCC[187]. Therefore, screening of all HCV positive patients for diabetes mellitus is recommended to reduce the adverse effects associated with diabetes on HCV infection, which may progress to liver fibrosis, cirrhosis, or even HCC.

The incidence of diabetes mellitus among HCV positive patients ranges from 23% to 62% in different parts of the world[183]. This incidence is 18.3% among Iranian HCV-infected patients, which is higher than that in the general population of Iran[183]. Compared to other parts of the world, the prevalence of diabetes mellitus among Iranian patients with HCV infection is low. Overall, there are no adequate studies in this field in Iran. Therefore, more surveys are recommended to clearly identify the frequency of diabetes mellitus among HCV-infected patients in Iran.

HCV in patients with autoimmune thyroid disorders

Autoimmune thyroid disorders (ATD), including Hashimoto's thyroiditis and Graves' disease, are the most prevalent endocrine problems worldwide[199,200]. Many investigators have investigated the possible association between chronic HCV infection and autoimmune thyroiditis. However, the exact role of HCV infection in the development of autoimmune thyroiditis remains unclear[201]. Investigations have suggested several mechanisms, including the following: (1) Non-autoimmune-mediated pathogenesis through direct cytopathic effect of HCV on thyrocytes, which results in destruction of thyroid follicular cells[201]; (2) Autoimmune-mediated pathogenesis due to the presence of homologous amino acid sequences between viral proteins and thyroidal proteins or molecular mimicry and over activation of autoreactive T-cells and B-cells during HCV infection, which results in production of anti-thyroid antibodies[200-202]; and (3) The adverse effects of IFN-therapy on thyroid gland through immune stimulatory and direct effects of IFN on the thyrocytes, which ultimately results in destructive thyroiditis[199,201,203]. Therefore, monitoring thyroid function is recommended during IFN-therapy in patients with HCV infection[201,204].

There are limited reports on the significance of HCV infection in patients with ATD in Iran. Ziaee *et al*[204] reported thyroid dysfunction in 10.3% of patients with chronic HCV infection in Tehran in 2002-2003, while, Rahimi *et al*[205] found no relationship between chronic HCV infection and autoimmune thyroiditis in Kermanshah in 2010. Similarly, Jadali *et al*[206,207] reported no relationship between HCV infection and Hashimoto's thyroiditis or Graves' disease in Tehran in 2005. Still, more studies are recommended to generate a clear epidemiological pattern of HCV infection among patients with thyroid disorders in Iran.

HCV in patients with lichen planus

Lichen planus (LP) is a chronic inflammatory disease of the skin and mucous membranes with unknown etiology[199,208,209]. Chronic HCV infection appears to have a role in the pathogenesis of LP through induction of host immune responses and immune dysregulation in susceptible patients[200,210,211]. This mechanism was confirmed by the presence of HCV-RNA and HCV-specific T lymphocytes in the skin and mucous membrane specimens of patients with LP[200,209]. Another possibility is the effect of IFN-therapy in the development of LP in patients with HCV infection[209]. However, HCV replicates in skin and mucous lesions of patients with LP, but no direct cytotoxic effect of HCV on skin and mucosa cells could be proposed in the development of LP[209]. The majority of patients with LP have not been infected with HCV[212]. In addition, the incidence of LP among patients with chronic HCV infection was estimated about 5% (1%-6%)[199,209]. Therefore, it seems that HCV contributes to the development of LP, with some unknown underlying factors also involved in this process[210].

According to the epidemiological data, the prevalence of HCV among LP patients varies considerably from 4% to 62% in different parts of the world, where this prevalence is higher in HCV endemic countries[209,210]. There are limited reports on the prevalence of HCV among patients with LP in Iran. Rabiei *et al*[213] reported high prevalence of oral lichen planus (OLP) in HCV-infected patients (4.7%) compared

to the general population (0.5%-2.0%) and suggested an association between HCV infection and OLP in Gilan in 2002. Similary, Khatibi *et al*[214] reported a higher prevalence of OLP in HCV-infected patients (4%) than the general population in Tehran. In contrast, Rahnama *et al*[215] reported no association between LP and HCV in Kerman in 2005. Similarly, Taghavi Zenouz *et al*[216] found no relationship between LP and HCV in Tabriz in 2009, and Ansar *et al*[208] reported a similar result in Hamedan province in 2011. Overall, Petti *et al*[212] reported a weak association between HCV and OLP in Iranian population. Further investigations are needed to clearly identify the association between HCV and LP in Iran.

HCV IN MALIGNANCY

HCV in patients with B-cell non-Hodgkin's lymphoma

HCV is not only primarily hepatotropic, but it can also affect lymphatic systems and lead to B cell lympho-proliferative disorders such as non-Hodgkin's lymphoma (NHL)[217]. Few studies have evaluated the relationship between HCV seropositivity and the incidence of NHL in Iran. Aledavood *et al*[218] reported low prevalence of HCV infection among patients with NHL (0.7%) compared to the general population (0.5%-1%) and found no relationship between HCV infection and NHL in Northeast of Iran in 2014. In contrast, Rezaeian *et al*[219] reported high prevalence of HCV in patients with NHL (15.7%) compared to the control group (0%) and suggested an association between HCV infection and NHL. Similarly, Rastin *et al*[217] found a HCV prevalence of 7.4% among patients with NHL in Mashhad city. NHL is prevalent worldwide and is the eighth and 11th most common cancer in males and females, respectively[220]. Although the exact risk factor for NHL has not yet been determined, it seems that HCV infection has a role in the pathogenesis of this lymphoproliferative disorder[178].

According to the results of a meta-analysis study, the global prevalence of HCV infection in NHL patients is approximately 15%, which is higher than the prevalence of HCV in general population (1.5%), suggesting a possible role of HCV infection in the development of NHL[221]. Although the role of other factors, such as genetic and environmental factors, should also be considered in the pathogenesis of NHL malignancy[217,221].

HCV in patients with HCC

HCC is the fifth most common malignancy and the second most fatal cancer, with approximately 600000 deaths annually worldwide[222]. HBV and HCV infections account for 50% and 25% of global HCC cases, respectively. However, HCV infection is the most predominant cause of HCC in Japan and the United States[222]. Iran is considered a low endemic area for HCC, with less than five cases per 100000 persons annually[26,223]. Kerman province, located in Southeast of Iran, has a higher incidence of HCC compared to other provinces. This may be due to higher frequency of HBV and HCV infections in this part of the country[224].

In Hajiani *et al*[225]'s study, the seroprevalence of HBV and HCV infections among patients with HCC in southern Iran were 52.1% and 8.5%, respectively. They pointed out that the prevalence of HCV infection among HCC patients may be underestimated due to the potential contribution of occult HCV infection in the development of HCC. Therefore, the prevalence of occult HCV infection among patients with HCC should be investigated in future surveys. Ansari *et al*[135] found a very low incidence of HCC (0.6%) among thalassemia patients with HCV infection due to the anti-HCV treatment in this group of patients. In Iran, HCV is the second most common cause of HCC after HBV infection[26,223]. However, it is predicted that chronic HCV infection will replace HBV infection as the main cause of HCC in the future[26].

DISTRIBUTION OF HCV GENOTYPES IN IRAN

HCV genotypes differ in their nucleotide sequence and biological properties, such as pathogenicity, infectivity, antigenicity, response to antiviral therapy, mode of transmission, as well as geographical distribution and age-distribution[226,227]. Distribution of HCV genotypes is variable in different regions of Iran (Table 8[101,102,128,131,228-251]). Subtypes 1a is more prevalent in southern and northern Iran, 3a is more prevalent in northern and central Iran, 1b is more prevalent in southern and western Iran, and genotype 2 is more prevalent in western regions of Iran[4,226,228]. Overall, the most frequent genotype in Iran is 1a, followed by 3a and 1b[4].

Distribution of HCV genotypes in Iran is different from other Middle Eastern countries with predominant genotype 4, but it is similar to the pattern seen in North America, with predominant genotypes 1, 2, and 3[4]. Genotype 2 is generally uncommon in Iran, therefore, the genotypic pattern differs from the United States, Europe, and Asia but is similar to Pakistan and India, where genotype 2 is very rare[226,229]. Genotype 4 is uncommon in Iran and only seen in special patient groups[226]. A similar pattern regarding genotype 4 is seen in Europe, the United States, and India. However, due to changes in immigration patterns, the prevalence of genotype 4 is increasing in western countries in recent years (Table 9)[4,229,230]. Overall, the worldwide distribution of HCV genotypes shows that the genotypes 1, 2, and 3 have a global prevalence, while genotypes 4, 5, and 6 have a restricted prevalence[4,226,229,231].

Different HCV genotypes may be associated with particular patient groups. Therefore, the genotypic patterns can be used to trace the routes of transmission[4]. Genotype 1 is more prevalent among thalassemia, hemophilia, hemodialysis, and solid organ

Table 8 Distribution of hepatitis C virus genotypes among hepatitis C virus -infected patients in Iran *n* (%)

Study group	City or province	Location	Year of study	Sample size	Genotype 1	Genotype 2	Genotype 3	Genotypes 4 and 5	Mixed genotype	Non typable	Method	Author	Ref.
Blood donors	Ahvaz	South-West	2007-2008	45	1a: 24 (53.3)		3a: 21 (46.7)				RFLP	Farshadpour	[233]
Blood donors	Tehran	North-Center	2006-2008	103	1a: 53 (51.5) 1b: 4 (3.9)		3a: 39 (37.9)			7 (6.8)	Type-specific primers	Sharifi	[234]
General population	Iran	Iran	2000-2005	116	1a: 71 (61.2) 1b: 16 (13.8)		3a: 29 (25.0)				RFLP	Amini	[235]
General population	Iran	Iran	2004-2007	206	1a: 53 (25.73) 1b: 36 (17.47)	2: 4 (1.95)	3a: 96 (46.60)		11 (5.34)	6 (2.91)	PCR kit	Hajia	[236]
General population	Isfahan	Center	2007-2009	97	1a: 29 (29.5) 1b: 5 (5.1)	2: 2 (2.0)	3a: 59 (61.2)		2 (2.0)		PCR based genotyping kit	Zarkesh-Esfahani	[237]
General population	Zanjan	West	2007-2013	ND	1a: 22.05% 1b: 25.73%	2: 5.14%	3a: 38.26%	4: 4.41%		4.41%	LiPA	Esmaeilzadeh	[238]
General population	Yazd	Center	2010-2013	191	1a: 74 (38.7) 1b: 13 (6.8)	2: 3 (1.6)	3: 96 (50.3)		5 (2.6)		PCR based genotyping kit	Hadinedoushan	[239]
General population	Mashhad	North-East	2009-2010	382	1a: 147 (39.2) 1b: 41(10.9)	2a: 9 (2.4)	3a: 150 (40.0)	5: 13(3.4)			Genotype specific primers	Vossughinia	[240]
General population	Tehran	North-Center	2007	2231	1a: 886 (39.7) 1b: 271 (12.1)		3a: 613 (27.5)		33 (1.6)	401 (18.0)	Genotype specific primers	Keyvani	[241]
General population	Golestan	North	2010	77	1a: 15 (19.5) 1b: 15(19.5)	2a: 2 (2.6)	3a: 12 (15.6) 3b: 19 (24.7)	4: 6 (7.8)	8 (6.5)		Genotype specific primers	Moradi	[242]
General population	Ahvaz	South-West	2009	80	1a: 43 (53.8)		3a: 37 (46.2)				RFLP	Hamidi-Fard	[243]
Thalassemia	Mazandaran	North	2009-2011	34	1a: 13 (38.24) 1b: 1 (2.94)		3a: 15 (44.12)		4 (11.76)	1 (2.94)	Type-specific primer	Rafiei	[244]
Thalassemia	Mazandaran and Guilan	North	2010	28	1a: 9 (32.1) 1b: 1 (3.6)		3a: 18 (64.3)				RFLP	Ghane	[245]
Thalassemia	Fars	South	2009-2012	38	1: 17 (44.7)		3: 6 (15.8)			15 (39.5)	Real-time PCR	Jamalidoust	[231]
Haemophilia	Mazandaran	North	2009-2011	33	1a: 7 (21.21)		3a: 25 (75.76)			1 (3.03)	Type-specific primer	Rafiei	[244]
Haemophilia	Fars	South	2009-2012	8	1: 5 (62.5)		3: 1 (12.5)			2 (25.0)	Real-time PCR	Jamalidoust	[231]
Haemophilia	Ahvaz	South-West	2008-2009	42	1a: 26 (61.9) 1b: 11 (26.1)		3a: 5 (11.9)				Genotype specific primers	Assarehzadegan	[131]
Haemophilia	Markazi	West-Center	2004	22	1: 6 (27.3) 1a: 3 (13.6) 1b: 2 (9.1)	2: 1 (4.54)	3a: 4 (18.2)		6 (27.3)		LiPA	Samimi-Rad	[128]
IDUs	Mazandaran	North	2009-2011	37	1a: 11 (29.73) 1b: 10 (27.03)		3a: 5 (13.51)		11 (29.73)		Type-specific primer	Rafiei	[244]
IDUs	Tehran	North-Center	2008-2009	36	1a: 9 (25) 1b: 6 (16.7)		3a: 21 (58.3)				Type-specific primers	Ranjbar Kermani	[246]
IDUs	Fars	South	2009-2012	550	1: 283 (51.5)		3: 192 (34.9)		8 (12.2)	67 (12.2)	Real-time PCR	Jamalidoust	[231]
IDUs	Tehran	North-Center	2008-2009	83	1a: 35 (42)		3a: 48 (58.0)				Sequencing	Samimi-Rad	[247]
Haemodialysis	Mazandaran	North	2009-2011	31	1a: 6 (19.36)		3a: 24 (77.42)		1 (3.22)		Type-specific primer	Rafiei	[244]
Haemodialysis	Markazi	West-Center	2005	8	1a: 4 (50) 1b: 1 (12.5)		3a: 1 (12.5)	4: 2 (25)			LiPA	Samimi-Rad	[102]
Haemodialysis	Fars	South	2009-2012	6	1: 4 (66.7)			4: 1(16.7)		1 (16.7)	Real-time PCR	Jamalidoust	[231]

Haemodialysis	East Azerbaijan	North-West	2006	55	1a: 42 (76.4) 1b: 3 (5.5)	3a: 3 (5.5)		1 (1.8)	4 (10.9)	Type-specific primer	somi	[248]
Haemodialysis	Gilan	North	2008	32	1a: 19 (59.4)	3a: 13 (40.6)				Genotype-specific primers	Joukar	[101]
Haemodialysis	Tehran	North-Center	2004	66	1a: 19 (28.8) 1b: 12 (18.2)	3a: 20 (30.3) 3b: 2 (3.0)	4:11(16.7)	2 (3.0)		RFLP	Hosseini-Moghaddam	[249]
HIV/HCV co-infection	Shiraz	South	2004-2005	50	1a: 20 (40) 1b: 13 (26)	3a: 17 (34.0)				RFLP	Davarpanah	[250]
Occult HCV infected patients	Tehran	North-Center	2007-2010	7	1a: 2 (29) 1b: 3 (43)	3a: 2 (29.0)				RFLP	Bokharaei-Salim	[251]

ND: Not defined.

recipient patients[231]. Subtype 1b is prevalent in individuals with a history of hospitalization, surgery, blood transfusion, and alcohol consumption[226]. Subtype 1a is frequently found in infection by blood and blood products[228]. High frequency of genotypes 3a and 1a are seen among IDUs in Iran[228], which is similar to the genotypic pattern among IDUs in Europe and the United States[226]. Genotype 4 is found in patients undergoing hemodialysis and piercing[226,228]. This might be due to communication by dialysis during the Hajj ceremony in Saudi Arabia[4]. The mixed infection with two or more genotypes is more common in patients with hemophilia and thalassemia and may lead to chronic infection, more severe disease, re-infection, and poor response to therapy[2,4].

There has been a shift in the distribution pattern of HCV genotypes over time[4,6,232]. Genotype 1a is the most prevalent genotype in Iran, but in recent years, an increase in the frequency of 3a and a decrease in 1a and 1b have been reported among HCV-infected patients in Iran. Genotype 1 with subtypes 1a and 1b are more prevalent in older patients and genotype 3a in younger patients and IDUs[4,228,232]. Therefore, it seems that injection drug use has contributed to the majority of new HCV infections in Iran[4,232].

Distribution of HCV genotypes is variable in different groups and geographic regions of Iran. This genotypic variability reflects differences in the routes of transmission, population and socioeconomic factors, and the presence of risk factors in the society. Thus, some genotypes are more frequent in certain regions or groups of patients[4,232]. Studies on the molecular epidemiology of HCV in Iran are needed to reveal the current genotypic pattern of HCV infection in the country[228], which can predict the dose, duration, and type of treatment as well as clinical outcome of the infection[2,228-231].

OCCULT HCV INFECTION IN IRAN

Occult HCV infection is described by the absence of detectable HCV-RNA and anti-HCV antibodies in serum or plasma with elevated liver enzymes or by the presence of anti-HCV antibodies but undetectable levels of HCV-RNA in serum or plasma with normal levels of liver enzymes[252-254]. In both cases, HCV-RNA is detectable in 100% of liver biopsy, up to 70% of peripheral blood mononuclear cells (PBMCs) specimens, and in nearly 60% of ultracentrifugated serum samples of infected patients[255]. Occult HCV can persist and replicate in hepatocytes and lymphoid cells for a long time even after an apparently spontaneous eradication or therapy-induced resolution of HCV infection[256]. In this condition, low copy numbers of HCV RNA are present in serum while it cannot be detected by conventional RT-PCR assays but remains potentially infectious[253,254].

Distribution of occult HCV infection has been reported all around the world, and it seems that all genotypes are involved in this infection[253]. A few studies are available regarding the prevalence of occult HCV in Iran. Bokharaei-Salim *et al*[251] found occult HCV in 10% (7/69) of patients with cryptogenic liver disease in Iran, while 43%, 29%, and 29% of these patients had genotypes 1b, 1a and 3a, respectively. Keyvani *et al*[257] described 8.9% occult HCV infection with genotypes 3a (50%) and 1b (50%) in patients with cryptogenic cirrhosis in Iran. Farahani *et al*[258] found 1.9% occult HCV infection with genotype 1a in patients with lymphoproliferative disorders in Iran. Makvandi *et al*[259] reported 32% occult HCV infection in patients with abnormal levels of alanine aminotransferase in Ahvaz city. Rezaee Zavareh *et al*[260] reported the absence of HCV-RNA in PBMC samples of 53 patients with autoimmune hepatitis in Iran. Ramezani *et al*[261], reported the absence of occult HCV

Table 9 Global distribution of hepatitis C virus genotypes[4,229,230]

Region/country	Predominant genotype/subtype	Uncommon genotype/subtype
Latin America		
Peru	1a	2
Chile and Colombia	1b	2, 1a
Brazil	1b, 1a, 3	4, 2
Argentina	1b, 2, 1a	4
North America		
United States	1a, 1b, 2	4, 3
Canada	1a, 3, 1b	4
Central Europe		
Albania	1b, 2, 4	1a, 3
Bosnia and Herzegovina, Czech Republic and Croatia	1b, 3	4, 2, 1a
Hungary	1b, 1a	2, 4
Romania	1b	3, 4
Western Europe		
Switzerland, Belgium, Germany, Spain and France	1b, 3, 1a	5, 2, 4
Italy	1b, 2	5, 3, 4
United Kingdom and Denmark	3, 1a	2
Eastern Europe		
Russia, Latvia, Lithuania and Estonia	1b, 3	1a, 2
Central Africa	4	2
South Africa	5	2
West Africa		
Guinea-Bissau, Ghana and Burkina Faso	2	1
East Africa		
Ethiopia	4, 2	1
North Africa		
Tunisia, Morocco, Algeria	1b, 2	4
Middle East		
Saudi Arabia, Bahrain, Yemen, Kuwait, Qatar, Iraq and Egypt	4	1, 3, 2
Jordan	1a, 1b, 4	-
Iran	1a, 3a, 1b	4, 2
Turkey	1b	4, 2, 3, 1a
Asia Pacific		
Japan and Korea	1b, 2	1a
Asia, Central		
Uzbekistan, Tajikistan, Turkmenistan and Georgia	1b	1a
East Asia		
China, Taiwan	1b, 2	1a, 3, 6
South East Asia		
Laos	6	1
Philippines	1a, 2	6, 4
Thailand	3	2
Myanmar	6	2
Malaysia	3	4
South Asia		
Pakistan and India	3	1b, 2, 4
Australasia		
Australia and New Zealand	3, 1a, 1b	4, 2

infection in 30 hemodialysis patients in Tehran.

Occult HCV infection has also been found in apparently healthy populations[253,255]. The possible presence of occult HCV infection in the general population or blood donors poses a real concern about undetectable transmission of HCV[255,262]. In a recent study in Italy, the prevalence of occult HCV infection was higher than the frequency of anti-HCV seropositivity in the general population[262]. Therefore, the prevalence of HCV infection may be underestimated in the society[253,255], and the risk of HCV transmission through blood donation may be higher than predicted. Although screening of blood reduces the risk of HCV transmission by blood transfusion, transmission of occult HCV cannot be prevented in this way[253,255].

Currently, the prevalence of occult HCV infection in the general population of Iran and even blood donors is unknown. Therefore, further studies on the prevalence and significance of occult HCV in different cities are needed to identify the real burden of this infection in the country and subsequently in healthy subjects, especially among blood donors, to prevent the most of unknown transmission of HCV.

CONCLUSION

HCV infects large proportion of the high-risk populations in almost all regions of Iran and has a role in occurrence of different immunological disorders and even malignancies. The distribution patterns of HCV infection are related to different status of public health and the presence of risk factors in the society. Available estimates emphasize that injecting drug use is the most important risk factor for HCV infection in Iran and due to the growth in the number of injecting drug users, the prevalence of HCV infection is growing in the country. In addition, it seems that injection drug use has contributed to the occurrence of the majority of new HCV infections in Iran. Even the recent changes in the distribution pattern of HCV genotypes in Iranian patients confirm this issue. In fact, the predominance of risk factors for transmission of HCV has changed over time, from blood transfusion to intravenous drug use. The possible presence of occult HCV infection among the apparently healthy general population or blood donors proposes a real concern about undetectable transmission of HCV. Therefore, it seems that the prevalence of HCV infection will increase in near future not only among high-risk groups but even in the general population and blood donors of Iran. However, by breaking the cycle of infection among drug users, the rate of HCV infection will decrease. To approach this goal, efforts to screen, prevent, and treat HCV infection as well as reduce the high-risk behaviors are required.

ACKNOWLEDGMENTS

We would like to express our sincere thanks to Manoochehr Makvandi, PhD, professor, Infectious and Tropical Disease Research Center, Ahvaz Jundishapur University of Medical Sciences, for editing the manuscript.

REFERENCES

1 **Wang Y**. Scotomas in molecular virology and epidemiology of hepatitis C virus. *World J Gastroenterol* 2013; **19**: 7910-7921 [PMID: 24307785 DOI: 10.3748/wjg.v19.i44.7910]
2 **Bokharaei Salim F**, Keyvani H, Amiri A, Jahanbakhsh Sefidi F, Shakeri R, Zamani F. Distribution of different hepatitis C virus genotypes in patients with hepatitis C virus infection. *World J Gastroenterol* 2010; **16**: 2005-2009 [PMID: 20419838 DOI: 10.3748/wjg.v16.i16.2005]
3 **El-Shamy A**, Hotta H. Impact of hepatitis C virus heterogeneity on interferon sensitivity: an overview. *World J Gastroenterol* 2014; **20**: 7555-7569 [PMID: 24976696 DOI: 10.3748/wjg.v20.i24.7555]
4 **Khodabandehloo M**, Roshani D. Prevalence of hepatitis C virus genotypes in Iranian patients: a systematic review and meta-analysis. *Hepat Mon* 2014; **14**: e22915 [PMID: 25685164 DOI: 10.5812/hepatmon.22915]
5 **Alter MJ**. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 2436-2441 [PMID: 17552026 DOI: 10.3748/wjg.v13.i17.2436]
6 **Andalibalshohada A**, Rezaii SA, Abedi F. HCV prevalence and predominant genotype in IV drug users. *Rev Clin Med* 2014; **1**: 200-206
7 **Alter MJ**. HCV routes of transmission: what goes around comes around. *Semin Liver Dis* 2011; **31**: 340-346 [PMID: 22189974 DOI: 10.1055/s-0031-1297923]
8 **Shaheen MA**, Idrees M. Evidence-based consensus on the diagnosis, prevention and management of hepatitis C virus disease. *World J Hepatol* 2015; **7**: 616-627 [PMID: 25848486 DOI: 10.4254/wjh.v7.i3.616]
9 **Zobeiri M**, Adibi P, Alavian SM. Intravenous drug use and hepatitis C virus in iran. *Hepat Mon* 2012; **12**: 9-10 [PMID: 22451838 DOI: 10.5812/kowsar.1735143X.797]
10 **Shepard CW**, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]
11 **Alavian S**, Fallahian F. Epidemiology of Hepatitis C in Iran and the World. *Shiraz E Medical J* 2009; **10**: 162-172
12 **Westbrook RH**, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; **61**: S58-S68 [PMID: 25443346 DOI: 10.1016/j.jhep.2014.07.012]
13 **Wilkins T**, Malcolm JK, Raina D, Schade RR. Hepatitis C: diagnosis and treatment. *Am Fam Physician* 2010; **81**: 1351-1357 [PMID: 20521755]
14 **WHO**. Hepatitis C, WHO fact sheet No. 164, updated April 2014. Available from: URL: http://www.who.int/mediacentre/factsheets/fs164/en/
15 **Jamali R**. Epidemiologic Studies on Viral Hepatitis: A Short Review. *Thrita* 2014; **3**: e15376 [DOI: 10.5812/thrita.15376]
16 **Mohd Hanafiah K**, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
17 **Wedemeyer H**, Dore GJ, Ward JW. Estimates on HCV disease burden worldwide - filling the gaps. *J Viral Hepat* 2015; **22** Suppl 1: 1-5 [PMID: 25560838 DOI: 10.1111/jvh.12371]
18 **Te HS**, Jensen DM. Epidemiology of hepatitis B and C viruses: a global overview. *Clin Liver Dis* 2010; **14**: 1-21, vii [PMID: 20123436 DOI: 10.1016/j.cld.2009.11.009]
19 **Pawlotsky JM**, Feld JJ, Zeuzem S, Hoofnagle JH. From non-A, non-B hepatitis to hepatitis C virus cure. *J Hepatol* 2015; **62**: S87-S99 [PMID: 25920094 DOI: 10.1016/j.jhep.2015.02.006]
20 **Barth H**. Hepatitis C virus: Is it time to say goodbye yet? Perspectives and challenges for the next decade. *World J Hepatol* 2015; **7**: 725-737 [PMID: 25914773 DOI: 10.4254/wjh.v7.i5.725]
21 **Hajiaghamohammadi A**, Samimi R, Miroliaee A, Kazemifar AM, Nazem M. Treatment outcome in chronic hepatitis C infection: a four years survey among Iranian patients. *Glob J Health Sci* 2015; **7**: 75-81 [PMID: 25948447 DOI: 10.5539/gjhs.v7n3p75]
22 **Ravi S**, Nasiri Toosi M, Karimzadeh I, Ahadi-Barzoki M, Khalili H. Adherence to chronic hepatitis C treatment regimen: first report from a referral center in iran. *Hepat Mon* 2013; **13**: e11038 [PMID: 24032043 DOI: 10.5812/hepatmon.11038]
23 **Jabbari H**, Zamani F, Hatami K, Sheikholeslami A, Fakharzadeh E, Shahzamani K, Zamini H, Merat S, Malekzadeh R, Sharfi AH. Pegaferon in hepatitis C: Results of a Multicenter Study. *Middle East J Dig Dis* 2011; **3**: 110-114 [PMID: 25197541]
24 **Namazee N**, Sali S, Asadi S, Shafiei M, Behnava B, Alavian SM. Real response to therapy in chronic hepatitis C virus patients: a study from iran. *Hepat Mon* 2012; **12**: e6151 [PMID: 23087759 DOI: 10.5812/hepatmon.6151]
25 **WHO**. Guidelines for the screening, care and treatment of persons with hepatitis C infection; Updated April 2014. Available from: URL: http://www.who.int/hiv/pub/hepatitis/hepatitis-c-guidelines/en/
26 **Zidan A**, Scheuerlein H, Schüle S, Settmacher U, Rauchfuss F. Epidemiological pattern of hepatitis B and hepatitis C as etiological agents for hepatocellular carcinoma in iran and worldwide. *Hepat Mon* 2012; **12**: e6894 [PMID: 23233864 DOI: 10.5812/hepatmon.6894]
27 **Alavian SM**, Adibi P, Zali MR. Hepatitis C virus in Iran: Epidemiology of an emerging infection. *Arch Iranian Med* 2005; **8**: 84-90
28 **Khodabandehloo M**, Roshani D, Sayehmiri K. Prevalence and trend of hepatitis C virus infection among blood donors in Iran: A systematic review and meta-analysis. *J Res Med Sci* 2013; **18**: 674-682 [PMID: 24379843]
29 **Kafi-abad SA**, Rezvan H, Abolghasemi H, Talebian A. Prevalence and trends of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus among blood donors in Iran, 2004 through 2007. *Transfusion* 2009; **49**: 2214-2220 [PMID: 19527477 DOI: 10.1111/j.1537-2995.2009.02245.x]
30 **Fallahian F**, Najafi A. Epidemiology of hepatitis C in the Middle East. *Saudi J Kidney Dis Transpl* 2011; **22**: 1-9 [PMID: 21196607]
31 **Mohamoud YA**, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. *BMC Infect Dis* 2013; **13**: 288 [PMID: 23799878 DOI: 10.1186/1471-2334-13-288]
32 **Dehesa-Violante M**, Nuñez-Nateras R. Epidemiology of hepatitis virus B and C. *Arch Med Res* 2007; **38**: 606-611 [PMID: 17613351 DOI: 10.1016/j.arcmed.2007.03.001]
33 **Wasley A**, Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis* 2000; **20**: 1-16 [PMID: 10895428 DOI: 10.1055/s-2000-9506]
34 **Soldan K**, Davison K, Dow B. Estimates of the frequency of HBV, HCV, and HIV infectious donations entering the blood supply in the United Kingdom, 1996 to 2003. *Euro Surveill* 2005; **10**: 17-19 [PMID: 15735312]
35 **Khedmat H**, Fallahian F, Abolghasemi H, Alavian SM, Hajibeigi B, Miri SM, Jafari AM. Seroepidemiologic study of hepatitis B virus, hepatitis C virus, human immunodeficiency virus and syphilis infections in Iranian blood donors. *Pak J Biol Sci* 2007; **10**: 4461-4466 [PMID: 19093512 DOI: 10.3923/pjbs.2007.4461.4466]
36 **Taheri Azbarmi Z**, Nouri S, Joukar F, Jafarshad R, Haajikarimian K, Alinejad S, Abdollahzadeh Estakhari GH, Mansour Ghanaei F. Transfusion transmitted diseases in Rasht blood donors (in Persian). *Sci J Iran Blood Transfus Org* 2008; **4**: 337-343
37 **Mansour-Ghanaei F**, Fallah M, Jafarshad R, Joukar F, Salari A, Tavafzadeh R. Prevalence of hepatitis B surface antigen and hepatitis C virus antibody and their risk factors among Guilan's volunteer blood donors (1998-2003). *Hepat Mon* 2007; **7**: 239-241
38 **Bani Aghil SS**, Abbasi S, Arab M, Seyedein MS. The Prevalence of HCV, HBV, HIV in Blood Donors of Golestan Province,(2006-2008) (in Persian). *Med Laboratory J* 2010; **3**: 1-5
39 **Khedmat H**, Alavian SM, Miri SM, Amini M, Abolghasemi H, Hajibeigi B, Alaeddini F, Fallahian F. Trends in seroprevalence of hepatitis B, hepatitis C, HIV, and syphilis infections in Iranian blood donors from 2003 to 2005. *Hepat Mon* 2009; **9**: 24-28
40 **Attarchi Z**, Ghafouri M, Hajibaygi B, Assari S, SM A. Donor deferral and blood-borne infections in blood donors of Tehran (in

Persian). *Sci J Iran Blood Transfus Org* 2006; **2**: 353-364
41 **Bozorgi S**, Ahmadzad Asl M, Ramezani H, Kargarfard H, S A. Study of viral infections prevalence in blood donors of Qazvin province in different time intervals and during Bam earthquake (in Persian). *Govaresh* 2006; **11**: 242-248
42 **Mahdaviani F**, Saremi S, Maghsoudlu M, AA P. Prevalence of blood transmitted viral infections in regular and non-regular donors of Arak Blood Center (in Persian). *Sci J Iran Blood Transfus Org* 2006; **2**: 343-351
43 **Bozorgi SH**, Ramezani H, Nooranipour M, Ahmadi M, Baghernejad A, Mostajeri A, Kargar-Fard H, Sadri M, Alavian SM. Risk factors of viral hepatitis: yet to explore. *Transfus Apher Sci* 2012; **47**: 145-149 [PMID: 22858443 DOI: 10.1016/j.transci.2012.06.023]
44 **Afzali H**, Ardakani AT, Vali GR. Seroepidemiology of hepatitis B and C in blood donors in Kashan, 1996-2001 (in Persian). *FEYZ* 2002; **6**: 43-50
45 **Moniri R**, Mosayebii Z, Mossavi G. Seroprevalence of cytomegalovirus, hepatitis B, hepatitis C and human immunodeficiency virus antibodies among volunteer blood donors. *Iran J Public Health* 2004; **33**: 38-42
46 **Karimi A**, Hoseini SM. Seroprevalence of hepatitis B and C virus and HIV markers among blood donors from Shahre-Kord, Iran (2004-2006). *Kuwait Med J* 2008; **40**: 279-281
47 **Masaeli Z**, Jaberi M, Magsudlu M. A comparison of seroprevalence of blood-borne infections among regular, sporadic, and first-time blood donors in Isfahan (in Persian). *Sci J Iran Blood Transfus Org* 2006; **2**: 301-307
48 **Esmaieli H**, Hajiani G, Mankhian A, Poumehdi Broujeni M. Seroepidemiological survey of hepatitis B, C, HIV and syphilis among blood donors in Bushehr-Iran (in Persian). *ISMJ* 2009; **11**: 183-190
49 **Ghavanini AA**, Sabri MR. Hepatitis B surface antigen and anti-hepatitis C antibodies among blood donors in the Islamic Republic of Iran. *East Mediterr Health J* 2000; **6**: 1114-1116 [PMID: 12197336]
50 **Emamghorashi F**, Fathi G, Mohtashami A. Evaluation of demographic characteristics and hepatitis B, C and HIV prevalence among blood donors in Jahrom (in Persian). *SJIBTO* 2006; **2**: 373-378
51 **Kasraian L**, Torab Jahromi SA. Prevalence of Major Transfusion-Transmissible Viral Infections in Blood Donors Attending Fars Blood Transfusion Center, Shiraz, Southern Iran: 2002-2005. *Iran J Med Sci* 2007; **32**: 114-117
52 **Kasraian L**, Tavassoli A. Prevalence of hepatitis C and its risk factors in blood donors at Shiraz transfusion center (in Persian). *Koomesh* 2008; **10**: 7-12
53 **Delavari M**, Tabatabaei SM. Frequency of hepatitis C and its related factors in blood donors in Kerman in 2003 (in Persian). *JAUMS* 2004; **2**: 323-358
54 **Tajbakhsh E**, Yaghobi R, Vahedi AR. A serological survey on hepatitis C virus Antibody in blood donors with an ELISA method (in Persian). *Tehran Univ Med J* 2007; **65**: 69-73
55 **Doosti A**, Amini-Bavil-Olyaee S, Tajbakhsh E, Adeli A, Mahboudi F. Prevalence of viral hepatitis and molecular analysis of HBV among voluntary blood donors in west Iran. *New Microbiol* 2009; **32**: 193-198 [PMID: 19579699]
56 **Ghafouri M**, Ameli M. Comparing prevalence of transfusion transmitted viral infections in various population groups of South Khorasan (in Persian). *Sci J Iran Blood Transfus Org* 2011; **7**: 242-248
57 **Merat S**, Poustchi H. Hepatitis C in Iran. How extensive of a problem is it? *Arch Iran Med* 2012; **15**: 268 [PMID: 22519372]
58 **Merat S**, Rezvan H, Nouraie M, Jafari E, Abolghasemi H, Radmard AR, Zaer-rezaii H, Amini-Kafiabad S, Maghsudlu M, Pourshams A, Malekzadeh R, Esmaili S. Seroprevalence of hepatitis C virus: the first population-based study from Iran. *Int J Infect Dis* 2010; **14** Suppl 3: e113-e116 [PMID: 20362479 DOI: 10.1016/j.ijid.2009.11.032]
59 **Karoney MJ**, Siika AM. Hepatitis C virus (HCV) infection in Africa: a review. *Pan Afr Med J* 2013; **14**: 44 [PMID: 23560127 DOI: 10.11604/pamj.2013.14.44.2199]
60 **Zamani F**, Sohrabi M, Poustchi H, Keyvani H, Saeedian FS, Ajdarkosh H, Khoonsari M, Hemmasi G, Moradilakeh M, Motamed N, Maadi M. Prevalence and risk factors of hepatitis C virus infection in amol city, north of iran: a population-based study (2008-2011). *Hepat Mon* 2013; **13**: e13313 [PMID: 24358039 DOI: 10.5812/hepatmon.13313]
61 **Mansour-Ghanaei F**, Fallah M, Jafarshad R, Joukar F, Pourtahmasbi A, Bahari-Moghaddam A. Seroprevalence of hepatitis B and C among residents of Guilan Nursing Home. *Hepat Mon* 2007; **7**: 139-141
62 **Shakeri MT**, Nomani H, Ghayour Mobarhan M, Sima HR, Gerayli S, Shahbazi S, Rostami S, Meshkat Z. The prevalence of hepatitis C virus in mashhad, iran: a population-based study. *Hepat Mon* 2013; **13**: e7723 [PMID: 23745128 DOI: 10.5812/hepatmon.7723]
63 **Ghadir M**, Jafari E, Amiriani M, Rezvan H, Aminikafiabad S, Pourshams A. Hepatitis C in Golestan province-Iran (in Persian). *Govaresh* 2006; **11**: 158-162
64 **Motlagh M**, Makvandi M, Jalali M. Prevalence of anti-HCV among pregnant women (in Persian). *J Qazvin Univ Med Sci* 2001; **18**: 59-63
65 **Nikbakht R**, Saadati N, Firoozian F. Prevalence of HBsAg, HCV and HIV Antibodies Among Infertile Couples in Ahvaz, South-West Iran. *Jundishapur J Microbiol* 2012; **5**: 393-397 [DOI: 10.5812/jjm.2809]
66 **Moradi A**, Mohagheghi AH, Shahraki S, Borji A, Marjani A, Sanei-Moghadam E, Kalavi K-B, Zangi-Abadi M. Seroepidemiology of Rubella, Measles, HBV, HCV and B19 Virus Within Women in Child Bearing Ages (Saravan City of Sistan and Bloochastan Province). *Res J Microbiol* 2007; **2**: 289-293 [DOI: 10.3923/jm.2007.289.293]
67 **Sayad B**, Shamsedin-Saeed F, Keyvani H, Rezaii M, Asadi T, Vaziri S, Janbakhsh A, Mansouri F, Afsharian M, Laghaii Z. Seroepidemiology of hepatitis C in Kermanshah (West of Iran, 2006). *Hepat Mon* 2008; **8**: 141-146
68 **Mohebbi SR**, Sanati A, Cheraghipour K, Rostami Nejad M, Shalmani HM, Zali MR. Hepatitis C and hepatitis B virus infection: epidemiology and risk factors in a large cohort of pregnant women in Lorestan, West of Iran. *Hepat Mon* 2011; **11**: 736-739 [PMID: 22235217 DOI: 10.5812/kowsar.1735143X.749]
69 **Aceijas C**, Rhodes T. Global estimates of prevalence of HCV infection among injecting drug users. *Int J Drug Policy* 2007; **18**: 352-358 [PMID: 17854722 DOI: 10.1016/j.drugpo.2007.04.004]
70 **Honarvar B**, Odoomi N, Moghadami M, Afsar Kazerooni P, Hassanabadi A, Zare Dolatabadi P, Farzanfar E, Lankarani KB. Blood-borne hepatitis in opiate users in iran: a poor outlook and urgent need to change nationwide screening policy. *PLoS One* 2013; **8**: e82230 [PMID: 24312645 DOI: 10.1371/journal.pone.0082230]
71 **Nelson PK**, Mathers BM, Cowie B, Hagan H, Des Jarlais D, Horyniak D, Degenhardt L. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet* 2011; **378**: 571-583 [PMID: 21802134 DOI: 10.1016/S0140-6736(11)61097-0]
72 **Mohtasham Amiri Z**, Rezvani M, Jafari Shakib R, Jafari Shakib A. Prevalence of hepatitis C virus infection and risk factors of drug using prisoners in Guilan province. *East Mediterr Health J* 2007; **13**: 250-256 [PMID: 17684845]
73 **Rahimi-Movaghar A**, Razaghi EM, Sahimi-Izadian E, Amin-Esmaeili M. HIV, hepatitis C virus, and hepatitis B virus co-infections among injecting drug users in Tehran, Iran. *Int J Infect Dis* 2010; **14**: e28-e33 [PMID: 19464218 DOI: 10.1016/j.ijid.2009.03.002]
74 **Hosseini M**, SeyedAlinaghi S, Kheirandish P, Esmaeli Javid G, Shirzad H, Karami N, Jahani M, Seyed Ahmadian M, Payvarmehr F, Mohraz M, Emadi Koochak H, McFarland W. Prevalence and correlates of co-infection with human immunodeficiency virus and hepatitis C virus in male injection drug users in Iran. *Arch Iran Med* 2010; **13**: 318-323 [PMID: 20597566]
75 **Zali MR**, Aghazadeh R, Nowroozi A, Amir-Rasouly H. Anti-HCV

antibody among Iranian IV drug users: is it a serious problem. *Arch Iran Med* 2001; **4**: 115-119
76 **Zamani S**, Ichikawa S, Nassirimanesh B, Vazirian M, Ichikawa K, Gouya MM, Afshar P, Ono-Kihara M, Ravari SM, Kihara M. Prevalence and correlates of hepatitis C virus infection among injecting drug users in Tehran. *Int J Drug Policy* 2007; **18**: 359-363 [PMID: 17854723 DOI: 10.1016/j.drugpo.2007.02.007]
77 **Hajinasrollah A**, Yeganeh R, Salehi N, Saheh M, Khoshkar A, Malekpour F, Ghaseminejad A, Hojati M. Prevalence of HIV, Hepatitis B, and Hepatitis C in Drug Abuser in Loghman Medical Center (in Persian). *IJS* 2006; **13**: 89-94
78 **Amin-Esmaeili M**, Rahimi-Movaghar A, Razaghi EM, Baghestani AR, Jafari S. Factors Correlated With Hepatitis C and B Virus Infections Among Injecting Drug Users in Tehran, IR Iran. *Hepat Mon* 2012; **12**: 23-31 [PMID: 22451840 DOI: 10.5812/kowsar.1735143X.806]
79 **Nokhodian Z**, Meshkati M, Adibi P, Ataei B, Kassaian N, Yaran M, Shoaei P, Hassannejad R. Hepatitis C among Intravenous Drug Users in Isfahan, Iran: a Study of Seroprevalence and Risk Factors. *Int J Prev Med* 2012; **3**: S131-S138 [PMID: 22826755]
80 **Zamani S**, Radfar R, Nematollahi P, Fadaie R, Meshkati M, Mortazavi S, Sedaghat A, Ono-Kihara M, Kihara M. Prevalence of HIV/HCV/HBV infections and drug-related risk behaviours amongst IDUs recruited through peer-driven sampling in Iran. *Int J Drug Policy* 2010; **21**: 493-500 [PMID: 20483578 DOI: 10.1016/j.drugpo.2010.04.006]
81 **Kassaian N**, Adibi P, Kafashaian A, Yaran M, Nokhodian Z, Shoaei P, Hassannejad R, Babak A, Ataei B. Hepatitis C Virus and Associated Risk Factors among Prison Inmates with History of Drug Injection in Isfahan, Iran. *Int J Prev Med* 2012; **3**: S156-S161 [PMID: 22826759]
82 **Nobari RF**, Meshkati M, Ataei B, Yazdani MR, Heidari K, Kassaian N, Nokhodian Z, Shoaei P, Yaran M, Adibi P. Identification of Patients with Hepatitis C Virus Infection in Persons with Background of Intravenous Drug Use: The First Community Announcement-based Study From Iran. *Int J Prev Med* 2012; **3**: S170-S175 [PMID: 22826761]
83 **Sofian M**, Aghakhani A, Banifazl M, Azadmanesh K, Farazi AA, McFarland W, Eslamifar A, Ramezani A. Viral hepatitis and HIV infection among injection drug users in a central Iranian City. *J Addict Med* 2012; **6**: 292-296 [PMID: 22895463 DOI: 10.1097/ADM.0b013e3182659928]
84 **Ramezani A**, Amirmoezi R, Volk JE, Aghakhani A, Zarinfar N, McFarland W, Banifazl M, Mostafavi E, Eslamifar A, Sofian M. HCV, HBV, and HIV seroprevalence, coinfections, and related behaviors among male injection drug users in Arak, Iran. *AIDS Care* 2014; **26**: 1122-1126 [PMID: 24499303 DOI: 10.1080/09540121.2014.882485]
85 **Davoodian P**, Dadvand H, Mahoori K, Amoozandeh A, Salavati A. Prevalence of selected sexually and blood-borne infections in Injecting drug abuser inmates of bandar abbas and roodan correction facilities, Iran, 2002. *Braz J Infect Dis* 2009; **13**: 356-358 [PMID: 20428635 DOI: 10.1590/S1413-86702009000500008]
86 **Sarkari B**, Eilami O, Khosravani A, Sharifi A, Tabatabaee M, Fararouei M. High prevalence of hepatitis C infection among high risk groups in Kohgiloyeh and Boyerahmad Province, Southwest Iran. *Arch Iran Med* 2012; **15**: 271-274 [PMID: 22519374]
87 **Imani R**, Karimi A, Rouzbahani R, Rouzbahani A. Seroprevalence of HBV, HCV and HIV infection among intravenous drug users in Shahr-e-Kord, Islamic Republic of Iran. *East Mediterr Health J* 2008; **14**: 1136-1141 [PMID: 19161086]
88 **Alavi SM**, Behdad F. Seroprevalence study of hepatitis C and Hepatitis B virus among hospitalized intravenous drug users in Ahvaz, Iran (2002-2006). *Hepat Mon* 2010; **10**: 101-104 [PMID: 22312381]
89 **Mohammad Alizadeh AH**, Alavian SM, Jafari K, Yazdi N. Prevalence of hepatitis C virus infection and its related risk factors in drug abuser prisoners in Hamedan--Iran. *World J Gastroenterol* 2005; **11**: 4085-4089 [PMID: 15996035]
90 **Keramat F**, Eini P, Majzoobi MM. Seroprevalence of HIV, HBV and HCV in Persons Referred to Hamadan Behavioral Counseling Center, West of Iran. *Iran Red Crescent Med J* 2011; **13**: 42-46 [PMID: 22946017]
91 **Souqiyyeh MZ**, Al-Attar MB, Zakaria H, Shaheen FA. Dialysis centers in the kingdom of saudi arabia. *Saudi J Kidney Dis Transpl* 2001; **12**: 293-304 [PMID: 18209376]
92 **Saxena AK**, Panhotra BR. The impact of nurse understaffing on the transmission of hepatitis C virus in a hospital-based hemodialysis unit. *Med Princ Pract* 2004; **13**: 129-135 [PMID: 15073424 DOI: 10.1159/000076951]
93 **Bdour S**. Hepatitis C virus infection in Jordanian haemodialysis units: serological diagnosis and genotyping. *J Med Microbiol* 2002; **51**: 700-704 [PMID: 12171303]
94 **Khokhar N**, Alam AY, Naz F, Mahmud SN. Risk factors for hepatitis C virus infection in patients on long-term hemodialysis. *J Coll Physicians Surg Pak* 2005; **15**: 326-328 [PMID: 15924834]
95 **Amin J**, Gidding H, Gilbert G, Backhouse J, Kaldor J, Dore G, Burgess M. Hepatitis C prevalence--a nationwide serosurvey. *Commun Dis Intell Q Rep* 2004; **28**: 517-521 [PMID: 15745402]
96 **Fissell RB**, Bragg-Gresham JL, Woods JD, Jadoul M, Gillespie B, Hedderwick SA, Rayner HC, Greenwood RN, Akiba T, Young EW. Patterns of hepatitis C prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. *Kidney Int* 2004; **65**: 2335-2342 [PMID: 15149347 DOI: 10.1111/j.1523-1755.2004.00649.x]
97 **Qadi AA**, Tamim H, Ameen G, Bu-Ali A, Al-Arrayed S, Fawaz NA, Almawi WY. Hepatitis B and hepatitis C virus prevalence among dialysis patients in Bahrain and Saudi Arabia: a survey by serologic and molecular methods. *Am J Infect Control* 2004; **32**: 493-495 [PMID: 15573057 DOI: 10.1016/j.ajic.2003.12.009]
98 **Alavian SM**. Hepatitis C infection in Iran: A review article. *Iran J Clin Infect Dis* 2009; **4**: 47-59
99 **Makhlough A**, Jamshidi M, Mahdavi MR. Hepatitis C prevalence studied by polymerase chain reaction and serological methods in haemodialysis patients in Mazandaran, Iran. *Singapore Med J* 2008; **49**: 921-923 [PMID: 19037560]
100 **Amiri ZM**, Shakib AJ, Toorchi M. Seroprevalence of hepatitis C and risk factors in haemodialysis patients in Guilan, Islamic Republic of Iran. *East Mediterr Health J* 2005; **11**: 372-376 [PMID: 16602456]
101 **Joukar F**, Khalesi AK, Jafarshad R, Rahimabadi MS, Mansour-Ghanaei F. Distribution of hepatitis C virus genotypes in haemodialysis patients of Guilan, northern Islamic Republic of Iran. *East Mediterr Health J* 2012; **18**: 236-240 [PMID: 22574476]
102 **Samimi-Rad K**, Hosseini M. Hepatitis C virus infection and HCV genotypes of hemodialysis patients. *Iran J Public Health* 2008; **37**: 146-152
103 **Bozorghi SH**, Ramezany H, Vahid T, Mostajeri A, Karegharfard H, Rezayi M, Ashayeri N, Alaviyan SM. Assessment of prevalence and risk factors of hepatitis C virus infection in haemodialysis patients in Ghazvin (in Persian). *Sci J Iran Blood Transfus Org* 2006; **2**: 331-337
104 **Somi MH**, Etemadi J, Ghojazadeh M, Farhang S, Faramarzi M, Foroutan S, Soleimanpour M. Risk factors of HCV seroconversion in hemodialysis patients in tabriz, iran. *Hepat Mon* 2014; **14**: e17417 [PMID: 24976839 DOI: 10.5812/hepatmon.17417]
105 **Zahedi MJ**, Darvish Moghaddam S, Alavian SM, Dalili M. Seroprevalence of Hepatitis Viruses B, C, D and HIV Infection Among Hemodialysis Patients in Kerman Province, South-East Iran. *Hepat Mon* 2012; **12**: 339-343 [PMID: 22783346 DOI: 10.5812/hepatmon.5969]
106 **Kalantari H**, Ebadi S, Yaran M, Maracy MR, Shahshahan Z. Prevalence and risk factors of hepatitis B and C viruses among hemodialysis patients in Isfahan, Iran. *Adv Biomed Res* 2014; **3**: 73 [PMID: 24627881 DOI: 10.4103/2277-9175.125869]
107 **Zamani F**, Ameli M, Razmjou S, Shakeri R, Amiri A, Darvish R. Incidence of hepatitis C infection in patients on hemodialysis: a multicenter study of northern part of Iran. *Saudi J Kidney Dis Transpl* 2010; **21**: 1169-1171 [PMID: 21060201]
108 **Assarehzadegan MA**, Shakerinejad G, Noroozkohnejad R, Amini

A, Rahim Rezaee SA. Prevalence of hepatitis C and B infection and HC V genotypes among hemodialysis patients in Khuzestan province, southwest Iran. *Saudi J Kidney Dis Transpl* 2009; **20**: 681-684 [PMID: 19587521]
109 **Nemati E**, Alavian SM, Taheri S, Moradi M, Pourfarziani V, Einollahi B. Hepatitis C virus infection among patients on hemodialysis: a report from a single center in Iran. *Saudi J Kidney Dis Transpl* 2009; **20**: 147-153 [PMID: 19112238]
110 **Sotoudehjahromi A**, Nejatollahi F, Hosseini MM. Prevalence of anti-HCV antibody in haemodialysis patients referring to haemodialysis unit of Jahrom (in Persian). *Jahrom Medica* 2007; **5**: 38-43
111 **Alavian SM**, Einollahi B, Hajarizadeh B, Bakhtiari S, Nafar M, Ahrabi S. Prevalence of hepatitis C virus infection and related risk factors among Iranian haemodialysis patients. *Nephrology* (Carlton) 2003; **8**: 256-260 [PMID: 15012714]
112 **Broumand B**, Shamshirsaz AA, Kamgar M, Hashemi R, Aiazi F, Bekheirnia M, Boozary N, Komeilian Z, Shamshirsaz AH, Tabatabaiee MR, Broumand V. Prevalence of hepatitis C infection and its risk factors in hemodialysis patients in tehran: preliminary report from "the effect of dialysis unit isolation on the incidence of hepatitis C in dialysis patients" project. *Saudi J Kidney Dis Transpl* 2002; **13**: 467-472 [PMID: 17660669]
113 **Nassiri-Toosi M**, Larti F, Rasteh M, Foroutan H, Salarieh N, Lessan-Pezeshki M, Abdollahi A, Seifi S, Razeghi E, Rahbar M. Risk factors and seroprevalence of hepatitis B and C infections among hemodialysis patients in Tehran. *Iran J Pathol* 2007; **2**: 181-186
114 **Mohammad-Alizadeh A**, Ranjbar M, Seyfoleslami S. The frequency of hepatitis C in dialyse patients in Hamadan Ekbatan hospital (in Persian). *Iran J Infect Dis Trop Med* 2002; **7**: 27-34
115 **Saboor B**, Boroomand P, Mehrabi Y, Ghanbari M, Zarrinfam H. Prevalence and risk factors of hepatitis C infection in hemodialysis patients (Kermanshah, 1999-2000) (in Persian). *Behbood* 2003; **7**: 60-66
116 **Jabbari A**, Besharat S, Khodabakhshi B, Gorgan I. Hepatitis C in hemodialysis centers of Golestan province, northeast of Iran (2005). *Hepat Mon* 2008; **8**: 61-65
117 **Ansari MHK**, Omrani M. Evaluation of diagnostic value of ELISA method (EIA) & PCR in diagnosis of hepatitis C virus in hemodialysis patients. *Hepatitis Monthly* 2006; **6**: 19-23
118 **Hassanshahi G**, Arababadi MK, Assar S, Hakimi H, Karimabad MN, Abedinzadeh M, Rafatpanah H, Derakhshan R. Post-transfusion-transmitted hepatitis C virus infection: a study on thalassemia and hemodialysis patients in southeastern Iran. *Arch Virol* 2011; **156**: 1111-1115 [PMID: 21340738 DOI: 10.1007/s00705-011-0950-y]
119 **Ansar MM**, Kooloobandi A. Prevalence of hepatitis C virus infection in thalassemia and haemodialysis patients in north Iran-Rasht. *J Viral Hepat* 2002; **9**: 390-392 [PMID: 12225335 DOI: 10.1046/j.1365-2893.2002.00368.x]
120 **Mansour-Ghanaei F**, Fallah MS, Shafaghi A, Yousefi-Mashhoor M, Ramezani N, Farzaneh F, Nassiri R. Prevalence of hepatitis B and C seromarkers and abnormal liver function tests among hemophiliacs in Guilan (northern province of Iran). *Med Sci Monit* 2002; **8**: CR797-CR800 [PMID: 12503038]
121 **Torabi SA**, Abedashtiani K, Dehkada R, Moghadam AN, Bahram M, Dolatkhah R, Babaei J, Taheri N. Prevalence of HCV, HBV and HIV in hemophiliac patients of East Azarbaijan in 2004 (in Persian). *SJIBTO* 2006; **2**: 291-299
122 **Valizadeh N**, Nateghi S, Noroozi M, Hejazi S, Aghanezhad F, Ali AA. Seroprevalence of hepatitis C, hepatitis B and HIV viruses in hemophiliacs born 1985-2010 in west Azarbaijan of Iran. *Asian J Transfus Sci* 2013; **7**: 55-58 [PMID: 23559767 DOI: 10.4103/0973-6247.106739]
123 **Mousavian S**, Mansouri F, Saraei A, Sadeghei A, Merat S. Seroprevalence of hepatitis C in hemophilia patients refering to Iran Hemophilia Society Center in Tehran (in Persian). *Govaresh* 2011; **16**: 169-174
124 **Kalantari H**, Mirzabaghi A, Akbari M, Shahshahan Z. Prevalence of hepatitis C virus, hepatitis B virus, human immunodeficiency virus and related risk factors among hemophilia and thalassemia patients In Iran. *Iran J Clin Infect Dis* 2011; **6**: 82-84
125 **Mobini G**, Hosseini M, Shahbaz B, Salari MH, Mokhtari-Azad T, Nategh R. Prevalence of anti-HCV antibody and related risk factors among bleeding disorder patients in Yazd province of Iran (in Persian). *J Shahrekord Univ Med Sci* 2010; **12**: 36-42
126 **Yazdani MR**, Kassaian N, Ataei B, Nokhodian Z, Adibi P. Hepatitis C virus infection in patients with hemophilia in Isfahan, Iran. *Int J Prev Med* 2012; **3**: S89-S93 [PMID: 22826775]
127 **Javadzadeh Shahshahani H**, Attar M, Yavari MT, Savabieh S. Study of the prevalence of hepatitis B, C and HIV infection in hemophilia and thalassemia population of Yazd (in Persian). *SJIBTO* 2006; **2**: 315-322
128 **Samimi-Rad K**, Shahbaz B. Hepatitis C virus genotypes among patients with thalassemia and inherited bleeding disorders in Markazi province, Iran. *Haemophilia* 2007; **13**: 156-163 [PMID: 17286768 DOI: 10.1111/j.1365-2516.2006.01415.x]
129 **Mahdaviani F**, Saremi S, Rafiee M. Prevalence of hepatitis B, C and HIV infection in thalassemic and hemophilic patients of Markazi province in 2004 (in Persian). *Blood Sci J Iran* 2008; **4**: 313-322
130 **Karimi M**, Ghavanini AA. Seroprevalence of HBsAg, anti-HCV, and anti-HIV among haemophiliac patients in Shiraz, Iran. *Haematologia* (Budap) 2001; **31**: 251-255 [PMID: 11855788 DOI: 10.1163/15685590152763809]
131 **Assarehzadegan MA**, Ghafourian Boroujerdnia M, Zandian K. Prevalence of hepatitis B and C infections and HCV genotypes among haemophilia patients in ahvaz, southwest iran. *Iran Red Crescent Med J* 2012; **14**: 470-474 [PMID: 23105982]
132 **Zahedi MJ**, Darvishmoghadam S. Frequency of Hepatitis B and C infection among Hemophiliac patients in Kerman (in Persian). *JKUMS* 2004; **11**: 131-135
133 **Sharifi-Mood B**, Eshghi P, Sanei-Moghaddam E, Hashemi M. Hepatitis B and C virus infections in patients with hemophilia in Zahedan, southeast Iran. *Saudi Med J* 2007; **28**: 1516-1519 [PMID: 17914511]
134 **Esfahani H**, Bazmamoun H. The Prevalence of Blood-Borne Viral Infection (HBV, HCV, HIV) among Hemophilia Patients in Hamedan Province of Iran. *IJBC* 2014; **6**: 209-211
135 **Ansari S**, Azarkivan A, Halagi F. Incidence of hepatocellular carcinoma in patients with thalassemia who had hepatitis C. *Acta Med Iran* 2013; **51**: 404-407 [PMID: 23852846]
136 **Alavian S**, Tabatabaei S, Lankarani K. Epidemiology of HCV infection among thalassemia patients in eastern Mediterranean countries: a quantitative review of literature. *Iran Red Crescent Med J* 2010; **12**: 365-376
137 **Mansouritorghabeh H**, Badiei Z. Transfusion-transmitted viruses in individuals with β thalassemia major at Northeastern Iran, a retrospective sero-epidemiological survey. *IJBC* 2008; **1**: 1-4
138 **Mirmomen S**, Alavian SM, Hajarizadeh B, Kafaee J, Yektaparast B, Zahedi MJ, Zand V, Azami AA, Hosseini MM, Faridi AR, Davari K, Hajibeigi B. Epidemiology of hepatitis B, hepatitis C, and human immunodeficiency virus infecions in patients with beta-thalassemia in Iran: a multicenter study. *Arch Iran Med* 2006; **9**: 319-323 [PMID: 17061602]
139 **Ghane M**, Eghbali M, Abdolahpour M. Prevalence of Hepatitis C Amongst Beta-thalassemia Patients in Gilan and Mazandaran Provinces, 2011 (in Persian). *Govaresh* 2011; **16**: 22-27
140 **Tamaddoni A**, Mohammadzadeh I, Ziaei O. Seroprevalence of HCV antibody among patients with beta-thalassemia major in Amirkola Thalassemia Center, Iran. *Iran J Allergy Asthma Immunol* 2007; **6**: 41 [PMID: 17303929]
141 **Alavi S**, Valeshabad AK, Sharifi Z, Nourbakhsh K, Arzanian MT, Navidinia M, Seraj SM. Torque teno virus and hepatitis C virus co-infection in Iranian pediatric thalassemia patients. *Turk J Haematol* 2012; **29**: 156-161 [PMID: 24744647 DOI: 10.5505/tjh.2012.20280]
142 **Alavian SM**, Kafaei J, Yektaparast B, Hajarizadeh B, Kamali A, Sadri M. The prevalence of Hepatitis B and C among Thalassemia

major patients in Ghazvin (in Persian). *Kowsar Med J* 2003; **7**: 319-326
143 **Bozorgi SH**, Ramezani H, Vahid T, Mostajeri A, Kargarfard H, Rezaei M, Ashayeri N, Alavian SM. The prevalence and risk factors of hepatitis C virus infection among thalassemic patients of Qazvin (2005) (in Persian). *JQUMS* 2008; **11**
144 **Azarkeivan A**, Toosi MN, Maghsudlu M, Kafiabad SA, Hajibeigi B, Hadizadeh M. The incidence of hepatitis C in patients with thalassemia after screening in blood transfusion centers: a fourteen-year study. *Transfusion* 2012; **52**: 1814-1818 [PMID: 22500658 DOI: 10.1111/j.1537-2995.2012.03652.x]
145 **Nakhaie S**, Talachian E. Prevalence and characteristic of liver involvement in thalassemia patients with HCV in Ali-Asghar children hospital, Tehran, Iran (in Persian). *JIUMS* 2003; **10**: 799-806
146 **Ataei B**, Hashemipour M, Kassaian N, Hassannejad R, Nokhodian Z, Adibi P. Prevalence of anti HCV infection in patients with Beta-thalassemia in isfahan-iran. *Int J Prev Med* 2012; **3**: S118-S123 [PMID: 22826753]
147 **Karimi M**, Ghavanini AA. Seroprevalence of hepatitis B, hepatitis C and human immunodeficiency virus antibodies among multitransfused thalassaemic children in Shiraz, Iran. *J Paediatr Child Health* 2001; **37**: 564-566 [PMID: 11903836 DOI: 10.1046/j.1440-1754.2001.00709.x]
148 **Kashef S**, Karimi M, Amirghofran Z, Ayatollahi M, Pasalar M, Ghaedian MM, Kashef MA. Antiphospholipid antibodies and hepatitis C virus infection in Iranian thalassemia major patients. *Int J Lab Hematol* 2008; **30**: 11-16 [PMID: 18190462 DOI: 10.1111/j.1751-553X.2007.00916.x]
149 **Kadivar M**, Mirahmadizadeh A, Karimi A, Hemmati A. The prevalence of HCV and HIV in thalassemia patients in Shiraz, Iran. *Med J Iran Hosp* 2001; **4**: 18-20
150 **Shahraki T**, Shahraki M, Moghaddam ES, Najafi M, Bahari A. Determination of hepatitis C genotypes and the viral titer distribution in children and adolescents with major thalassemia. *Iran J Pediatr* 2010; **20**: 75-81 [PMID: 23056686]
151 **Ghafourian Boroujerdnia M**, Assarehzadegan M, Haghirizadeh Rodany M, Zandian K, Noroozkohnejad R. Detection of molecular markers of hepatitis B, hepatitis C and human immunodeficiency virus (HIV) in thalassemic patients referring to Shafa hospital (in Persian). *Jundishapur Sci Med J* 2009; **7**: 454-462
152 **Alavi SM**, Hajiani E. Hepatitis C infection: a review on epidemiology and management of occupational exposure in health care workers for general physicians working in Iranian health network setting. *Jundishapur J Microbiol* 2011; **4**: 1-9
153 **Shoaei P**, Lotfi N, Hassannejad R, Yaran M, Ataei B, Kassaian N, Foroughifar M, Adibi P. Seroprevalence of Hepatitis C Infection among Laboratory Health Care Workers in Isfahan, Iran. *Int J Prev Med* 2012; **3**: S146-S149 [PMID: 22826757]
154 **Yarmohammadi M**. Investigating the Serologic Status and Epidemiological Aspects of Health Care Workers' Exposure to HBV and HCV Viruses (in Persian). *Knowledge & Health* 2011; **5**: 37-42
155 **Hadadi A**, Afhami S, Kharbakhsh M, Hajabdoulbaghi M, Rasoolinejad M, Emadi H, Esmaeelpour N, Sadeghi A, Ghorashi L. Epidemiological determinants of occupational exposure to HIV, HBV and HCV in health care workers (in Persian). *TUMJ* 2007; **65**: 59-66
156 **Askarian M**, Yadollahi M, Kuochak F, Danaei M, Vakili V, Momeni M. Precautions for health care workers to avoid hepatitis B and C virus infection. *Int J Occup Environ Med* 2011; **2**: 191-198 [PMID: 23022838]
157 **Amiri FB**, Gouya MM, Saifi M, Rohani M, Tabarsi P, Sedaghat A, Fahimfar N, Memarnejadian A, Aghasadeghi MR, Haghdoost AA, Jahanbakhsh F, Nasehi M, Mostafavi E. Vulnerability of homeless people in Tehran, Iran, to HIV, tuberculosis and viral hepatitis. *PLoS One* 2014; **9**: e98742 [PMID: 24896247 DOI: 10.1371/journal.pone.0098742]
158 **Vahdani P**, Hosseini-Moghaddam SM, Family A, Moheb-Dezfouli R. Prevalence of HBV, HCV, HIV and syphilis among homeless subjects older than fifteen years in Tehran. *Arch Iran Med* 2009; **12**: 483-487 [PMID: 19722771]
159 **Beijer U**, Wolf A, Fazel S. Prevalence of tuberculosis, hepatitis C virus, and HIV in homeless people: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; **12**: 859-870 [PMID: 22914343 DOI: 10.1016/S1473-3099(12)70177-9]
160 **Fallah F**, Karimi A, Eslami G, Tabatabaii S, Goudarzi H, Moradi RRA, Malekan M, Navidinia M, Golnabi A, Gholinejad Z. The Homeless youth and their exposure to Hepatitis B and Hepatitis C among in Tehran, Iran. *Gene Ther Mol Biol* 2008; **12**: 95-100
161 **Ataei B**, Nokhodian Z, Babak A, Shoaei P, Mohhammadzadeh M, Sadeghi R. Seroprevalence of Hepatitis C (HCV) and Human Immunodeficiency Virus (HIV) infection among street children in Isfahan, Iran (in Persian). *TUMJ* 2010; **67**: 811-816
162 **Sharifi-Mood B**, Alavi-Naini R, Salehi M, Hashemi M, Rakhshani F. Spectrum of clinical disease in a series of hospitalized HIV-infected patients from southeast of Iran. *Saudi Med J* 2006; **27**: 1362-1366 [PMID: 16951774]
163 **Mohammadi M**, Talei G, Sheikhian A, Ebrahimzade F, Pournia Y, Ghasemi E, Boroun H. Survey of both hepatitis B virus (HBsAg) and hepatitis C virus (HCV-Ab) coinfection among HIV positive patients. *Virol J* 2009; **6**: 202 [PMID: 19922624 DOI: 10.1186/1743-422X-6-202]
164 **SeyedAlinaghi S**, Valiollahi P, Paydary K, Emamzadeh-Fard S, Mohraz M. Prevalence of hepatitis B (HBV) and C (HCV) viruses coinfections among HIV infected people in Iran. *J AIDS & HIV Res* 2012; **4**: 181-186
165 **Zahedi MJ**, Moghaddam SD, Abasi MH, Parnian M, Shokoohi M. Hepatitis B, C virus co-infection and behavioral risks in HIV-positive patients in southern Iran. *J Pak Med Assoc* 2014; **64**: 134-137 [PMID: 24640799]
166 **Davarpanah MA**, Khademolhosseini F, Rajaeefard A, Tavassoli A, Yazdanfar SK, Rezaianzadeh A. Hepatitis C Virus Infection in HIV Positive Attendees of Shiraz Behavioral Diseases Consultation Center in Southern Iran. *Indian J Community Med* 2013; **38**: 86-91 [PMID: 23878420 DOI: 10.4103/0970-0218.112437]
167 **Ramezani A**, Mohraz M, Gachkar L. Epidemiologic situation of human immunodeficiency virus (HIV/AIDS patients) in a private clinic in Tehran, Iran. *Arch Iran Med* 2006; **9**: 315-318 [PMID: 17061601]
168 **Ataei B**, Tayeri K, Kassaian N, Farajzadegan Z, Babak A. Hepatitis B and C among patients infected with human immunodeficiency virus in Isfahan, Iran: seroprevalence and associated factors. *Hepat Mon* 2010; **10**: 188-192 [PMID: 22308138]
169 **Alipour A**, Rezaianzadeh A, Hasanzadeh J, Rajaeefard A, Davarpanah MA, Hasanabadi M. High prevalence of HCV coinfection in HIV-infected individuals in Shiraz, Islamic Republic of Iran. *East Mediterr Health J* 2013; **19**: 975-981 [PMID: 24684094]
170 **SeyedAlinaghi S**, Jam S, Mehrkhani F, Fattahi F, Sabzvari D, Kourorian Z, Jabbari H, Mohraz M. Hepatitis-C and hepatitis-B co-infections in patients with human immunodeficiency virus in Tehran, Iran. *Acta Med Iran* 2011; **49**: 252-257 [PMID: 21713737]
171 **Alavi SM**, Etemadi A. HIV/HBV, HIV/HCV and HIV/HTLV-1 co infection among injecting drug user patients hospitalized at the infectious disease ward of a training hospital in Iran. *Pak J Med Sci* 2007; **23**: 510-513
172 **Khosravi A**, Bahmani M, Ghezel-Sofla I. Co-infection by hepatitis C virus in human immunodeficiency virus infected patients in southwest of Iran. *IJCID* 2010; **5**: 223-227
173 **Babamahmoodi F**, Heidari Gorji MA, Mahdi Nasehi M, Delavarian L. The prevalence rate of hepatitis B and hepatitis C co-infection in HIV positive patients in Mazandaran province, Iran. *Med Glas* (Zenica) 2012; **9**: 299-303 [PMID: 22926367]
174 **Saleh F**, Azizi H, Kheirandish F, Rashnou F, Mousavi Nasab SD, Movahedi F, Azizi M. Frequency of HCV and HBV Co-infections in HIV Positive Patient in City of Iran: A Cross-Sectional Study. *IJTDH* 2015; **6**: 14-19 [DOI: 10.9734/IJTDH/2015/13282]
175 **Tampaki M**, Koskinas J. Extrahepatic immune related manifestations in chronic hepatitis C virus infection. *World J Gastroenterol*

2014; **20**: 12372-12380 [PMID: 25253938 DOI: 10.3748/wjg.v20.i35.12372]
176 **El Baki AMA**, Arab MAE, El Mageed NA. Chronic Hepatitis C Virus (HCV)-associated Cryoglobulinemia and its possible impact on the skin in Egyptian Patients. *EJHM* 2010; **39**: 197-207
177 **Gragnani L**, Fognani E, Piluso A, Zignego AL. Hepatitis C virus-related mixed cryoglobulinemia: is genetics to blame? *World J Gastroenterol* 2013; **19**: 8910-8915 [PMID: 24379615 DOI: 10.3748/wjg.v19.i47.8910]
178 **Jadali Z**. Hepatitis C virus cryoglobulinemia and non-hodgkin lymphoma. *Hepat Mon* 2012; **12**: 85-91 [PMID: 22509184 DOI: 10.5812/hepatmon.818]
179 **Anis S**, Muzaffar R, Ahmed E, Ali S, Nadir A, Naqvi A, Rizvi AH. Cryoglobulinaemia and autoimmune markers in hepatitis C virus infected patients on renal replacement therapy. *J Pak Med Assoc* 2007; **57**: 225-229 [PMID: 17571476]
180 **Morcos NY**, Hassanein MH, Eliase NY, Bayoumi Eel-D, Mustafa IM. Chronic hepatitis C virus infection: prevalence of cryoglobulinemia and renal affection in the Egyptian patients. *J Egypt Soc Parasitol* 2010; **40**: 539-550 [PMID: 21246960]
181 **Gharagozloo S**, Khoshnoodi J, Shokri F. Hepatitis C virus infection in patients with essential mixed cryoglobulinemia, multiple myeloma and chronic lymphocytic leukemia. *Pathol Oncol Res* 2001; **7**: 135-139 [PMID: 11458277 DOI: 10.1007/BF03032580]
182 **Owlia MB**, Sami R, Akhondi M, Salimzadeh A. Cryoglobulinaemia in hepatitis C-positive patients in Iran. *Singapore Med J* 2007; **48**: 1136-1139 [PMID: 18043843]
183 **Larijani B**, Bandarian F. On Diabetes Mellitus and Hepatitis C Infection: Should the Patients be Screened? *Hepat Mon* 2009; **9**: 92-94
184 **Allison ME**, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. *J Hepatol* 1994; **21**: 1135-1139 [PMID: 7699240 DOI: 10.1016/S0168-8278(05)80631-2]
185 **Negro F**, Alaei M. Hepatitis C virus and type 2 diabetes. *World J Gastroenterol* 2009; **15**: 1537-1547 [PMID: 19340895 DOI: 10.3748/wjg.15.1537]
186 **Mason A**, Nair S. Is type II diabetes another extrahepatic manifestation of HCV infection? *Am J Gastroenterol* 2003; **98**: 243-246 [PMID: 12591036 DOI: 10.1111/j.1572-0241.2003.07269.x]
187 **Hwang SJ**, Chen LK. Chronic hepatitis C and diabetes mellitus. *J Chin Med Assoc* 2006; **69**: 143-145 [PMID: 16689193 DOI: 10.1016/S1726-4901(09)70194-7]
188 **Abenavoli L**, Masarone M, Peta V, Milic N, Kobyliak N, Rouabhia S, Persico M. Insulin resistance and liver steatosis in chronic hepatitis C infection genotype 3. *World J Gastroenterol* 2014; **20**: 15233-15240 [PMID: 25386071 DOI: 10.3748/wjg.v20.i41.15233]
189 **Greca LF**, Pinto LC, Rados DR, Canani LH, Gross JL. Clinical features of patients with type 2 diabetes mellitus and hepatitis C infection. *Braz J Med Biol Res* 2012; **45**: 284-290 [PMID: 22286533 DOI: 10.1590/S0100-879X2012007500013]
190 **Olokoba A**, Badung L, Abdulrahman M, Salawu F, Danburam A, Aderibigbe S, Midala J, Tidi S. Hepatitis C virus infection in Nigerians with diabetes mellitus. *Am J Sci Ind Res* 2010; **1**: 135-138 [DOI: 10.5251/ajsir.2010.1.2.135.138]
191 **Naing C**, Mak JW, Ahmed SI, Maung M. Relationship between hepatitis C virus infection and type 2 diabetes mellitus: meta-analysis. *World J Gastroenterol* 2012; **18**: 1642-1651 [PMID: 22529694 DOI: 10.3748/wjg.v18.i14.1642]
192 **Aghamohammadzadeh N**, Ghotaslou R, Javadi M, Najafipour F, Niafar M. Prevalence of hepatitis C infection among type 2 diabetic patients (in Persian). *MJTUMS* 2010; **32**: 7-11
193 **Alavian SM**, Hajarizadeh B, Nematizadeh F, Larijani B. Prevalence and determinants of diabetes mellitus among Iranian patients with chronic liver disease. *BMC Endocr Disord* 2004; **4**: 4 [PMID: 15555059 DOI: 10.1186/1472-6823-4-4]
194 **Janbakhsh A**, Mansouri F, Vaziri S, Sayad B, Afsharian M, Soleiman Meigouni S. Prevalence and coexistence of diabetes in HIV, HCV and HIV/HCV co-infection in Kermanshah-Iran (in Persian). *Behbood* 2012; **15**: 473-480
195 **Metanat M**, Sharifi-Mood B, Sanei-Moghaddam E, Alavi-Naini R, Naderi M, Khosravi S. Prevalence of hepatitis C among diabetes mellitus patients in Zahedan (in Persian). *Tabibe Shargh Res J* 2006; **8**: 179-186
196 **Bahar A**, Azizi F. Insulin Resistance and β Cell Function in Patients with Chronic Hepatitis and Impaired Glucose Tolerance. *Int J Endocrinol Metab* 2007; **4**: 125-133
197 **Persico M**, Masarone M, La Mura V, Persico E, Moschella F, Svelto M, Bruno S, Torella R. Clinical expression of insulin resistance in hepatitis C and B virus-related chronic hepatitis: differences and similarities. *World J Gastroenterol* 2009; **15**: 462-466 [PMID: 19152451 DOI: 10.3748/wjg.15.462]
198 **Rouabhia S**, Malek R, Bounecer H, Dekaken A, Bendali Amor F, Sadelaoud M, Benouar A. Prevalence of type 2 diabetes in Algerian patients with hepatitis C virus infection. *World J Gastroenterol* 2010; **16**: 3427-3431 [PMID: 20632447 DOI: 10.3748/wjg.v16.i27.3427]
199 **Himoto T**, Masaki T. Extrahepatic manifestations and autoantibodies in patients with hepatitis C virus infection. *Clin Dev Immunol* 2012; **2012**: 871401 [PMID: 22988469 DOI: 10.1155/2012/871401]
200 **Jadali Z**, Alavian SM. Autoimmune diseases co-existing with hepatitis C virus infection. *Iran J Allergy Asthma Immunol* 2010; **9**: 191-206 [PMID: 21131699]
201 **Jadali Z**. Autoimmune thyroid disorders in hepatitis C virus infection: Effect of interferon therapy. *Indian J Endocrinol Metab* 2013; **17**: 69-75 [PMID: 23776855 DOI: 10.4103/2230-8210.107856]
202 **Yang DH**, Ho LJ, Lai JH. Useful biomarkers for assessment of hepatitis C virus infection-associated autoimmune disorders. *World J Gastroenterol* 2014; **20**: 2962-2970 [PMID: 24659887 DOI: 10.3748/wjg.v20.i11.2962]
203 **Chong VH**. Autoimmune thyroiditis and delayed onset psoriasis in association with combination therapy for chronic hepatitis C infection. *Singapore Med J* 2011; **52**: e20-e22 [PMID: 21373724]
204 **Ziaee A**, Esfehanian F, Sarreshtedari M. Thyroid dysfunction in patients with chronic viral hepatitis B and C during alpha interferon therapy. *Hepat Mon* 2009; **9**: 110-113
205 **Rahimi MA**, Sayad B, Tahamoli Roudsari A, Shahebrahimi K, Shirvani M, Rezaei M. Autoimmune thyroid disorder in patient with chronic hepatitis C before treatment (in Persian). *Behbood* 2011; **15**: 208-212
206 **Jadali Z**, Esfahanian F, Farhud DD, Alavian SM, Soltan Dallal MM. Hashimoto's Thyroiditis and Its Association with Hepatitis C Virus Infection. *Int J Endocrinol Metab* 2005; **3**: 116-120
207 **Jadali Z**, Esfahanian F, Eslami MB, Sanati MH. Serum Antibodies against Hepatitis C Virus in Iranian Patients with Graves' Disease. *Iran J Allergy Asthma Immunol* 2005; **4**: 91-94 [PMID: 17301428]
208 **Ansar A**, Zamanian A, Farschian M, Sorouri R, Mobaien AR. Comparison of seropositivity of HCV between oral lichen planus and healthy control group in Hamedan province (west of Iran). *Dermatol* 2011; **2**: 181-184
209 **Alavian SM**, Mahboobi N, Mahboobi N, Karayiannis P. Oral conditions associated with hepatitis C virus infection. *Saudi J Gastroenterol* 2013; **19**: 245-251 [PMID: 24195977 DOI: 10.4103/1319-3767.121032]
210 **Asaad T**, Samdani AJ. Association of lichen planus with hepatitis C virus infection. *Ann Saudi Med* 2005; **25**: 243-246 [PMID: 16119527]
211 **Carrozzo M**, Scally K. Oral manifestations of hepatitis C virus infection. *World J Gastroenterol* 2014; **20**: 7534-7543 [PMID: 24976694 DOI: 10.3748/wjg.v20.i24.7534]
212 **Petti S**, Rabiei M, De Luca M, Scully C. The magnitude of the association between hepatitis C virus infection and oral lichen planus: meta-analysis and case control study. *Odontology* 2011; **99**: 168-178 [PMID: 21505737 DOI: 10.1007/s10266-011-0008-3]
213 **Rabiei M**, Mohtasham Amiri Z. Prevalence of Lichen Planus in HCV infected patients ofGilan province, 2002 (in Persian). *Beheshti Univ Dent J* 2003; **21**: 193-200

214 **Khatibi M**, Ahmadinejad Z, Nasiri-Toosi M, Hajibaygi B, Zahedipour H. Prevalence of oral lichen planus in HCV infected patients: the effective factors (in Persian). *Tehran Univ Med J* 2008; **66**: 585-589

215 **Rahnama Z**, Esfandiarpour I, Farajzadeh S. The relationship between lichen planus and hepatitis C in dermatology outpatients in Kerman, Iran. *Int J Dermatol* 2005; **44**: 746-748 [PMID: 16135143 DOI: 10.1111/j.1365-4632.2004.02176.x]

216 **Taghavi Zenouz A**, Mehdipour M, Gholizadeh N, Naghili B, Jafari Heydarlou M. Evaluation of Relationship between Lichen Planus and HCV Antibody. *J Dent Res Dent Clin Dent Prospects* 2010; **4**: 10-13 [PMID: 22991587]

217 **Rastin M**, Khoee AR, Tabasi N, Sheikh A, Ziaolhagh S, Esmaeeli E, Zamani S, Khazaee M, Mahmoudi M. Evaluation of HTLV-I and HCV Prevalence in Non-Hodgkin's Lymphoma. *Iran J Basic Med Sci* 2013; **16**: 242-246 [PMID: 24470870]

218 **Aledavood SA**, Ghavam-Nasiri MR, Ghaffarzadegan K, Raziee HR, Saboori G, Anvari K, Mohtashami S, Ahadi M, Memar B. Hepatitis-C Infection Incidence Among the non-Hodgkin's B-cell Lymphoma Patients in the Northeast of Iran. *Iran J Cancer Prev* 2014; **7**: 147-151 [PMID: 25250166]

219 **Rezaeian AA**, Yaghobi R, Nia MR, Mirzaee M, Ramzi M, Shaheli M. Etiology of hepatitis G virus (HGV) and hepatitis type C virus (HCV) infections in non-Hodgkin's lymphoma patients in Southern Iran. *Afr J Biotechnol* 2012; **11**: 11659-11664

220 **Datta S**, Chatterjee S, Policegoudra RS, Gogoi HK, Singh L. Hepatitis viruses and non-Hodgkin's lymphoma: A review. *World J Virol* 2012; **1**: 162-173 [PMID: 24175222 DOI: 10.5501/wjv.v1.i6.162]

221 **Gisbert JP**, García-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in B-cell non-Hodgkin's lymphoma: systematic review and meta-analysis. *Gastroenterology* 2003; **125**: 1723-1732 [PMID: 14724825 DOI: 10.1053/j.gastro.2003.09.025]

222 **Civan J**, Hann HW. Hepatitis C virus mediated hepatocellular carcinoma: a focused review for a time of changing therapeutic options. *N AJ Med Sci* 2014; **7**: 8-16 [DOI: 10.7156/najms.2014.0701008]

223 **Fazeli Z**, Pourhoseingholi MA, Vahedi M, Zali MR. Burden of hepatocellular carcinoma in Iran. *Asian Pacific J Cancer Prev* 2012; **13**: 5955-5958 [DOI: 10.7314/APJCP.2012.13.12.5955]

224 **Saiedi Hosseini SY**. Risk factors and incidence of hepatocellular carcinoma in Southeast Iran. *Hepat Mon* 2011; **11**: 666-667 [PMID: 22140394 DOI: 10.5812/kowsar.1735143X.710]

225 **Hajiani E**, Masjedizadeh R, Hashemi J, Azmi M, Rajabi T. Risk factors for hepatocellular carcinoma in Southern Iran. *Saudi Med J* 2005; **26**: 974-977 [PMID: 15983686]

226 **Kabir A**, Alavian SM, Keyvani H. Distribution of hepatitis C virus genotypes in patients infected by different sources and its correlation with clinical and virological parameters: a preliminary study. *Comp Hepatol* 2006; **5**: 4 [PMID: 17014721 DOI: 10.1186/1476-5926-5-4]

227 **Dusheiko G**, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S, McIntyre N, Simmonds P. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology* 1994; **19**: 13-18 [PMID: 8276349 DOI: 10.1002/hep.1840190104]

228 **Samimi-Rad K**, Nategh R, Malekzadeh R, Norder H, Magnius L. Molecular epidemiology of hepatitis C virus in Iran as reflected by phylogenetic analysis of the NS5B region. *J Med Virol* 2004; **74**: 246-252 [PMID: 15332273 DOI: 10.1002/jmv.20170]

229 **Gower E**, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol* 2014; **61**: S45-S57 [PMID: 25086286 DOI: 10.1016/j.jhep.2014.07.027]

230 **Ramia S**, Eid-Fares J. Distribution of hepatitis C virus genotypes in the Middle East. *Int J Infect Dis* 2006; **10**: 272-277 [PMID: 16564719 DOI: 10.1016/j.ijid.2005.07.008]

231 **Jamalidoust M**, Namayandeh M, Asaei S, Aliabadi N, Ziyaeyan M. Determining hepatitis C virus genotype distribution among high-risk groups in Iran using real-time PCR. *World J Gastroenterol* 2014; **20**: 5897-5902 [PMID: 24914351 DOI: 10.3748/wjg.v20.i19.5897]

232 **Jahanbakhsh Sefidi F**, Keyvani H, Monavari SH, Alavian SM, Fakhim S, Bokharaei-Salim F. Distribution of hepatitis C virus genotypes in Iranian chronic infected patients. *Hepat Mon* 2013; **13**: e7991 [PMID: 23550108 DOI: 10.5812/hepatmon.7991]

233 **Farshadpour F**, Makvandi M, Samarbafzadeh AR, Jalalifar MA. Determination of hepatitis C virus genotypes among blood donors in Ahvaz, Iran. *Indian J Med Microbiol* 2010; **28**: 54-56 [PMID: 20061766 DOI: 10.4103/0255-0857.58731]

234 **Sharifi Z**, Shooshtari MM, Kermani FR. Identification of HCV genotypes in HCV infected blood donors. *Indian J Microbiol* 2010; **50**: 275-279 [PMID: 23100841 DOI: 10.1007/s12088-010-0059-0]

235 **Amini S**, Mahmoodi Farahani Majd Abadi M, Joulaie M, MH A. Distribution of hepatitis C virus genotypes in Iran: a population-based study. *Hepat Mon* 2009; **9**: 95-102

236 **Hajia M**, Amirzargar A, Khedmat H, Shahrokhi N, Farzanehkhah M, Ghorishi S, Biglari S, Salehinodeh A, Sarafnejad A. Genotyping Pattern among Iranian HCV Positive Patients. *Iran J Public Health* 2010; **39**: 39-44 [PMID: 23113005]

237 **Zarkesh-Esfahani SH**, Kardi MT, Edalati M. Hepatitis C virus genotype frequency in Isfahan province of Iran: a descriptive cross-sectional study. *Virol J* 2010; **7**: 69 [PMID: 20331907 DOI: 10.1186/1743-422X-7-69]

238 **Esmaeilzadeh A**, Erfanmanesh M, Ghasemi S, Mohammadi F. Serological assay and genotyping of hepatitis C virus in infected patients in zanjan province. *Hepat Mon* 2014; **14**: e17323 [PMID: 25368655 DOI: 10.5812/hepatmon.17323]

239 **Hadinedoushan H**, Salmanroghani H, Amirbaigy MK, Akhondi-Meybodi M. Hepatitis C virus genotypes and association with viral load in yazd, central province of iran. *Hepat Mon* 2014; **14**: e11705 [PMID: 24693314 DOI: 10.5812/hepatmon.11705]

240 **Vossughinia H**, Goshayeshi L, Bayegi HR, Sima H, Kazemi A, Erfani S, Abedini S, Goshayeshi L, Ghaffarzadegan K, Nomani H, Jamehdar S. Prevalence of Hepatitis C Virus Genotypes in Mashhad, Northeast Iran. *Iran J Public Health* 2012; **41**: 56-61 [PMID: 23193507]

241 **Keyvani H**, Alizadeh AH, Alavian SM, Ranjbar M, Hatami S. Distribution frequency of hepatitis C virus genotypes in 2231 patients in Iran. *Hepatol Res* 2007; **37**: 101-103 [PMID: 17300704 DOI: 10.1111/j.1872-034X.2007.00015.x]

242 **Moradi A**, Semnani S, Keshtkar A, Khodabakhshi B, Kazeminejad V, Molana A, Roshandel G, Besharat S. Distribution of hepatitis C virus genotype among HCV infected patients in Golestan province, Iran (in Persian). *Govaresh* 2010; **15**: 7-13

243 **Hamidi-Fard M**, Samarbaf-Zadeh A, Makvandi M, Hajiani E. Determination of HCV Genotypes among Chronic Hepatic Patients in Ahvaz. *Iran J virol* 2010; **3**: 12-16

244 **Rafiei A**, Darzyani AM, Taheri S, Haghshenas MR, Hosseinian A, Makhlough A. Genetic diversity of HCV among various high risk populations (IDAs, thalassemia, hemophilia, HD patients) in Iran. *Asian Pac J Trop Med* 2013; **6**: 556-560 [PMID: 23768829 DOI: 10.1016/S1995-7645(13)60096-6]

245 **Ghane M**, Eghbali M, Nejad HR, Saeb K, Farahani M. Distribution of hepatitis C virus genotypes amongst the beta-thalassemia patients in North of Iran. *Pak J Biol Sci* 2012; **15**: 748-753 [PMID: 24171261 DOI: 10.3923/pjbs.2012.748.753]

246 **Ranjbar Kermani F**, Sharifi Z, Ferdowsian F, Paz Z, Zamanian M. Distribution of Hepatitis C Virus Genotypes Among Chronic Infected Injecting Drug Users in Tehran, Iran. *Jundishapur J Microbiol* 2013; **6**: 265-268 [DOI: 10.5812/jjm.5191]

247 **Samimi-Rad K**, Nasiri Toosi M, Masoudi-Nejad A, Najafi A, Rahimnia R, Asgari F, Shabestari AN, Hassanpour G, Alavian SM, Asgari F. Molecular epidemiology of hepatitis C virus among injection drug users in Iran: a slight change in prevalence of HCV genotypes over time. *Arch Virol* 2012; **157**: 1959-1965 [PMID: 22695769 DOI: 10.1007/s00705-012-1369-9]

248 **Somi MH**, Keivani H, Ardalan MR, Farhang S, Pouri AA. Hepatitis C virus genotypes in patients with end-stage renal disease in East Azerbaijan, Iran. *Saudi J Kidney Dis Transpl* 2008; **19**: 461-465 [PMID: 18445914]

249 **Hosseini-Moghaddam SM**, Keyvani H, Kasiri H, Kazemeyni SM, Basiri A, Aghel N, Alavian SM. Distribution of hepatitis C virus genotypes among hemodialysis patients in Tehran--a multicenter study. *J Med Virol* 2006; **78**: 569-573 [PMID: 16555284 DOI: 10.1002/jmv.20577]

250 **Davarpanah MA**, Saberi-Firouzi M, Bagheri Lankarani K, Mehrabani D, Behzad Behbahani A, Serati A, Ardebili M, Yousefi M, Khademolhosseini F, Keyvani-Amineh H. Hepatitis C virus genotype distribution in Shiraz, southern Iran. *Hepat Mon* 2009; **9**: 122-127

251 **Bokharaei-Salim F**, Keyvani H, Monavari SH, Alavian SM, Madjd Z, Toosi MN, Mohammad Alizadeh AH. Occult hepatitis C virus infection in Iranian patients with cryptogenic liver disease. *J Med Virol* 2011; **83**: 989-995 [PMID: 21503911 DOI: 10.1002/jmv.22044]

252 **Jain P**, Nijhawan S. Occult hepatitis C virus infection is more common than hepatitis B infection in maintenance hemodialysis patients. *World J Gastroenterol* 2008; **14**: 2288-2289 [PMID: 18407613 DOI: 10.3748/wjg.14.2288]

253 **Carreño V**, Bartolomé J, Castillo I, Quiroga JA. New perspectives in occult hepatitis C virus infection. *World J Gastroenterol* 2012; **18**: 2887-2894 [PMID: 22736911 DOI: 10.3748/wjg.v18.i23.2887]

254 **De Marco L**, Manzini P, Trevisan M, Gillio-Tos A, Danielle F, Balloco C, Pizzi A, De Filippo E, D'Antico S, Violante B, Valfrè A, Curti F, Merletti F, Richiardi L. Prevalence and follow-up of occult HCV infection in an Italian population free of clinically detectable infectious liver disease. *PLoS One* 2012; **7**: e43541 [PMID: 22927986 DOI: 10.1371/journal.pone.0043541]

255 **Carreño V**. Seronegative occult hepatitis C virus infection: clinical implications. *J Clin Virol* 2014; **61**: 315-320 [PMID: 25304062 DOI: 10.1016/j.jcv.2014.09.007]

256 **Pham TN**, Michalak TI. Occult hepatitis C virus infection and its relevance in clinical practice. *J Clin Exp Hepatol* 2011; **1**: 185-189 [PMID: 25755384 DOI: 10.1016/S0973-6883(11)60130-8]

257 **Keyvani H**, Bokharaei-Salim F, Monavari SH, Esghaei M, Nassiri Toosi M, Fakhim S, Sadigh ZA, Alavian SM. Occult hepatitis C virus infection in candidates for liver transplant with cryptogenic cirrhosis. *Hepat Mon* 2013; **13**: e11290 [PMID: 24082889 DOI: 10.5812/hepatmon.11290]

258 **Farahani M**, Bokharaei-Salim F, Ghane M, Basi A, Meysami P, Keyvani H. Prevalence of occult hepatitis C virus infection in Iranian patients with lymphoproliferative disorders. *J Med Virol* 2013; **85**: 235-240 [PMID: 23168913 DOI: 10.1002/jmv.23460]

259 **Makvandi M**, Khalafkhany D, Rasti M, Neisi N, Omidvarinia A, Mirghaed AT, Masjedizadeh A, Shyesteh AA. Detection of Hepatitis C virus RNA in peripheral blood mononuclear cells of patients with abnormal alanine transaminase in Ahvaz. *Indian J Med Microbiol* 2014; **32**: 251-255 [PMID: 25008816 DOI: 10.4103/0255-0857.136553]

260 **Rezaee Zavareh MS**, Alavian SM, Karimisari H, Shafiei M, Saiedi Hosseini SY. Occult hepatitis C virus infection in patients with autoimmune hepatitis. *Hepat Mon* 2014; **14**: e16089 [PMID: 25337141 DOI: 10.5812/hepatmon.16089]

261 **Ramezani A**, Eslamifar A, Banifazl M, Keyvani H, Razeghi E, Ahmadi F, Amini M, Gachkar L, Bavand A, Aghakhani A. Occult HCV infection in Hemodialysis Patients with Elevated Liver Enzymes (in Persian). *AMUJ* 2014; **16**: 34-40

262 **De Marco L**, Gillio-Tos A, Fiano V, Ronco G, Krogh V, Palli D, Panico S, Tumino R, Vineis P, Merletti F, Richiardi L, Sacerdote C. Occult HCV infection: an unexpected finding in a population unselected for hepatic disease. *PLoS One* 2009; **4**: e8128 [PMID: 19956542 DOI: 10.1371/journal.pone.0008128]

P- Reviewer: Hu ZJ, Lo SY, Stahmeyer JT **S- Editor**: Yu J
L- Editor: Filipodia **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10811

World J Gastroenterol 2015 October 14; 21(38): 10811-10823
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

Injecting drug use: A vector for the introduction of new hepatitis C virus genotypes

Simona Ruta, Costin Cernescu

Simona Ruta, Carol Davila University of Medicine and Pharmacy, Stefan S. Nicolau Institute of Virology, 030304 Bucharest, Romania

Costin Cernescu, Stefan S. Nicolau Institute of Virology, 030304 Bucharest, Romania

Author contributions: Ruta S and Cernescu C contributed equally to this work, designed the study, analyzed the data, and wrote the paper.

Supported by NIH grant 5P30 AI036211-19 (partially, subcontract PO 5600167489, through the Baylor International Pediatric AIDS Initiative); and the Romanian Academy Programme for the Stefan S. Nicolau Institute of Virology.

Conflict-of-interest statement: We undersigned, Simona Ruta and Costin Cernescu, authors of the review entitled "Injecting drug use: A vector for introduction of new hepatitis C virus genotypes", submitted to World Journal of Gastroenterology declare no competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work.

Correspondence to: Simona Ruta, MD, PhD, Professor, Virology Chair, Carol Davila University of Medicine and Pharmacy, Stefan S. Nicolau Institute of Virology, 285 sos Mihai Bravu, 030304 Bucharest, Romania. simona@simonaruta.ro
Telephone: +40-321-3242590
Fax: +40-213-241471

Received: April 27, 2015
Peer-review started: April 29, 2015
First decision: June 2, 2015
Revised: June 19, 2015
Accepted: September 15, 2015

Article in press: September 15, 2015
Published online: October 14, 2015

Abstract

Hepatitis C virus (HCV) genotypes' monitoring allows real-time insight into the dynamic changes that occur in the global epidemiological picture of HCV infection. Intravenous drug use is currently the primary driver for HCV transmission in developed and developing countries. The distribution of HCV genotypes/subtypes differs significantly between people who inject drugs (PWID) and the general population. HCV genotypes that previously exhibited a limited geographical distribution (3a, 4) are becoming more prevalent in this high-risk group. Immigration from HCV-endemic countries and the evolving networks of HCV transmission in PWID influence HCV genotypes distribution in Europe. Social vulnerabilities (*e.g.*, unemployment, homelessness, and limited access to social and healthcare insurances systems) are important triggers for illicit drug use, which increases the associated risks of HCV infection and the frequent emergence of less prevalent genotypes. Genotype/subtype determination bears important clinical consequences in the progression of liver disease, susceptibility to antiviral therapies and the emergence of resistance-associated variants. An estimated half of the chronically HCV-infected PWID are unaware of their infection, and only one in ten of those diagnosed enter treatment. Nevertheless, PWID exhibit high response rates to new antiviral regimens, and the level of HCV reinfection is unexpectedly low. The focus of the healthcare system must be on the early detection and treatment of infection, to avoid late presentations that are associated with high levels of viremia and liver fibrosis, which may diminish the therapeutic success rate.

Key words: Hepatitis C; Hepatitis C virus genotypes;

Intravenous drug use; People who inject drugs; Direct-acting antivirals

Core tip: Careful surveillance of circulating hepatitis C virus (HCV) genotypes/subtypes is compulsory to reconstruct the natural history of HCV epidemics and viral transmission chains in high-risk populations, such as people who inject drugs (PWIDs). Genotypes 1a and 3a predominate among PWID worldwide, but genotype 4 has been reported with increased frequency. This review analyzes the factors that underlie the different distributions of HCV genotypes in PWID relative to the general population and highlights the need for early diagnosis and care in this vulnerable group, which responds well to new antiviral therapies and exhibits unexpectedly low reinfection rates.

Ruta S, Cernescu C. Injecting drug use: A vector for the introduction of new hepatitis C virus genotypes. *World J Gastroenterol* 2015; 21(38): 10811-10823 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10811.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10811

INTRODUCTION

Non-communicable diseases have replaced infectious diseases as the most important causes of morbidity in the general population in the last two decades. Communicable diseases accounted for 24.9% of the total 52.8 million deaths reported worldwide in 2010, which is an important decrease relative to 1990, when these diseases were responsible for 34.1% of 46.5 million deaths[1]. Human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), tuberculosis and chronic viral hepatitis are important exceptions. There are significant regional variations in this trend[2], which highlight the importance of continuous epidemiological monitoring of all diseases with public health relevance. Chronic hepatitis C is a significant cause of liver-related morbidity and mortality. There are more than 180 million persons chronically infected with the hepatitis C virus (HCV) worldwide who are at risk of developing liver cirrhosis, end-stage liver disease and hepatocellular carcinoma. An additional 3-4 million persons are newly infected each year[3]. It is estimated that 57% and 78% of patients with active viral replication will develop cirrhosis and hepatocellular carcinoma, respectively, within two or three decades in the absence of antiviral treatment, with 500000 deaths reported annually[4]. A large community-based Australian study demonstrated that people with hepatitis C exhibited a significantly increased risk of liver-related deaths compared with the general population[5].

HCV belongs to the Flaviviridae family, Hepacivirus genus. Humans are the only reservoir for HCV, but experimental infection in chimpanzees is possible. New members of the Hepacivirus and the related Pegivirus genera (pathogens for dogs and horses) have been recently discovered in rodents and bats, which serve as models for HCV biological studies[6,7]. HCV is an enveloped, positive single-stranded RNA virus, and its genome encodes three structural (core and envelope E1 and E2) and seven non-structural (NS) genes. Three NS genes are essential for the viral replicative cycle, and these genes are targets for direct-acting antivirals (DAA)[8]: (1) NS3-4A protease, which is involved in post-translational viral protein processing; (2) NS5B viral polymerase, which directs nucleic acid replication; and (3) NS5A, which encodes a phosphoprotein that participates in genome replication and the assembly of progeny virions.

The error-prone nature of the HCV NS5B polymerase and the accumulation of mutations in a small hypervariable region in the envelope-encoding genes generate a high level of variability. This variability is translated in the existence of 7 major HCV genotypes (with 30%-35% variation at the nucleotide level); 67 subtypes (with less than 15% difference at the nucleotides level), each composed by a myriad of viral quasispecies; and 9 recombinant forms (*e.g*., the most frequently reported, G2k/1b, which is represented by multiple isolates)[9,10]. Each genotype exhibits a different degree of variability: 7 subtypes in G1; 11 subtypes in G2; 6 subtypes in G3; 17 subtypes in G4; 24 subtypes in G6; and only 1 subtype in G5 and 7. There are multiple consequences related to this enormous viral heterogeneity: (1) reinfections with a different genotype are possible because of the very limited cross-antigenicity; (2) the emergence of immune-escape mutants, which accounts for the high rate of chronic infections; (3) the therapeutic response is genotype- and subtype-specific; and (4) the selection of viral-resistant strains contribute to the need for combination therapies.

The most important method of HCV spreading is parenteral transmission *via* intravenous drug use, unsafe medical procedures, including breaches in injection safety and infection prevention practices in hospitals, and the administration of unscreened blood products[11,12]. Approximately 80% of all HCV cases are concentrated in low- and middle-income countries in the Middle East, North Africa, South and East Asia (Table 1). The prevalence of HCV in North America is generally low (< 1.5%), with an increase to 5.4%-20% in military veterans[13]. The estimated mean prevalence of HCV infection is 1.03% in Europe, but large geographical variations are registered, from less than 0.2% in the Northern countries to approximately 1% in the Western countries. The highest rates are reported in Romania (3.3%) and rural areas in Greece and Italy[14,15]. The most affected age group is 25-34 years, the notification rates are 22.3 in men *vs* 13.3

Table 1 The burden of hepatitis C virus infection in the WHO regions and the proportion of people who inject drugs

WHO regions	Population (millions)[1]	Estimated HCV seroprevalence[2] (%)	Estimated prevalence of viremic persons[3] (%)	Proportion of PWID lowest-highest estimates[4]
Africa	1396	1.0-5.3	0.6-4.1	55-97
Latin America	572	0.9-1.3	0.6-1.0	69-96
North America	355	1.3	0.8-1.0	
Europe	751	0.9-3.3	0.6-2.3	36-69
Asia	3985	1.1-5.4	0.7-2.3	50-53

Data sources: [1]World health statistics, 2014 (available from: www.who.int/world_health_statistics); [2]Mohd Hanafiah K, 2013[3]; [3]Gower E, 2014[10] and Global Health Observatory Data Repository (http://apps.who.int/gho/data); [4]World Drug report 2014[12]. PWID: Proportion of people who inject drugs; HCV: Hepatitis C virus.

in women per 100000 population. However, the male-to-female ratio varies considerably between countries and ranges from 0.6 in Romania to 17.7 in the Netherlands[15,16].

The seroprevalence data must be interpreted cautiously, because the methodology for HCV screening is not uniform across different regions. National estimates in Europe sometimes derive from targeted studies in specific regions of a single country, in non-clinical settings, or in selected populations[17]. A targeted screening strategy involves the testing of persons who are at risk for acquiring HCV infection (*e.g.*, drug users, HIV-infected subjects, inmates, migrants from endemic countries, *etc.*) or persons with clinical signs or biochemical modifications that are suggestive of liver disease. The Center for Diseases Control (CDC) in the United States recommended HCV birth-cohort screening for persons born from 1945 to 1965[18,19]. A strategy aimed at reducing the discrepancies in reporting and promoting early HCV diagnosis and access to treatment is needed in Europe. Persons who exhibit positive HCV antibody results must be tested for active viral replication to confirm the diagnosis and assess the need for HCV therapy[20-22].

INTRAVENOUS DRUG USE - THE PRIMARY DRIVER OF HCV TRANSMISSION

People who inject drugs (PWID) account for 0.2%-0.5% of the world's population, but represent approximately 6.8% of persons infected with HCV[12,23]. The global seroprevalence of HCV infection in PWID is approximately 51%, which means that at least 7.2 million PWID are living with HCV[12].

It is estimated that 1980000 years of life were lost because of drug dependence in 2010, and 494000 years of life were lost because of HCV infection associated with unsafe intravenous drug use (IDU)[23].

China, Russia, the United States and Brazil are home to the largest drug-injecting populations[24], with an estimated 1.3-1.6 million PWID infected with HCV per country[25]. High HCV seroprevalence rates that reach 80% are also reported in PWID from Mexico, Pakistan and Thailand[24,25]. Almost half of the 590000 people aged 18-29 years who reported intravenous drug use in the US are HCV infected[26,27], and the seroprevalence rates reach 98.7% in people who have used drugs for more than 30 years[28,29].

IDU is the most commonly reported HCV transmission route in Europe, and it represents the main risk factor for acute (33.3%) and chronic hepatitis C cases (83.7%)[14,30]. IDU is becoming prevalent in Northern and Southern European countries, where it is replacing the iatrogenic transmission that was recorded for decades[31,32]. Almost all European countries exhibit high HCV seroprevalence rates in PWID, with only the Czech Republic, Hungary and Slovenia reporting levels under 30%[33]. Table 2 presents a more detailed picture of the current levels of HCV infection in the top ten most populated European countries that are representative of this geographical region.

An alarming rising trend in HCV seroprevalence in PWID was observed in several European countries in 2005, including Austria, Bulgaria, Cyprus, Greece and Romania. Very high levels in the incidence and prevalence of drug-associated HCV infection were also reported in 2013 in Latvia, Portugal, Turkey and Cyprus[33,34]. In contrast, the figures for Germany, France, the United Kingdom and Italy exhibited a downward trend from previous years[31,33], which reflects good performances in case-finding and case-screening approaches.

An upsurge in the prevalence of HCV infection is an epidemiological indicator of injection-related HIV infection risk in PWID[35,36]. For example, high rates of HCV infection in PWID preceded by several years important HIV outbreaks in Greece and Romania[37]. A recent meta-analysis demonstrated that the incidence of HCV infection in PWID in the European Union (EU) was as high as 66/100 person-years, and half of the chronically infected PWID were unaware of their infection status[34].

Significant risk factors for drug-associated infectious diseases have been identified in many European countries[33,38-42]: (1) a switch to drugs that allow a higher injection frequency, such as new psychotropic substances; (2) decreases in needle and syringe

Table 2 Hepatitis C virus infection prevalence in the general population and in people who inject drugs in the most populated European countries

Country	Population (millions)[1]	Injecting drug use (rate/1000 inhabitants)[2]	HCV prevalence in the general population[3]	HCV prevalence in PWID[4]
Germany	80.4	4.25-5.04	0.7	51
France	65.6	6.7-8.8	0.85	73
United Kingdom	63.7	3.3	0.6	47.9
Italy	59.5	10	3.0	61.0-64.8
Spain	41.0	0.2	1.5	73.3-85.9
Poland	38.5	2.9	1.5	44.3 72.4
Romania	20.0	NA	2.1-2.4	82.4
Netherlands	16.7	0.2	0.2	50-86
Greece	10.9	1.1	> 1.5	60-73
Sweden	9.5	4.9	0.5	83

Data sources: [1]World health statistics 2014 (available from: www.who.int/world_health_statistics; [2,4]ECMDDA, 2013[33]; [3]Mühlberger N, 2009[30] and Cornberg M, 2011[16]. NA: Not available. PWID: People who inject drugs; HCV: Hepatitis C virus.

Table 3 The worldwide prevalence of hepatitis C virus genotypes

Area	The most prevalent genotype	Frequency of other genotypes	First author
North America	G1 (80%) 1a- the most common	G2 (11.1%) G3 (7.4%) G4 (1.2%)	Thomas *et al*[13], 2012
Europe	G1 (60%) 1b- the most common	G3 (20%); G4 (18%)	Messina *et al*[43], 2015
South-East Asia	G3 (65%)	G1 (25%) G1 prevails in China, G6 also reported	Mao *et al*[44], 2014 Li *et al*[45], 2015
Middle East and North Africa	G4 (70%)	G1, G2, G6	Ray *et al*[46], 2000 Ramia *et al*[47], 2012
Sub-Saharan and Central Africa	G4	G5 and G6, G1a, 1b, 2a, 2b	Papastergiou *et al*[48], 2015
South Africa	G 5	G1, 2, 3, 4	Gededzha *et al*[49], 2014
Asia Pacific and Latin America	G1a	G 1b, 2a, 2b	Messina *et al*[43], 2015 Ohno *et al*[50], 1997 Villar *et al*[51], 2015

Table 4 Hepatitis C virus genotypes prevalence in the European regions

European regions	The most prevalent genotype	Other genotypes	Comments	First author
Northern Europe	1a	1b, 2, 4	G1a frequent among PWID	Bruggmann *et al*[52], 2014
Western Europe	1b	3a (France) 4a (United Kingdom, The Netherlands, Germany)	G1b-common in older age groups	Messina *et al*[43], 2015 Payan *et al*[53], 2005
Southern Europe	1b	2a, 2b, 2c, 4	G4 is becoming more frequent	Gower *et al*[10], 2014 Cifuentes *et al*[54], 2015
Eastern Europe	1a	1b, 2, 3, 4	Non G1 genotypes reported in migrants	Cornberg *et al*[16], 2011 Messina *et al*[43], 2015

coverage (< 100 syringes per PWID per year, which represents a low coverage level even for HIV transmission) were reported in Romania, Greece, Cyprus, Slovakia, Hungary, Belgium and Norway; and (3) low levels (< 30%) of substitution treatment coverage, reported in Cyprus, Latvia, Lithuania, Hungary, Poland and Slovakia.

These data highlight the continuous potential for HCV-HIV epidemics to spread throughout Europe and jeopardize the efforts to decrease or stabilize the seroprevalence of blood-borne infections.

THE DISTRIBUTION OF HCV GENOTYPES IN THE GENERAL POPULATION

HCV genotypes and subtypes exhibit a distinct geographical distribution, illustrated in Tables 3 (worldwide[13,43-51]) and 4 (European Regions[10,16,43,52-54]).

HCV genotype 1 is the most prevalent genotype worldwide; subtype 1a prevails in Northern America, Japan and Northern Europe, and subtype 1b is dominant in Southern Europe and Japan[43,50] and exhibits a high frequency in Northern Africa.

HCV genotype 2 is reported in North America, Japan, Western Africa[55] and Europe (*e.g.*, 2a/c has been isolated in Northern Italy[56] and 2c has been isolated in Southern Italy[57]). Genotype 2a and 1b were identified as the major HCV genotypes circulating in former blood donors from rural China[58].

HCV subtype 3a is endemic in South Eastern Asia, but it is spreading in PWID in United States and Europe, with Germany, France, Italy, and Portugal reporting an increased prevalence of genotypes 1a and 3a[59-62]. Mixed infections have been reported in Italy (1b/3a), Germany (2a/3b), and Sweden (1a/1b)[10].

HCV genotype 4 dominates in the Middle East and Africa. Genotype 4 is responsible for 90% of the

Table 5 Hepatitis C virus genotypes prevalence among people who inject drugs in the most populated European countries[1]

Country	G1	G2	G3	G4	G1 + G4
Germany	63	3.8	31	2.6	61
France	46	2.5	37	9.1	55
United Kingdom	49	5.7	42	0.8	50
Italy	45	3.3	38	13.0	58
Spain	54	2.3	27	16.0	69
Poland	35	0.0	57	8.7	44
Romania[2]	73	0.0	7	12.0	85
Netherlands	53	6.0	32	9.0	66
Greece[2]	24	2.8	61	11.0	36
Sweden	36	8.7	34	0.9	38

[1]Data are adapted from Wiessing *et al*[34], 2014, with figures for [2]Greece and Romania, corrected according to more recent estimates, after the recent human immunodeficiency virus/hepatitis C virus outbreaks in people who inject drugs.

nosocomially transmitted HCV infections in Egypt[63] (the country with the highest rate of HCV infections worldwide - 15% of the population, associated with parenteral treatments for schistosomiasis) and most infections in the Democratic Republic of Congo, Central African Republic, Liberia, Uganda, Rwanda and Gabon[64,65]. Infections with genotype 4 are reported with increasing frequency in PWID in Europe.

HCV genotypes 5, 6 and 7 are rather limited in their distribution. The highest prevalence of genotypes 5 and 6 is reported in South Africa[49] and Asia[67], respectively, and genotype 7 was isolated from an emigrant from Congo[68]. A cluster of genotype 5a infections was also recently reported in the Rhodes island of Greece[69].

HCV GENOTYPES CIRCULATING IN PWID

A careful surveillance of circulating genotypes and subtypes is compulsory to reconstruct the natural history of HCV epidemics and viral transmission chains in this high-risk population. Genotypes 1a and 3a predominate in PWID worldwide. Russia and Estonia reported high rates of genotype 3a, especially in young drug users[70,71]. Genotype 3a is also increasing in frequency in Eastern and Central European countries, with growing rates in Bulgaria[72], Serbia and Montenegro[73], Poland[74] and Romania[75]. PWID in England are more likely to harbor genotype 3a relative to other risk groups, in which genotype 1a is prevalent[76].

An increasing proportion of new infections with genotype 4, which predominates in the Middle East and Africa, was identified primarily in Southern European countries, with distinct subtypes prevailing in different geographic regions: 4a in Greece[77], 4d in Italy[78,79], 4c and 4d in Spain[80], and a local spread of subtype 4d in the Netherlands[81]. France reported increased rates of genotype 4 (from 15% in 2003 to 22% in 2012) in persons coinfected with HCV/HIV: PWID and men having sex with men[82].

Table 5 presents the overall prevalence of HCV genotypes in PWID in the most populated countries in Europe.

FACTORS INVOLVED IN THE DIFFERENT PREVALENCE OF HCV GENOTYPES IN PWID

HCV genotype monitoring allows a real-time insight into the dynamic changes that occur in the global epidemiological picture of HCV infection.

Social vulnerabilities

The spreading of HCV genotypes/subtypes differs significantly between and within countries, between urban and rural settings, and according to the burden of risk-groups and economic status. There is a direct correlation between the gross national income per capita (GNI) and the so-called hepatitis index[83], which represents a comprehensive assessment of public health performances in the handling and treatment of HCV infections (Figure 1). Five main elements compose the hepatitis index: prevention (public awareness), case identification (screening programs), access to treatment (funding and waiting time), treatment outcomes (sustained virological response and adherence to treatment) and the national health strategy[83]. Figure 1 demonstrates that Germany, France and the United Kingdom are the top three performers, whereas the Baltic States, Hungary and Romania exhibit the lowest scores. A national plan for viral hepatitis has been implemented in France, and similar initiatives are ongoing in Scotland, Germany, Bulgaria and Croatia.

Case studies: Recent HCV/HIV outbreaks in PWID in Greece and Romania

The impact of the economic crisis on HCV seroprevalence and the distribution of circulating genotypes was recently illustrated by HCV/HIV outbreaks that evolved in PWID in the capital cities of Greece (Athens) and Romania (Bucharest) between 2011 and 2013[84-86]. The gross domestic product per capita in Romania (a country with 20.02 millions inhabitants) and Greece (a country with 11.06 millions inhabitants) is lower than the European Union (EU-27) average (representing only 50 and 75 Purchasing Power Standards, respectively)[87]. Greece has higher unemployment rates than the EU average: 27.3% *vs* 10.8% of the total labor force, and 58.3% *vs* 23.4% in persons under 25 years of age. Romania reports a slightly higher unemployment rate in young persons (23.6% in persons aged under 25 years), but a moderate rate of 7.3% in the total labor force[87]. Both countries exhibit higher percentages of people who

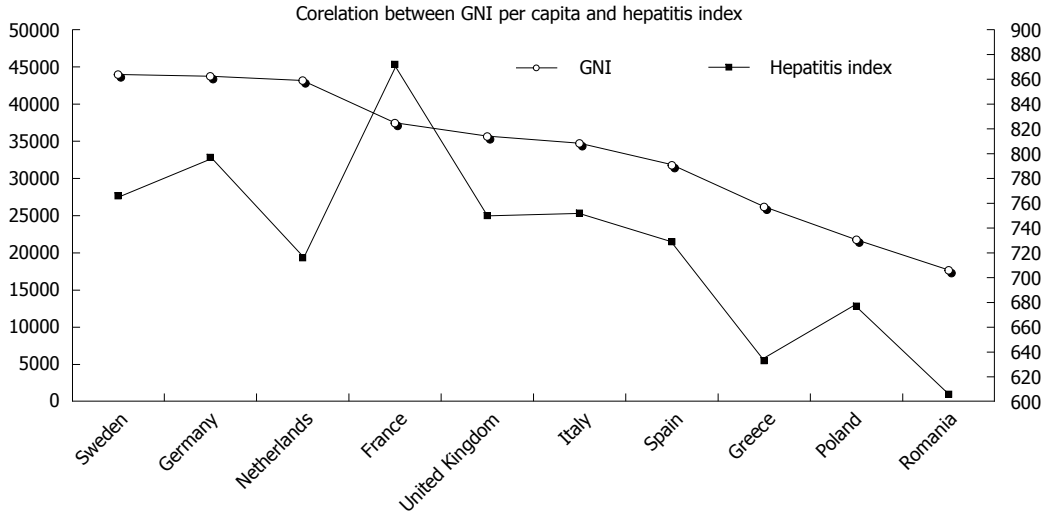


Figure 1 Correlation between the gross national income and the hepatitis index in the top 10 most populated European countries. The gross national income per capita (GNI) directly correlates with the public health performances in the handling and treatment of hepatitis C virus infections (evaluated using the hepatitis index), as calculated by the Euro Hepatitis Report (2012) elaborated by Health Consumer Powerhouse[83].

are at risk of poverty: 22.6% in Romania and 23.1% in Greece relative to the EU average of 17%[33]. The HIV/HCV outbreaks in both countries were associated with financial restrictions in harm-reduction programs, and the persons affected were primarily young males who are unemployed, frequently homeless, and without medical insurances[86,88]. These social vulnerabilities are important triggers for illicit drug use, which increases the associated risk of drug-related infectious diseases and the emergence of different genotypes than the genotypes circulating in the general population. HCV genotype 1b[89] and HIV subtype F[90] predominate in Romania, but the introduction of new viral strains was documented during a recent outbreak in PWID: HCV subtypes 1a, 3a, 4 (Ruta S, unpublished data) and HIV subtype G, with the particular recombinant form CRF14_BG[90]. HCV genotype 3[91] and HIV CRF14_BG and CRF_35AD[92] prevail in PWID in Greece. Assessments of the evolution of HCV infection in older patients (infected with genotype 1, primarily through nosocomial procedures) *vs* younger patients (infected with newly introduced genotypes, primarily through IDU) will be interesting. Younger patients are candidates for shorter durations of therapy, with important implications for treatment-related costs and patient quality of life.

Immigration from HCV endemic countries and the evolving networks of HCV transmission in PWID influence the genotype distribution. European countries with the highest number of migrants (Germany: 12.3%, Italy, Spain, Netherlands: each 10%-12%, and France: 10%)[93] exhibit a high prevalence of HCV infection and increased frequencies of less common genotypes. One recent study demonstrated that more than one third of the patients with chronic hepatitis C from Germany were born abroad[13], and an increased prevalence of HCV infection was reported in migrants in Italy[94]. Many cases of HCV infection in PWID from Cyprus are diagnosed in foreign nationals[95]. The increasing prevalence of non-1b genotypes in France, Spain, Italy and Greece was primarily attributed to a large flow of immigrants, but some limited molecular epidemiology studies argue against this hypothesis[96,97].

Phylogenetic analyses recently identified HCV transmission clusters associated with injection relationships in Melbourne, Australia[98] and Vancouver, Canada[99].

WHAT ARE THE CONSEQUENCES OF THE DISTINCT PREVALENCE OF HCV GENOTYPES IN HIGH-RISK POPULATIONS?

HCV variability triggers important clinical consequences. The emergence of immune response escape mutants accounts for the high level of chronic infection, and the infecting genotype is critical for the natural and on-treatment evolution of the infection. These data are especially significant for PWID, who are frequently infected with genotypes 1a, 3 and 4 that tend to exhibit less favorable responses to therapies, as discussed below.

Interferon-based therapy

HCV genotype was one of the primary predictors of the response rate to the classic pegylated interferon-ribavirin (P/R) therapy, which is the only affordable therapy in developing countries. HCV subtype 1b exhibits the most unfavorable response profile, and genotypes 2 and 3 are "easy-to-treat" and exhibit a sustained virological response (SVR) in up to 80% of treated patients[100]. The reported SVR rates for genotype 4 are 60%-69% in Egypt and 40%-50%

in countries outside endemic areas[64]. Genotype 3, initially correlated with a very high response rate to the classic P/R treatment, is associated with a higher rate of liver fibrosis and steatosis (unlinked to insulin resistance) and a more rapidly progressive end-stage liver disease[101]. Subsequently, many genotype 3-infected patients, including PWID, exhibit cirrhosis at the initiation of P/R treatment, and the overall response rate has been disappointing.

Direct-acting antiviral-based regimens

Treatment regimens for chronic hepatitis C and the inclusion criteria have largely changed in the last 4 years with the approval of new DAAs. However, the HCV genotype matters for therapeutic responses[102]. Novel treatments for HCV are highly cost-effective for HCV genotype 1. The current World Health Organization[103], American Association for the Study of Liver[104] and European Association for the Study of Liver[105] guidelines for HCV treatment are genotype-dependent, with several available options for each genotype, including IFN-free regimens, considered the most suitable ones in genotype 2-infected patients, and recommended for genotypes 1, 3 and 4. However, the triple combination of pegylated IFN-α, ribavirin and sofosbuvir (a NS5B inhibitor) administered for 12 wk is still favored in terms of efficacy, for patients infected with HCV genotypes 1, 3, 4, 5 and 6, as well as for those infected with genotype 2 that are cirrhotic and/or treatment-experienced[105,106]. This regimen also avoids resistance selection in cases of treatment failure. A combination of sofosbuvir and ledipasvir (an NS5A inhibitor), administered as a single pill, is currently recommended by the AASL as a first-line agent for patients without cirrhosis[104,107]. The new DAAs are less effective for patients infected with genotype 3 who have advanced liver disease, which is frequently observed in PWID. Phase Ⅲ clinical studies of sofosbuvir and ribavirin revealed a sustained virological response in only 60% of patients with genotype 3 and cirrhosis who had previously failed P/R treatment, even in the case of a longer therapy duration[108,109]. Even the newly approved NS5A inhibitors, including ledipasvir, are less active against HCV genotype 3 than against other genotypes[110-112]. Therefore, genotype 3, which is prevalent in PWID, is currently considered one of the "difficult to treat" genotypes. Few studies have addressed the efficacy of the new oral regimens in patients infected with HCV genotypes 4, 5, and 6[48,113], which are less prevalent in Europe and North America.

The impact of HCV genotype on the development of viral resistance

Viral breakthrough during or after DAA treatment (especially with the first generation protease inhibitors, telaprevir and boceprevir) was associated with the selection of resistance-associated variants, which preexist as minority populations[114-116]. Differences in the genetic barrier to resistance exist between subtypes; resistance mutations arise more quickly in patients who are infected with genotype 1a[115,116]. Moreover, a series of natural HCV polymorphisms that are found with different frequencies according to the HCV subtype, can influence treatment outcomes[117]. A second generation protease inhibitor (Simeprevir) exhibits reduced efficacy on subtype 1a strains because of the high prevalence of a specific mutation (Q80K) at baseline[118,119]. The activity of an NS5 inhibitor, which was recently approved for the treatment of HCV infection (Daclatasvir), is inhibited in the presence of a natural polymorphism (Q30R), which is found in more than 50% of genotype 4 strains[117,120]. Notably, resistant HCV variants are not archived (because HCV, unlike HIV or HBV, does not establish reservoirs), and reversion to the wild-type strain is observed 10-29 mo after treatment interruption[121] (faster for subtype 1b compared with 1a[114]). These differences are likely important for the treatment of PWID.

Toward a patient-tailored therapy for chronic hepatitis C in PWID

Important barriers to care and treatment are present in vulnerable populations, such as PWID[122], and it is estimated that only one in ten diagnosed patients enter treatment for hepatitis C. Delays in diagnosis lead to late presentations, with associated high viral loads and significant fibrosis, that represent unfavorable predictors for treatment efficacy. Decisions to treat are taken on a case-by-case basis, and treatments are accompanied by active counseling to decrease or cease drug and alcohol intake and the promotion of comprehensive harm-reduction programs, including in prisons[123-125].

The same therapeutic regimens based on DAAs are recommended for PWID, and a history of drug use or recent drug use is not associated with a reduced response rate[105]. The perceived risk of reinfection is not a reason for treatment denial, but instead a possibility that must be actively monitored after the achievement of SVR. The estimated rate of reinfection in PWID with persistent risk behaviors following successful HCV treatment is approximately 1%-5%[126-128].

However, the prohibitive costs of highly efficient therapeutic DAA options have prevented their use outside countries with high incomes. The NS5B polymerase inhibitor Sofosbuvir costs $1000 per day, the combination of sofosbuvir with ledipasvir costs $1125 per day, and a short 12-wk IFN-free regimen can reach a price of $150000 per patient[129]. Therefore, many countries that have high seroprevalence rates of HCV infection in the general population and vulnerable risk groups, continue to rely on the classic dual P/R therapy or triple therapy that combines P/R with first-generation protease inhibitors.

Changes in circulating genotypes suggest the necessity of different clinical approaches, including the

choice of the most suitable and cost-effective antiviral combination therapy for patients who are "difficult to reach, manage and treat"[130]. Therefore, the deferral of a P/R-based treatment that has several challenges (*e.g*., administration, monitoring and management of related side-effects), may be an option for patients with an early fibrosis stage[131], while waiting for highly effective, pangenotypic-active combinations to become available at more reasonable prices in the foreseeable future. Other factors will likely influence the final decision, including: the cost-effectiveness of the IFN-free regimens (treatment duration plotted against the SVR rate), the adherence to treatment and the cumulative toxicities (which are important factors in PWID, especially in HIV-coinfected patients), and the extent of clinically relevant viral resistance[131-134].

CONCLUSION

Many PWID who are infected with HCV remain undiagnosed. The distribution of circulating genotypes in this vulnerable group is distinct from the general population. Transmission networks associated with drug use, increased global travel and immigration are the primary factors behind this different epidemiological picture. PWID are critical epidemiological connectors to the general populations and drug use is a key vector for the diversification of circulating viral genotypes. The determination of circulating HCV genotypes in high-risk groups, such as PWID, who frequently have additional risk factors (poverty, imprisonment, and HIV coinfections) will provide a further understanding of the global viral epidemiology. HCV genetic diversity has a major impact on viral persistence, evolution to cirrhosis and hepatocellular carcinoma and potential resistance to antiviral agents. Therefore, knowledge of HCV genotypes will likely remain an essential factor for the correct design of national health programs, even with the introduction of new antivirals.

ACKNOWLEDGMENTS

The authors thank Claudia Dita, MD from the Stefan S. Nicolau Institute of Virology, Bucharest, for technical support.

REFERENCES

1 **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, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León 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/S01406736(12)61728-0]

2 **GBD 2013 Mortality and Causes of Death Collaborators**. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015; **385**: 117-171 [PMID: 25530442 DOI: 10.1016/S0140-6736(14)61682-2]

3 **Mohd Hanafiah K**, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]

4 **Cooke GS**, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, DuCros P, Ford N. Viral hepatitis and the Global Burden of Disease: a need to regroup. *J Viral Hepat* 2013; **20**: 600-601 [PMID: 23910643 DOI: 10.1111/jvh.12123]

5 **Amin J**, Law MG, Bartlett M, Kaldor JM, Dore GJ. Causes of death after diagnosis of hepatitis B or hepatitis C infection: a large community-based linkage study. *Lancet* 2006; **368**: 938-945 [PMID: 16962883]

6 **Quan PL**, Firth C, Conte JM, Williams SH, Zambrana-Torrelio CM, Anthony SJ, Ellison JA, Gilbert AT, Kuzmin IV, Niezgoda M, Osinubi MO, Recuenco S, Markotter W, Breiman RF, Kalemba L, Malekani J, Lindblade KA, Rostal MK, Ojeda-Flores R, Suzan G, Davis LB, Blau DM, Ogunkoya AB, Alvarez Castillo DA, Moran D, Ngam S, Akaibe D, Agwanda B, Briese T, Epstein JH, Daszak P, Rupprecht CE, Holmes EC, Lipkin WI. Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proc Natl Acad Sci USA* 2013; **110**: 8194-8199 [PMID: 23610427 DOI: 10.1073/pnas.1303037110]

7 **Kapoor A**, Simmonds P, Scheel TK, Hjelle B, Cullen JM, Burbelo PD, Chauhan LV, Duraisamy R, Sanchez Leon M, Jain K, Vandegrift KJ, Calisher CH, Rice CM, Lipkin WI. Identification of rodent homologs of hepatitis C virus and pegiviruses. *MBio* 2013; **4**: e00216-e00213 [PMID: 23572554 DOI: 10.1128/mBio.00216-13]

8 **Moradpour D**, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007; **5**: 453-463 [PMID: 17487147 DOI: 10.1038/nrmicro1645]

9 **Smith DB**, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology* 2014; **59**: 318-327 [PMID: 24115039 DOI: 10.1002/hep.26744]

10 **Gower E**, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global

epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol* 2014; **61**: S45-S57 [PMID: 25086286 DOI: 10.1016/j.jhep.2014.07.027]
11 **Alter MJ**. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 2436-2441 [PMID: 17552026 DOI: 10.3748/wjg.v13.i17.2436]
12 United Nations Office on Drugs and Crime. World Drug Report 2014. United Nations Publications, eISBN: 978-92-1-056752-7. Available from: URL: http://www.unodc.org/documents/wdr2014/World_Drug_Report_2014_web.pdf
13 **Thomas LB**, Foulis PR, Mastorides SM, Djilan YA, Skinner O, Borkowski AA. Hepatitis C genotype analysis: results in a large veteran population with review of the implications for clinical practice. *Ann Clin Lab Sci* 2012; **42**: 355-362 [PMID: 23090730]
14 **Esteban JI**, Sauleda S, Quer J. The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol* 2008; **48**: 148-162 [PMID: 18022726 DOI: 10.1016/j.jhep.2007.07.033]
15 European Centre for Disease Prevention and Control. Hepatitis B and C surveillance in Europe, 2012. Stockholm: ECDC, 2014 TQ-AU-14-001-EN-N. Available from: URL: http://ecdc.europa.eu/en/hepatitis-b-c-surveillance-europe-2012-july-2014.pdf
16 **Cornberg M**, Razavi HA, Alberti A, Bernasconi E, Buti M, Cooper C, Dalgard O, Dillion JF, Flisiak R, Forns X, Frankova S, Goldis A, Goulis I, Halota W, Hunyady B, Lagging M, Largen A, Makara M, Manolakopoulos S, Marcellin P, Marinho RT, Pol S, Poynard T, Puoti M, Sagalova O, Sibbel S, Simon K, Wallace C, Young K, Yurdaydin C, Zuckerman E, Negro F, Zeuzem S. A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. *Liver Int* 2011; **31** Suppl 2: 30-60 [PMID: 21651702 DOI: 10.1111/j.1478-3231.2011.02539.x]
17 **Mathurin P**. HCV burden in Europe and the possible impact of current treatment. *Dig Liver Dis* 2013; **45** Suppl 5: S314-S317 [PMID: 24091109 DOI: 10.1016/j.dld.2013.07.009]
18 **McGarry LJ**, Pawar VS, Panchmatia HR, Rubin JL, Davis GL, Younossi ZM, Capretta JC, O'Grady MJ, Weinstein MC. Economic model of a birth cohort screening program for hepatitis C virus. *Hepatology* 2012; **55**: 1344-1355 [PMID: 22135116 DOI: 10.1002/hep.25510]
19 **Kabiri M**, Jazwinski AB, Roberts MS, Schaefer AJ, Chhatwal J. The changing burden of hepatitis C virus infection in the United States: model-based predictions. *Ann Intern Med* 2014; **161**: 170-180 [PMID: 25089861 DOI: 10.7326/M14-0095]
20 **Lewis H**, Burke K, Begum S, Ushiro-Limb I, Foster G. What is the best method of case finding for chronic viral Hepatitis in at-risk migrant communities? *J Hepatology* 2012; **56**: S351 [DOI: 10.1016/S0168-8278(12)60915-5]
21 **Litwin AH**, Smith BD, Drainoni ML, McKee D, Gifford AL, Koppelman E, Christiansen CL, Weinbaum CM, Southern WN. Primary care-based interventions are associated with increases in hepatitis C virus testing for patients at risk. *Dig Liver Dis* 2012; **44**: 497-503 [PMID: 22342471 DOI: 10.1016/j.dld.2011.12.014]
22 **Hahné SJ**, Veldhuijzen IK, Wiessing L, Lim TA, Salminen M, Laar Mv. Infection with hepatitis B and C virus in Europe: a systematic review of prevalence and cost-effectiveness of screening. *BMC Infect Dis* 2013; **13**: 181 [PMID: 23597411 DOI: 10.1186/1471-2334-13-181]
23 **Degenhardt L**, Whiteford HA, Ferrari AJ, Baxter AJ, Charlson FJ, Hall WD, Freedman G, Burstein R, Johns N, Engell RE, Flaxman A, Murray CJ, Vos T. Global burden of disease attributable to illicit drug use and dependence: findings from the Global Burden of Disease Study 2010. *Lancet* 2013; **382**: 1564-1574 [PMID: 23993281 DOI: 10.1016/S0140-6736(13)61530-5]
24 **World Health Organization**. A strategy to halt and reverse the HIV epidemic among people who inject drugs in Asia and the Pacific: 2010–2015, WHO Library Cataloguing 2015. Available from: URL: http://iris.wpro.who.int/handle/10665.1/5506
25 **Nelson PK**, Mathers BM, Cowie B, Hagan H, Des Jarlais D, Horyniak D, Degenhardt L. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet* 2011; **378**: 571-583 [PMID: 21802134 DOI: 10.1016/S0140-6736(11)61097-0]
26 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714 [PMID: 16702586 DOI: 10.7326/0003-4819-144-10-200605160-00004]
27 **Page K**, Morris MD, Hahn JA, Maher L, Prins M. Injection drug use and hepatitis C virus infection in young adult injectors: using evidence to inform comprehensive prevention. *Clin Infect Dis* 2013; **57** Suppl 2: S32-S38 [PMID: 23884063 DOI: 10.1093/cid/cit300]
28 **Tseng FC**, O'Brien TR, Zhang M, Kral AH, Ortiz-Conde BA, Lorvick J, Busch MP, Edlin BR. Seroprevalence of hepatitis C virus and hepatitis B virus among San Francisco injection drug users, 1998 to 2000. *Hepatology* 2007; **46**: 666-671 [PMID: 17657818 DOI: 10.1002/hep.21765]
29 **Ly KN**, Xing J, Klevens RM, Jiles RB, Ward JW, Holmberg SD. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. *Ann Intern Med* 2012; **156**: 271-278 [PMID: 22351712 DOI: 10.7326/0003-4819-156-4-201202210-00004]
30 **Mühlberger N**, Schwarzer R, Lettmeier B, Sroczynski G, Zeuzem S, Siebert U. HCV-related burden of disease in Europe: a systematic assessment of incidence, prevalence, morbidity, and mortality. *BMC Public Health* 2009; **9**: 34 [PMID: 19161623 DOI: 10.1186/1471-2458-9-34]
31 **Sarna A**, Panda S. HCV in people who inject drugs: a neglected epidemic. *Lancet Infect Dis* 2015; **15**: 4-5 [PMID: 25486850 DOI: 10.1016/S1473-3099(14)71054-0]
32 **Hope VD**, Eramova I, Capurro D, Donoghoe MC. Prevalence and estimation of hepatitis B and C infections in the WHO European Region: a review of data focusing on the countries outside the European Union and the European Free Trade Association. *Epidemiol Infect* 2014; **142**: 270-286 [PMID: 23714072 DOI: 10.1017/S0950268813000940]
33 **European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)**. European Drug Report. Trends and developments 2014. Luxembourg: Publications Office of the European Union, 2014. Available from: URL: http://www.ab.gov.tr/files/ardb/evt/european_drug_report_2014.pdf
34 **Wiessing L**, Ferri M, Grady B, Kantzanou M, Sperle I, Cullen KJ, Hatzakis A, Prins M, Vickerman P, Lazarus JV, Hope VD, Matheï C. Hepatitis C virus infection epidemiology among people who inject drugs in Europe: a systematic review of data for scaling up treatment and prevention. *PLoS One* 2014; **9**: e103345 [PMID: 25068274 DOI: 10.1371/journal.pone.0103345]
35 **Pilon R**, Leonard L, Kim J, Vallee D, De Rubeis E, Jolly AM, Wylie J, Pelude L, Sandstrom P. Transmission patterns of HIV and hepatitis C virus among networks of people who inject drugs. *PLoS One* 2011; **6**: e22245 [PMID: 21799802 DOI: 10.1371/journal.pone.0022245]
36 **Vickerman P**, Martin NK, Roy A, Beattie T, Jarlais DD, Strathdee S, Wiessing L, Hickman M. Is the HCV-HIV co-infection prevalence amongst injecting drug users a marker for the level of sexual and injection related HIV transmission? *Drug Alcohol Depend* 2013; **132**: 172-181 [PMID: 23453261 DOI: 10.1016/j.drugalcdep.2013.01.020]
37 **Wiessing L**, Likatavicius G, Hedrich D, Guarita B, van de Laar MJ, Vicente J. Trends in HIV and hepatitis C virus infections among injecting drug users in Europe, 2005 to 2010. *Euro Surveill* 2011; **16**: pii 20031 [PMID: 22172300]
38 **Lidman C**, Norden L, Kåberg M, Käll K, Franck J, Aleman S, Birk M. Hepatitis C infection among injection drug users in Stockholm Sweden: prevalence and gender. *Scand J Infect Dis* 2009; **41**: 679-684 [PMID: 19521924 DOI: 10.1080/00365540903062143]
39 **García-Fulgueiras A**, García-Pina R, Morant C, de Larrea-Baz NF, Alvarez E. Burden of disease related to hepatitis C and hepatitis B in Spain: a methodological challenge of an unfolding health problem. *J Viral Hepat* 2011; **18**: e453-e460 [PMID: 21914063 DOI: 10.1111/j.1365-2893.2011.01467.x]

40 **Vermehren J**, Schlosser B, Domke D, Elanjimattom S, Müller C, Hintereder G, Hensel-Wiegel K, Tauber R, Berger A, Haas N, Walcher F, Möckel M, Lehmann R, Zeuzem S, Sarrazin C, Berg T. High prevalence of anti-HCV antibodies in two metropolitan emergency departments in Germany: a prospective screening analysis of 28,809 patients. *PLoS One* 2012; **7**: e41206 [PMID: 22848445 DOI: 10.1371/journal.pone.0041206]

41 **Flisiak R**, Halota W, Tomasiewicz K, Kostrzewska K, Razavi HA, Gower EE. Forecasting the disease burden of chronic hepatitis C virus in Poland. *Eur J Gastroenterol Hepatol* 2015; **27**: 70-76 [PMID: 25426979 DOI: 10.1097/MEG.0000000000000237]

42 **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]

43 **Messina JP**, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]

44 **Mao XR**, Zhang LT, Chen H, Xiao P, Zhang YC. Correlation between the genetic variations in interleukin 28B and chronic hepatitis C virus genotypes in the Chinese population. *Mol Med Rep* 2014; **10**: 1037-1045 [PMID: 24840747 DOI: 10.3892/mmr.2014.2242]

45 **Li C**, Barnes E, Newton PN, Fu Y, Vongsouvath M, Klenerman P, Okamoto H, Abe K, Pybus OG, Lu L. An expanded taxonomy of hepatitis C virus genotype 6: Characterization of 22 new full-length viral genomes. *Virology* 2015; **476**: 355-363 [PMID: 25589238 DOI: 10.1016/j.virol.2014.12.025]

46 **Ray SC**, Arthur RR, Carella A, Bukh J, Thomas DL. Genetic epidemiology of hepatitis C virus throughout egypt. *J Infect Dis* 2000; **182**: 698-707 [PMID: 10950762 DOI: 10.1086/315786]

47 **Ramia S**, Melhem NM, Kreidieh K. Hepatitis C virus infection in the Middle East and North Africa "MENA" region: injecting drug users (IDUs) is an under-investigated population. *Infection* 2012; **40**: 1-10 [PMID: 22237470 DOI: 10.1007/s15010-011-0236-z]

48 **Papastergiou V**, Karatapanis S. Current status and emerging challenges in the treatment of hepatitis C virus genotypes 4 to 6. *World J Clin Cases* 2015; **3**: 210-220 [PMID: 25789294 DOI: 10.12998/wjcc.v3.i3.210]

49 **Gededzha MP**, Selabe SG, Blackard JT, Kyaw T, Mphahlele MJ. Near full-length genome analysis of HCV genotype 5 strains from South Africa. *Infect Genet Evol* 2014; **21**: 118-123 [PMID: 24220189 DOI: 10.1016/j.meegid.2013.10.022]

50 **Ohno O**, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, Mukaide M, Williams R, Lau JY. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *J Clin Microbiol* 1997; **35**: 201-207 [PMID: 8968908]

51 **Villar LM**, Ó KM, Scalioni LP, Cruz HM, Portilho MM, Mendonça AC, Miguel JC, Figueiredo AS, Almeida AJ, Lampe E. Prevalence of hepatitis B and C virus infections among military personnel. *Braz J Infect Dis* 2015; **19**: 285-290 [PMID: 25769737 DOI: 10.1016/j.bjid.2015.02.002]

52 **Bruggmann P**, Berg T, Øvrehus AL, Moreno C, Brandão Mello CE, Roudot-Thoraval F, Marinho RT, Sherman M, Ryder SD, Sperl J, Akarca U, Balık I, Bihl F, Bilodeau M, Blasco AJ, Buti M, Calinas F, Calleja JL, Cheinquer H, Christensen PB, Clausen M, Coelho HS, Cornberg M, Cramp ME, Dore GJ, Doss W, Duberg AS, El-Sayed MH, Ergör G, Esmat G, Estes C, Falconer K, Félix J, Ferraz ML, Ferreira PR, Frankova S, García-Samaniego J, Gerstoft J, Giria JA, Gonçales FL, Gower E, Gschwantler M, Guimarães Pessôa M, Hézode C, Hofer H, Husa P, Idilman R, Kåberg M, Kaita KD, Kautz A, Kaymakoglu S, Krajden M, Krarup H, Laleman W, Lavanchy D, Lázaro P, Marotta P, Mauss S, Mendes Correa MC, Müllhaupt B, Myers RP, Negro F, Nemecek V, Örmeci N, Parkes J, Peltekian KM, Ramji A, Razavi H, Reis N, Roberts SK, Rosenberg WM, Sarmento-Castro R, Sarrazin C, Semela D, Shiha GE, Sievert W, Stärkel P, Stauber RE, Thompson AJ, Urbanek P, van Thiel I, Van Vlierberghe H, Vandijck D, Vogel W, Waked I, Wedemeyer H, Weis N, Wiegand J, Yosry A, Zekry A, Van Damme P, Aleman S, Hindman SJ. Historical epidemiology of hepatitis C virus (HCV) in selected countries. *J Viral Hepat* 2014; **21** Suppl 1: 5-33 [PMID: 24713004 DOI: 10.1111/jvh.12247]

53 **Payan C**, Roudot-Thoraval F, Marcellin P, Bled N, Duverlie G, Fouchard-Hubert I, Trimoulet P, Couzigou P, Cointe D, Chaput C, Henquell C, Abergel A, Pawlotsky JM, Hezode C, Coudé M, Blanchi A, Alain S, Loustaud-Ratti V, Chevallier P, Trepo C, Gerolami V, Portal I, Halfon P, Bourlière M, Bogard M, Plouvier E, Laffont C, Agius G, Silvain C, Brodard V, Thiefin G, Buffet-Janvresse C, Riachi G, Grattard F, Bourlet T, Stoll-Keller F, Doffoel M, Izopet J, Barange K, Martinot-Peignoux M, Branger M, Rosenberg A, Sogni P, Chaix ML, Pol S, Thibault V, Opolon P, Charrois A, Serfaty L, Fouqueray B, Grange JD, Lefrère JJ, Lunel-Fabiani F. Changing of hepatitis C virus genotype patterns in France at the beginning of the third millenium: The GEMHEP GenoCII Study. *J Viral Hepat* 2005; **12**: 405-413 [PMID: 15985012 DOI: 10.1111/j.1365-2893.2005.00605.x]

54 **Cifuentes C**, Mancebo-Hernández M, Pérez-Navarro E, Recio E, Monje-Agudo P, Valiente A, Pineda JA. [Prevalence and genotype distribution changes in hepatitis C virus co-infection among human immunodeficiency virus-infected patients]. *Enferm Infecc Microbiol Clin* 2015; **33**: 110-112 [PMID: 25510595 DOI: 10.1016/j.eimc.2014.05.010]

55 **Candotti D**, Temple J, Sarkodie F, Allain JP. Frequent recovery and broad genotype 2 diversity characterize hepatitis C virus infection in Ghana, West Africa. *J Virol* 2003; **77**: 7914-7923 [PMID: 12829831 DOI: 10.1128/JVI.77.14.7914-7923.2003]

56 **Osella AR**, Misciagna G, Guerra V, Elba S, Buongiorno G, Cavallini A, Di Leo A, Sonzogni L, Mondelli MU, Silini EM. Hepatitis C virus genotypes and risk of cirrhosis in southern Italy. *Clin Infect Dis* 2001; **33**: 70-75 [PMID: 11389497 DOI: 10.1086/320887]

57 **Ansaldi F**, Bruzzone B, Salmaso S, Rota MC, Durando P, Gasparini R, Icardi G. Different seroprevalence and molecular epidemiology patterns of hepatitis C virus infection in Italy. *J Med Virol* 2005; **76**: 327-332 [PMID: 15902713 DOI: 10.1002/jmv.20376]

58 **Yin W**, Huang C, Qiu F, Liu L, Wang F, Zhou J, Zhang Y, Bi S. Risk factors of hepatitis C virus transmission and genotype distribution in former blood donors from Chinese rural area. *BMC Public Health* 2015; **15**: 184 [PMID: 25884321 DOI: 10.1186/s12889-015-1535-6]

59 **Morice Y**, Cantaloube JF, Beaucourt S, Barbotte L, De Gendt S, Goncales FL, Butterworth L, Cooksley G, Gish RG, Beaugrand M, Fay F, Fay O, Gonzalez JE, Martins RM, Dhumeaux D, Vanderborght B, Stuyver L, Sablon E, de Lamballerie X, Pawlotsky JM. Molecular epidemiology of hepatitis C virus subtype 3a in injecting drug users. *J Med Virol* 2006; **78**: 1296-1303 [PMID: 16927280 DOI: 10.1002/jmv.20692]

60 **Stroffolini T**, Fiumeb A, Fataleb G, Regnib F, Ciccozzia M, Marzolinia A, Mele A. Hepatitis C virus among intravenous drug users in Italy. *Hepatology Research* 1997; **9**: 20-27 [DOI: 10.1016/S1386-6346(97)00084-3]

61 **Calado RA**, Rocha MR, Parreira R, Piedade J, Venenno T, Esteves A. Hepatitis C virus subtypes circulating among intravenous drug users in Lisbon, Portugal. *J Med Virol* 2011; **83**: 608-615 [PMID: 21328374 DOI: 10.1002/jmv.21955]

62 **Sereno S**, Perinelli P, Laghi V. Changes in the prevalence of hepatitis C virus genotype among Italian injection drug users-relation to period of injection started. *J Clin Virol* 2009; **45**: 354-357 [PMID: 19497783 DOI: 10.1016/j.jcv.2009.04.022]

63 **Mohamoud YA**, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. *BMC Infect Dis* 2013; **13**: 288 [PMID: 23799878 DOI: 10.1186/1471-2334-13-288]

64 **Kamal SM**, Nasser IA. Hepatitis C genotype 4: What we know and what we don't yet know. *Hepatology* 2008; **47**: 1371-1383 [PMID: 18240152 DOI: 10.1002/hep.22127]

65 **Xu LZ**, Larzul D, Delaporte E, Bréchot C, Kremsdorf D. Hepatitis

C virus genotype 4 is highly prevalent in central Africa (Gabon). *J Gen Virol* 1994; **75** (Pt 9): 2393-2398 [PMID: 8077938 DOI: 10.1099/0022-1317-75-9-2393]

66 **Iles JC**, Raghwani J, Harrison GL, Pepin J, Djoko CF, Tamoufe U, LeBreton M, Schneider BS, Fair JN, Tshala FM, Kayembe PK, Muyembe JJ, Edidi-Basepeo S, Wolfe ND, Simmonds P, Klenerman P, Pybus OG. Phylogeography and epidemic history of hepatitis C virus genotype 4 in Africa. *Virology* 2014; **464-465**: 233-243 [PMID: 25105489 DOI: 10.1016/j.virol.2014.07.006]

67 **Thong VD**, Akkarathamrongsin S, Poovorawan K, Tangkijvanich P, Poovorawan Y. Hepatitis C virus genotype 6: virology, epidemiology, genetic variation and clinical implication. *World J Gastroenterol* 2014; **20**: 2927-2940 [PMID: 24659883 DOI: 10.3748/wjg.v20.i11.2927]

68 **Murphy DG**, Willems B, Deschênes M, Hilzenrat N, Mousseau R, Sabbah S. Use of sequence analysis of the NS5B region for routine genotyping of hepatitis C virus with reference to C/E1 and 5' untranslated region sequences. *J Clin Microbiol* 2007; **45**: 1102-1112 [PMID: 17287328 DOI: 10.1128/jcm.02366-06]

69 **Karatapanis S**, Tsoplou P, Papastergiou V, Vasiageorgi A, Stampori M, Saitis I, Tsitsopoulos E, Lisgos P, Skorda L, Ketikoglou I, Goulis I. Hepatitis C virus genotyping in Greece: unexpected high prevalence of genotype 5a in a Greek island. *J Med Virol* 2012; **84**: 223-228 [PMID: 22170541 DOI: 10.1002/jmv.22249]

70 **Paintsil E**, Verevochkin SV, Dukhovlinova E, Niccolai L, Barbour R, White E, Toussova OV, Alexander L, Kozlov AP, Heimer R. Hepatitis C virus infection among drug injectors in St Petersburg, Russia: social and molecular epidemiology of an endemic infection. *Addiction* 2009; **104**: 1881-1890 [PMID: 19712125 DOI: 10.1111/j.1360-0443.2009.02687.x]

71 **Tallo T**, Norder H, Tefanova V, Krispin T, Schmidt J, Ilmoja M, Orgulas K, Pruunsild K, Priimägi L, Magnius LO. Genetic characterization of hepatitis C virus strains in Estonia: fluctuations in the predominating subtype with time. *J Med Virol* 2007; **79**: 374-382 [PMID: 17311333 DOI: 10.1002/jmv.20828]

72 **Ciccozzi M**, Zehender G, Cento V, Lo Presti A, Teoharov P, Pavlov I, Bogdanova V, Perno CF, Ciotti M. Molecular analysis of hepatitis C virus infection in Bulgarian injecting drug users. *J Med Virol* 2011; **83**: 1565-1570 [PMID: 21739447 DOI: 10.1002/jmv.22154]

73 **Svirtlih N**, Delic D, Simonovic J, Jevtovic D, Dokic L, Gvozdenovic E, Boricic I, Terzic D, Pavic S, Neskovic G, Zerjav S, Urban V. Hepatitis C virus genotypes in Serbia and Montenegro: the prevalence and clinical significance. *World J Gastroenterol* 2007; **13**: 355-360 [PMID: 17230602 DOI: 10.3748/wjg.v13.i3.355]

74 **Chlabicz S**, Flisiak R, Kowalczuk O, Grzeszczuk A, Pytel-Krolczuk B, Prokopowicz D, Chyczewski L. Changing HCV genotypes distribution in Poland--relation to source and time of infection. *J Clin Virol* 2008; **42**: 156-159 [PMID: 18353714 DOI: 10.1016/j.jcv.2008.02.001]

75 **Sultana C**, Vagu C, Temereanca A, Grancea C, Slobozeanu J, Ruta S. Hepatitis C Virus Genotypes in Injecting Drug Users from Romania. *Cent Eur J Med* 2011; **6**: 672-678 [PMID: 23585824 DOI: 10.2478/s11536-011-0073-6]

76 **May S**, Ngui SL, Collins S, Lattimore S, Ramsay M, Tedder RS, Ijaz S. Molecular epidemiology of newly acquired hepatitis C infections in England 2008-2011: genotype, phylogeny and mutation analysis. *J Clin Virol* 2015; **64**: 6-11 [PMID: 25728071 DOI: 10.1016/j.jcv.2014.12.01410.1016/j.jcv.2014.12.014]

77 **Katsoulidou A**, Sypsa V, Tassopoulos NC, Boletis J, Karafoulidou A, Ketikoglou I, Tsantoulas D, Vafiadi I, Hatzis G, Skoutelis A, Akriviadis E, Vasiliadis T, Kitis G, Magiorkinis G, Hatzakis A. Molecular epidemiology of hepatitis C virus (HCV) in Greece: temporal trends in HCV genotype-specific incidence and molecular characterization of genotype 4 isolates. *J Viral Hepat* 2006; **13**: 19-27 [PMID: 16364078 DOI: 10.1111/j.1365-2893.2005.00649.x]

78 **Matera G**, Lamberti A, Quirino A, Focà D, Giancotti A, Barreca GS, Guadagnino V, Liberto MC. Changes in the prevalence of hepatitis C virus (HCV) genotype 4 in Calabria, Southern Italy. *Diagn Microbiol Infect Dis* 2002; **42**: 169-173 [PMID: 11929687 DOI: 10.1016/S0732-8893(01)00350-9]

79 **Liberto MC**, Marascio N, Zicca E, Matera G. Epidemiological features and specificities of HCV infection: a hospital-based cohort study in a university medical center of Calabria region. *BMC Infect Dis* 2012; **12** Suppl 2: S4 [PMID: 23173638 DOI: 10.1186/1471-2334-12-S2-S4]

80 **Sánchez-Quijano A**, Abad MA, Torronteras R, Rey C, Pineda JA, Leal M, Macias J, Lissen E. Unexpected high prevalence of hepatitis C virus genotype 4 in Southern Spain. *J Hepatol* 1997; **27**: 25-29 [PMID: 9252069 DOI: 10.1016/S0168-8278(97)80275-9]

81 **de Bruijne J**, Schinkel J, Prins M, Koekkoek SM, Aronson SJ, van Ballegooijen MW, Reesink HW, Molenkamp R, van de Laar TJ. Emergence of hepatitis C virus genotype 4: phylogenetic analysis reveals three distinct epidemiological profiles. *J Clin Microbiol* 2009; **47**: 3832-3838 [PMID: 19794040 DOI: 10.1128/JCM.01146-09]

82 **Cacoub P**, Dabis F, Costagliola D, Almeida K, Lert F, Piroth L, Semaille C. Burden of HIV and hepatitis C co-infection: the changing epidemiology of hepatitis C in HIV-infected patients in France. *Liver Int* 2015; **35**: 65-70 [PMID: 25040895 DOI: 10.1111/liv.12639]

83 **Cebolla B**, Björnberg A, editors. Health Consumer Powerhouse: Euro Hepatitis Index Report. 2012. Available from: URL: http://www.healthpowerhouse.com

84 **Pharris A**, Wiessing L, Sfetcu O, Hedrich D, Botescu A, Fotiou A, Nikolopoulos GK, Malliori M, Salminen M, Suk JE, Griffiths P, van de Laar MJ. Human immunodeficiency virus in injecting drug users in Europe following a reported increase of cases in Greece and Romania, 2011. *Euro Surveill* 2011; **16**: pii 20032 [PMID: 22172301]

85 **Paraskevis D**, Nikolopoulos G, Tsiara C, Paraskeva D, Antoniadou A, Lazanas M, Gargalianos P, Psychogiou M, Malliori M, Kremastinou J, Hatzakis A. HIV-1 outbreak among injecting drug users in Greece, 2011: a preliminary report. *Euro Surveill* 2011; **16**: pii 19962 [PMID: 21924120]

86 **Oprea C**, Ceausu E, Ruta S. Ongoing outbreak of multiple blood-borne infections in injecting drug users in Romania. *Public Health* 2013; **127**: 1048-1050 [PMID: 24239282 DOI: 10.1016/j.puhe.2013.08.018]

87 European Commision. Eurostat Yearbook, 2014. Available from: URL: http://ec.europa.eu/eurostat

88 **Sypsa V**, Paraskevis D, Malliori M, Nikolopoulos GK, Panopoulos A, Kantzanou M, Katsoulidou A, Psichogiou M, Fotiou A, Pharris A, Van De Laar M, Wiessing L, Jarlais DD, Friedman SR, Hatzakis A. Homelessness and Other Risk Factors for HIV Infection in the Current Outbreak Among Injection Drug Users in Athens, Greece. *Am J Public Health* 2015; **105**: 196-204 [PMID: 24524508 DOI: 10.2105/AJPH.2013.301656]

89 **Sultana C**, Oprisan G, Szmal C, Vagu C, Temereanca A, Dinu S, Teleman MD, Ruta S. Molecular epidemiology of hepatitis C virus strains from Romania. *J Gastrointestin Liver Dis* 2011; **20**: 261-266 [PMID: 21961093]

90 **Niculescu I**, Paraschiv S, Paraskevis D, Abagiu A, Batan I, Banica L, Otelea D. Recent HIV-1 Outbreak Among Intravenous Drug Users in Romania: Evidence for Cocirculation of CRF14_BG and Subtype F1 Strains. *AIDS Res Hum Retroviruses* 2015; **31**: 488-495 [PMID: 25369079 DOI: 10.1089/aid.2014.0189]

91 **Gigi E**, Sinakos E, Sykja A, Androulakis G, Tanis C, Stayridou V, Tsirogianni E, Zouridakis K, Bellou AL, Orfanou E, Raptopoulou-Gigi M. Epidemiology, clinical data, and treatment of viral hepatitis in a large cohort of intravenous drug users. *J Addict Med* 2013; **7**: 52-57 [PMID: 23340710 DOI: 10.1097/ADM.0b013e318279756f]

92 **Paraskevis D**, Nikolopoulos G, Fotiou A, Tsiara C, Paraskeva D, Sypsa V, Lazanas M, Gargalianos P, Psichogiou M, Skoutelis A, Wiessing L, Friedman SR, Jarlais DC, Terzidou M, Kremastinou J, Malliori M, Hatzakis A. Economic recession and emergence of an HIV-1 outbreak among drug injectors in Athens metropolitan area: a longitudinal study. *PLoS One* 2013; **8**: e78941 [PMID: 24265730 DOI: 10.1371/journal.pone.0078941]

93 **International Organization for Migration**. The World Migration Report 2013: Migrant Well-being and Development - the 7th report in IOM's World Migration Report series, 2013, Geneva. Available from: URL: http://www.iom.int

94 **Marascio N**, Liberto M, Barreca G, Zicca E, Quirino A, Lamberti A, Bianco G, Matera G, Surace L, Berardelli G, Surace L, De Maria V, Giancotti F, Leone R, Villella V, Nisticò S, Borelli A, Caruso V, Calderazzo M, Griffo G, Masciari R, Minchella P, Cosco L, Laganà C, Oliva A, Foti G, Fiorillo M, Bocchiaro G, Surace P, Ciccaglione A, Ciccozzi M, Cesario F, Torti C, Focà A. Update on epidemiology of HCV in Italy: focus on the Calabria Region. *BMC Infect Dis* 2014; **14** Suppl 5: S2 [PMID: 25236184 DOI: 10.1186/1471-2334-14-S5-S2]

95 **Zehender G**, Sorrentino C, Lai A, Ebranati E, Gabanelli E, Lo Presti A, Vujoševic D, Lauševic D, Terzić D, Shkjezi R, Bino S, Vratnica Z, Mugosa B, Galli M, Ciccozzi M. Reconstruction of the evolutionary dynamics of hepatitis C virus subtypes in Montenegro and the Balkan region. *Infect Genet Evol* 2013; **17**: 223-230 [PMID: 23603418 DOI: 10.1016/j.meegid.2013.04.003]

96 **Di Lello FA**, Neukam K, Parra-Sanchez M, Plaza Z, Soriano V, Cifuentes C, Mira JA, Poveda E, Pineda JA. Hepatitis C virus genotype 4 in Southern and Central Spain does not originate from recent foreign migration waves. *J Med Virol* 2013; **85**: 1734-1740 [PMID: 23861220 DOI: 10.1002/jmv.23657]

97 **Ciccozzi M**, Equestre M, Costantino A, Marascio N, Quirino A, Lo Presti A, Cella E, Bruni R, Liberto MC, Focà A, Pisani G, Zehender G, Ciccaglione AR. Hepatitis C virus genotype 4d in Southern Italy: reconstruction of its origin and spread by a phylodynamic analysis. *J Med Virol* 2012; **84**: 1613-1619 [PMID: 22930510 DOI: 10.1002/jmv.23384]

98 **Sacks-Davis R**, Daraganova G, Aitken C, Higgs P, Tracy L, Bowden S, Jenkinson R, Rolls D, Pattison P, Robins G, Grebely J, Barry A, Hellard M. Hepatitis C virus phylogenetic clustering is associated with the social-injecting network in a cohort of people who inject drugs. *PLoS One* 2012; **7**: e47335 [PMID: 23110068 DOI: 10.1371/journal.pone.0047335]

99 **Jacka B**, Applegate T, Krajden M, Olmstead A, Harrigan PR, Marshall BD, DeBeck K, Milloy MJ, Lamoury F, Pybus OG, Lima VD, Magiorkinis G, Montoya V, Montaner J, Joy J, Woods C, Dobrer S, Dore GJ, Poon AF, Grebely J. Phylogenetic clustering of hepatitis C virus among people who inject drugs in Vancouver, Canada. *Hepatology* 2014; **60**: 1571-1580 [PMID: 25042607 DOI: 10.1002/hep.27310]

100 **Lim SG**. Chronic hepatitis C genotype 1 treatment roadmap for resource constrained settings. *World J Gastroenterol* 2015; **21**: 1972-1981 [PMID: 25684966 DOI: 10.3748/wjg.v21.i6.1972]

101 **Abenavoli L**, Masarone M, Peta V, Milic N, Kobyliak N, Rouabhia S, Persico M. Insulin resistance and liver steatosis in chronic hepatitis C infection genotype 3. *World J Gastroenterol* 2014; **20**: 15233-15240 [PMID: 25386071 DOI: 10.3748/wjg.v20.i41.15233]

102 **Quer J**, Gregori J, Rodríguez-Frias F, Buti M, Madejon A, Perez-del-Pulgar S, Garcia-Cehic D, Casillas R, Blasi M, Homs M, Tabernero D, Alvarez-Tejado M, Muñoz JM, Cubero M, Caballero A, del Campo JA, Domingo E, Belmonte I, Nieto L, Lens S, Muñoz-de-Rueda P, Sanz-Cameno P, Sauleda S, Bes M, Gomez J, Briones C, Perales C, Sheldon J, Castells L, Viladomiu L, Salmeron J, Ruiz-Extremera A, Quiles-Pérez R, Moreno-Otero R, López-Rodríguez R, Allende H, Romero-Gómez M, Guardia J, Esteban R, Garcia-Samaniego J, Forns X, Esteban JI. High-resolution hepatitis C virus subtyping using NS5B deep sequencing and phylogeny, an alternative to current methods. *J Clin Microbiol* 2015; **53**: 219-226 [PMID: 25378574 DOI: 10.1128/JCM.02093-14]

103 **World Health Organization (WHO)**. Guidelines for the screening, care and treatment of persons with hepatitis C infection. WHO Library Cataloguing-in-Publication 2014. Available from: URL: http://www.who.int/hepatitis/hepatitis-c-guidelines/

104 **American Association for the Study of Liver Diseases (AASLD) and Infectious Diseases Society of America**. Recommendations for testing, managing, and treating hepatitis C. 2014. Available from: URL: http://www.hcvguidelines.org/full-report-view

105 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]

106 **Kohli A**, Shaffer A, Sherman A, Kottilil S. Treatment of hepatitis C: a systematic review. *JAMA* 2014; **312**: 631-640 [PMID: 25117132 DOI: 10.1001/jama.2014.7085]

107 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]

108 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]

109 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]

110 **Ferenci P**. Treatment of hepatitis C in difficult-to-treat patients. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 284-292 [PMID: 25895822 DOI: 10.1038/nrgastro.2015.53]

111 **Koff RS**. Review article: the efficacy and safety of sofosbuvir, a novel, oral nucleotide NS5B polymerase inhibitor, in the treatment of chronic hepatitis C virus infection. *Aliment Pharmacol Ther* 2014; **39**: 478-487 [PMID: 24387618 DOI: 10.1111/apt.12601]

112 **Lawitz E**, Poordad F, Brainard DM, Hyland RH, An D, Dvory-Sobol H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir with peginterferon-ribavirin for 12 weeks in previously treated patients with hepatitis C genotype 2 or 3 and cirrhosis. *Hepatology* 2015; **61**: 769-775 [PMID: 25322962 DOI: 10.1002/hep.27567]

113 **Akkarathamrongsin S**, Payungporn S, Thong VD, Poovorawan K, Prapunwattana P, Poovorawan Y, Tangkijvanich P. Early viral kinetics during hepatitis C virus genotype 6 treatment according to IL28B polymorphisms. *World J Gastroenterol* 2014; **20**: 10599-10605 [PMID: 25132781 DOI: 10.3748/wjg.v20.i30.10599]

114 **Sullivan JC**, De Meyer S, Bartels DJ, Dierynck I, Zhang EZ, Spanks J, Tigges AM, Ghys A, Dorrian J, Adda N, Martin EC, Beumont M, Jacobson IM, Sherman KE, Zeuzem S, Picchio G, Kieffer TL. Evolution of treatment-emergent resistant variants in telaprevir phase 3 clinical trials. *Clin Infect Dis* 2013; **57**: 221-229 [PMID: 23575197 DOI: 10.1093/cid/cit226]

115 **Ogert RA**, Howe JA, Vierling JM, Kwo PY, Lawitz EJ, McCone J, Schiff ER, Pound D, Davis MN, Gordon SC, Ravendhran N, Rossaro L, Jacobson IM, Ralston R, Chaudhri E, Qiu P, Pedicone LD, Brass CA, Albrecht JK, Barnard RJ, Hazuda DJ, Howe AY. Resistance-associated amino acid variants associated with boceprevir plus pegylated interferon-α2b and ribavirin in patients with chronic hepatitis C in the SPRINT-1 trial. *Antivir Ther* 2013; **18**: 387-397 [PMID: 23406826 DOI: 10.3851/IMP2549]

116 **Barnard RJ**, Howe JA, Ogert RA, Zeuzem S, Poordad F, Gordon SC, Ralston R, Tong X, Sniukiene V, Strizki J, Ryan D, Long J, Qiu P, Brass CA, Albrecht J, Burroughs M, Vuocolo S, Hazuda DJ. Analysis of boceprevir resistance associated amino acid variants (RAVs) in two phase 3 boceprevir clinical studies. *Virology* 2013; **444**: 329-336 [PMID: 23876458 DOI: 10.1016/j.virol.2013.06.029]

117 **Poveda E**, Wyles DL, Mena A, Pedreira JD, Castro-Iglesias A, Cachay E. Update on hepatitis C virus resistance to direct-acting antiviral agents. *Antiviral Res* 2014; **108**: 181-191 [PMID: 24911972 DOI: 10.1016/j.antiviral.2014.05.015]

118 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky

VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]

119 **Vidal LL**, Santos AF, Soares MA. Worldwide distribution of the NS3 gene 80K polymorphism among circulating hepatitis C genotype 1 viruses: implication for simeprevir usage. *J Antimicrob Chemother* 2015; **70**: 2024-2027 [PMID: 25835991 DOI: 10.1093/jac/dkv081]

120 **Nakamoto S**, Kanda T, Wu S, Shirasawa H, Yokosuka O. Hepatitis C virus NS5A inhibitors and drug resistance mutations. *World J Gastroenterol* 2014; **20**: 2902-2912 [PMID: 24659881 DOI: 10.3748/wjg.v20.i11.2902]

121 **McPhee F**, Hernandez D, Yu F, Ueland J, Monikowski A, Carifa A, Falk P, Wang C, Fridell R, Eley T, Zhou N, Gardiner D. Resistance analysis of hepatitis C virus genotype 1 prior treatment null responders receiving daclatasvir and asunaprevir. *Hepatology* 2013; **58**: 902-911 [PMID: 23504694 DOI: 10.1002/hep.26388]

122 **Grebely J**, Dore GJ. Can hepatitis C virus infection be eradicated in people who inject drugs? *Antiviral Res* 2014; **104**: 62-72 [PMID: 24468275 DOI: 10.1016/j.antiviral.2014.01.002]

123 **McGowan C**, Harris M, Rhodes T. Hepatitis C avoidance in injection drug users: a typology of possible protective practices. *PLoS One* 2013; **8**: e77038 [PMID: 24194855 DOI: 10.1371/journal.pone.0077038]

124 **Reimer J**, Schmidt CS, Schulte B, Gansefort D, Gölz J, Gerken G, Scherbaum N, Verthein U, Backmund M. Psychoeducation improves hepatitis C virus treatment during opioid substitution therapy: a controlled, prospective multicenter trial. *Clin Infect Dis* 2013; **57** Suppl 2: S97-104 [PMID: 23884073 DOI: 10.1093/cid/cit307]

125 **Korthuis PT**, Feaster DJ, Gomez ZL, Das M, Tross S, Wiest K, Douaihy A, Mandler RN, Sorensen JL, Colfax G, McCarty D, Cohen SE, Penn PE, Lape D, Metsch LR. Injection behaviors among injection drug users in treatment: the role of hepatitis C awareness. *Addict Behav* 2012; **37**: 552-555 [PMID: 22209655 DOI: 10.1016/j.addbeh.2011.12.001]

126 **Backmund M**, Meyer K, Edlin BR. Infrequent reinfection after successful treatment for hepatitis C virus infection in injection drug users. *Clin Infect Dis* 2004; **39**: 1540-1543 [PMID: 15546094 DOI: 10.1086/425361]

127 **Grebely J**, Knight E, Ngai T, Genoway KA, Raffa JD, Storms M, Gallagher L, Krajden M, Dore GJ, Duncan F, Conway B. Reinfection with hepatitis C virus following sustained virological response in injection drug users. *J Gastroenterol Hepatol* 2010; **25**: 1281-1284 [PMID: 20594256 DOI: 10.1111/j.1440-1746.2010.06238.x]

128 **Grebely J**, Pham ST, Matthews GV, Petoumenos K, Bull RA, Yeung B, Rawlinson W, Kaldor J, Lloyd A, Hellard M, Dore GJ, White PA. Hepatitis C virus reinfection and superinfection among treated and untreated participants with recent infection. *Hepatology* 2012; **55**: 1058-1069 [PMID: 22031335 DOI: 10.1002/hep.24754]

129 **Hagan LM**, Yang Z, Ehteshami M, Schinazi RF. All-oral, interferon-free treatment for chronic hepatitis C: cost-effectiveness analyses. *J Viral Hepat* 2013; **20**: 847-857 [PMID: 24304454 DOI: 10.1111/jvh.12111]

130 **Zanini B**, Benini F, Pigozzi MG, Furba P, Giacò E, Cinquegrana A, Fasoli M, Lanzini A. Addicts with chronic hepatitis C: difficult to reach, manage or treat? *World J Gastroenterol* 2013; **19**: 8011-8019 [PMID: 24307794 DOI: 10.3748/wjg.v19.i44.8011.]

131 **Tovo CV**, de Mattos AA, de Almeida PR. Chronic hepatitis C genotype 1 virus: who should wait for treatment? *World J Gastroenterol* 2014; **20**: 2867-2875 [PMID: 24659878 DOI: 10.3748/wjg.v20.i11.2867]

132 **Calvo-Cidoncha E**, González-Bueno J, Almeida-González CV, Morillo-Verdugo R. Influence of treatment complexity on adherence and incidence of blips in HIV/HCV coinfected patients. *J Manag Care Spec Pharm* 2015; **21**: 153-157 [PMID: 25615004]

133 **Razavi H**, Waked I, Sarrazin C, Myers RP, Idilman R, Calinas F, Vogel W, Mendes Correa MC, Hézode C, Lázaro P, Akarca U, Aleman S, Balık I, Berg T, Bihl F, Bilodeau M, Blasco AJ, Brandão Mello CE, Bruggmann P, Buti M, Calleja JL, Cheinquer H, Christensen PB, Clausen M, Coelho HS, Cramp ME, Dore GJ, Doss W, Duberg AS, El-Sayed MH, Ergör G, Esmat G, Falconer K, Félix J, Ferraz ML, Ferreira PR, Frankova S, García-Samaniego J, Gerstoft J, Giria JA, Gonçales FL, Gower E, Gschwantler M, Guimarães Pessôa M, Hindman SJ, Hofer H, Husa P, Kåberg M, Kaita KD, Kautz A, Kaymakoglu S, Krajden M, Krarup H, Laleman W, Lavanchy D, Marinho RT, Marotta P, Mauss S, Moreno C, Murphy K, Negro F, Nemecek V, Örmeci N, Øvrehus AL, Parkes J, Pasini K, Peltekian KM, Ramji A, Reis N, Roberts SK, Rosenberg WM, Roudot-Thoraval F, Ryder SD, Sarmento-Castro R, Semela D, Sherman M, Shiha GE, Sievert W, Sperl J, Stärkel P, Stauber RE, Thompson AJ, Urbanek P, Van Damme P, van Thiel I, Van Vlierberghe H, Vandijck D, Wedemeyer H, Weis N, Wiegand J, Yosry A, Zekry A, Cornberg M, Müllhaupt B, Estes C. The present and future disease burden of hepatitis C virus (HCV) infection with today's treatment paradigm. *J Viral Hepat* 2014; **21** Suppl 1: 34-59 [PMID: 24713005 DOI: 10.1111/jvh.12248]

134 **Aspinall EJ**, Corson S, Doyle JS, Grebely J, Hutchinson SJ, Dore GJ, Goldberg DJ, Hellard ME. Treatment of hepatitis C virus infection among people who are actively injecting drugs: a systematic review and meta-analysis. *Clin Infect Dis* 2013; **57** Suppl 2: S80-S89 [PMID: 23884071 DOI: 10.1093/cid/cit306]

P- Reviewer: Chuang WL **S- Editor**: Yu J **L- Editor**: A
E- Editor: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10824

World J Gastroenterol 2015 October 14; 21(38): 10824-10829
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Laparoscopic Surgery

Review of single incision laparoscopic surgery in colorectal surgery

Nisreen Madhoun, Deborah S Keller, Eric M Haas

Nisreen Madhoun, Deborah S Keller, Eric M Haas, Colorectal Surgical Associates, Houston, TX 77054, United States

Eric M Haas, Division of Minimally Invasive Colorectal Surgery, Department of Surgery, University of Texas Medical School at Houston, TX 77054, United States

Eric M Haas, Houston Methodist Hospital, Houston, Texas, TX 77054, United States

Author contributions: All authors contributed to this work.

Correspondence to: Eric M Haas, MD, FACS, FASCRS, Division of Minimally Invasive Colorectal Surgery, Department of Surgery, University of Texas Medical School at Houston, 7900 Fannin, Suite 2700, Houston, TX 77054, United States. ehaasmd@houstoncolon.com
Telephone: +1-713-7900600
Fax: +1-713-7900616

Received: April 9, 2015
Peer-review started: April 9, 2015
First decision: June 23, 2015
Revised: July 8, 2015
Accepted: September 14, 2015
Article in press: September 14, 2015
Published online: October 14, 2015

Abstract

As surgical techniques continue to move towards less invasive techniques, single incision laparoscopic surgery (SILS), a hybrid between traditional multiport laparoscopy and natural orifice transluminal endoscopic surgery, was introduced to further the enhanced outcomes of multiport laparoscopy. The safety and feasibility of SILS for both benign and malignant colorectal disease has been proven. SILS provides the potential for improved cosmesis, postoperative pain, recovery time, and quality of life at the drawback of higher technical skill required. In this article, we review the history, describe the available technology and techniques, and evaluate the benefits and limitations of SILS for colorectal surgery in the published literature.

Key words: Laparoscopic colectomy; Minimally invasive colorectal surgery; Single-incision laparoscopic surgery

Core tip: As surgical techniques continue to move towards less invasive techniques, single incision laparoscopic surgery (SILS) is a valuable platform with distinct advantages and comparable or better outcomes than other minimally invasive platforms. The safety and feasibility of SILS for both benign and malignant colorectal disease has been proven, and this review of the history, current state, available technology, limitations to widespread use, and their solutions will be a valuable addition to the published literature. It will draw attention to the benefits and potentially increase use of the platform and minimally invasive surgery as a whole.

Madhoun N, Keller DS, Haas EM. Review of single incision laparoscopic surgery in colorectal surgery. *World J Gastroenterol* 2015; 21(38): 10824-10829 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10824.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10824

INTRODUCTION

Since the first laparoscopic colectomy was described in 1991, minimally invasive colorectal surgery has continued to grow[1]. The expanding use of laparoscopy has been the greatest technical and clinical advance in the field of colorectal surgery. Laparoscopic colorectal surgery has been proven to improve patient outcomes, including faster return of gastrointestinal function, less postoperative pain, shorter length of stay, lower complication and readmission rates, and lower total healthcare utilization compared to open surgery[1-14]. Despite proven benefits, use of laparoscopy is estimated at only 50% of all colorectal procedures, 20% of colon cancer, and 10% of rectal cancer procedures in the United States[15,16]. Thus, there is room to increase utilization and the benefits. In addition, there is the continued drive towards reduced port and "scarless" surgery, and great efforts have been made to minimize surgical trauma, improving cosmesis and surgery-related pain and morbidity. Techniques, such as natural orifice transluminal endoscopic surgery (NOTES) and single-incision laparoscopy surgery (SILS) have been developed to reach the goals. NOTES is still in the experimental stages, but SILS is ready for incorporation into routine practice and currently regarded as the next major advance in the progression of minimally invasive surgical approaches feasible in generalized use[17,18].

SINGLE INCISION LAPAROSCOPIC SURGERY

SILS was developed to further the outcomes of multiport laparoscopy. The SILS technique was first reported for colorectal surgery in 2008, when both Remzi *et al*[19] and Bucher *et al*[20] reported use for right colectomy. Since that time, multiple studies have proven SILS is safe and feasible for the full array of benign and malignant colorectal disease, and its applications continue to grow[21-35]. SILS is currently regarded as the next major advance in the progression of minimally invasive surgical approaches to colorectal disease suitable for generalized use[17] (Figure 1).

TECHNICAL NOTES

Most SILS procedures enter the peritoneum at the umbilicus, creating a "hidden" incision and allowing the fascial incision to be lengthened without extending the overlying skin incision[36]. If a stoma is planned, the ostomy site may be used for access, allowing "scarless" surgery[23,37,38].

For access, there are several commercially produced SILS ports, as well as a homemade glove port. The most common ports are the SILS™ Port (Covidien, Mansfield, Massachusetts, United States), the GelPOINT® platform (Applied Medical, Rancho Santa Margarita, CA, United States), and the TriPort or QuadPort (Olympus Medical, Center Valley, PA, United States). All devices have three or more working channels in the single port to introduce the laparoscopic instruments and a camera into the operative field through a solitary incision. The single incision helps reduce fascial defects, abdominal wall trauma, and their associated postoperative pain and hernia risk[39-41]. Each port is introduced through a 2-4 cm skin and fascial incision, and has costs and benefits. The SILS™ Port (Covidien, Mansfield, MA, United States) is pliable elastomeric foam that creates a seal with the skin to maintain pneumoperitoneum, offers enhanced mobility, and allows the surgeon to interchange 5-mm and 12-mm ports. However, the SILS™ port is limited to 3 trocars and has no wound protector for specimen extraction. The GelPOINT® uses a wound protector sleeve inserted into the peritoneum and GelSeal® cap that trocars are inserted into per surgeon preference. The port offers a low internal profile, which may help accommodate various abdominal wall sizes, and the sleeve offers protection during specimen extraction from tumor seeding and superficial wound infections[42,43]. The GelPOINT® has a larger profile on the abdominal wall, and may lose pneumoperitoneum with extreme torque. The TriPort and QuadPort channels have three or four instrument channels, respectively, a similar to the GelPOINT®, and a lower external profile. However, the assembly, insertion, and extracorporealization are reported more difficult than other platforms. The glove port uses a sterile, non-latex glove secured to a small wound protector, with the glove's fingers used for instrument and camera access. This approach is simple, inexpensive, and easily reproducible, but there is a poor seal and lack of rigidity provided from the finger ports compared to commercially available devices[35,44-47].

Standard laparoscopic tools are commonly used with SILS, but straight, curved, and articulating instruments are available. Straight instruments offer rigidity, but when working in a parallel, fixed space, there can be collisions between the working ports and the camera. Curved instruments were introduced to remedy collisions, but they cannot be passed through conventional, straight trocars. Articulating instruments were designed to overcome the lack of triangulation, as they articulate at the tip, rotating 360° around the instrument axis. However, there is a loss of rigidity and tactile feedback with the flexible tools[48-50]. It is generally agreed upon that straight laparoscopic instruments are preferred and the curved or articulating instruments are not required or commonly used in practice.

OUTCOMES WITH SILS

In all clinical and quality metrics, SILS has comparable outcomes to traditional laparoscopy[34,39]. Studies

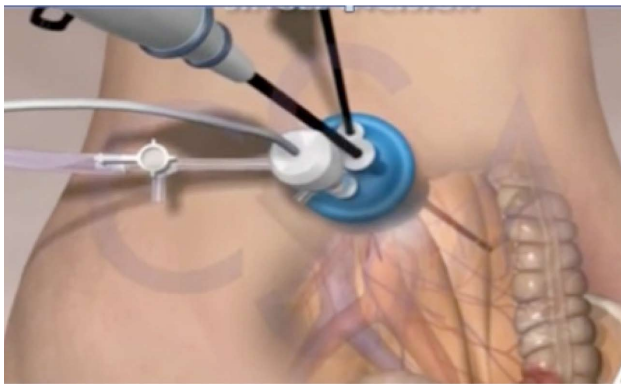


Figure 1 Single incision laparoscopic surgery animation.

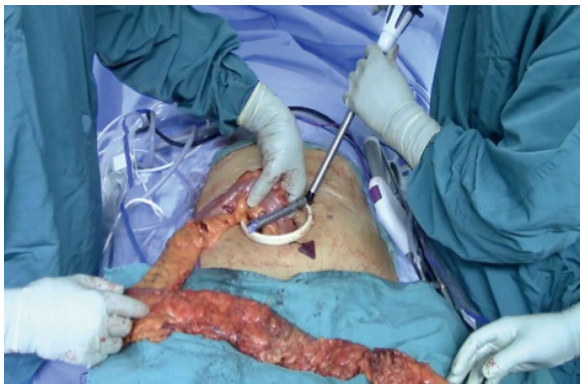


Figure 2 Specimen removal through single incision platform.

have proven SILS is feasible and safe for benign and malignant colorectal disease[22,28-30,32,33]. From early reports, SILS had similar postoperative outcomes, including complication, intraoperative conversion, and readmission rates[39]. Oncologic outcomes, including the feasibility of R0 resection, specimen length, number of lymph nodes harvested, and proximal and distal margins were comparable to multiport laparoscopy[51-54]. SILS has been shown safe and feasible specifically in rectal resections[29]. Initial studies reported an increased operative time with SILS, but failed to take into account the learning curve and need for experience[52,55]. The operative time decreases with accumulating experience, with a learning curve defined between 30 to 36 cases[56].

SILS has distinct benefits over traditional laparoscopic surgery. Using a single port with multiple incorporated working channels, SILS has reduced the number of incisions and tissue trauma required for surgery, improved cosmesis, and lowered the rate of port-site related complications and incisional hernias[39-41,57] (Figure 2). Reduced perioperative pain is another reported advantage of SILS over traditional multiport laparoscopy, with the reduction in pain translating to lower pain scores and opioid use from the immediate post-operative period up to post-operative day 2[39,41]. SILS has also shown a significantly shorter length of stay (LOS); studies have demonstrated LOS more than 1 d shorter for SILS compared to multiport laparoscopy[28,39]. A recent meta-analysis reviewing 14 studies comparing SILS to traditional multiport laparoscopy concluded SILS had lower blood loss, decreased blood transfusion requirement, shorter time to flatus, shorter hospital stay, and smaller incision[51].

TECHNICAL CHALLENGES

The use of SILS introduced several new technical challenges, which may limit widespread use of the platform[58]. The ergonomic and technical requirements of SILS are distinct from those used in conventional multiport laparoscopy, initially adding difficulty even for experienced laparoscopic surgeons[59,60]. The technical challenges are further amplified in colorectal procedures, where there is the need to work in more than one quadrant[58,61]. First, there is the challenge from the in-line orientation of the working trocars through the single access port causes the visual axis to become more in-line, with camera movement resulting in inadvertent movement of the adjacent instrument[17,62]. Working through a small single incision with multiple parallel, instruments competing for the same space at the fulcrum of the entry port decreases the range of motion and external working space, increasing instrument collisions[28,58,63]. These collisions are experienced both intra-corporeally, creating difficulty maintaining pneumoperitoneum, and extra-corporeally, complicating the role of the assistant holding the camera[28]. This forces the surgeon to operate with crossed hands to acclimate[17,62]. The proximity of the trocars at a fixed position, restricted freedom, and clashing of the instruments is contradictory to the traditional teaching of triangulation in laparoscopy[64]. These problems in exposure and "crowding" add to the difficulty in the SILS technique and can result in restricted visualization, inadequate dissection and mobilization, and the potential for inadvertent injury[55,65].

With increasing operator experience, these ergonomic and technical challenges can be readily overcome. Technical instruments and procedural adaptations have been developed to help work through these challenges. To improve surgeon efficiency and decrease collisions, it has also been recommended to keep the laparoscope away from the surgeon's hands, such as with a flexible-tipped or bariatric-length laparoscope[24,28,66,67]. Articulating or curved instruments can be used to help recreate triangulation familiar with multiport laparoscopy[58]. For assistance in pelvic and multi-quadrant cases, a SILS +1 technique has been developed and validated[65]. With SILS +1, the single access device is introduced through a Pfannenstiel incision and an additional 5-mm port is placed through the umbilicus for the laparoscopic camera, allowing access to more than one abdominal quadrant and minimizing "sword fighting" between the surgeon

and the camera holder[65]. To become proficient at SILS, one idea is to become proficient at reduced port laparoscopy - using 3 ports; then the transition to SILS will be more natural.

CURRENT STATE AND MOVING FORWARD WITH SILS

Despite evidence supporting the use and proven benefits, SILS has not been widely adopted. The main reason cited is the ergonomic demands and additional time, costs, and skills required, especially in early cases[55,59,64,68]. Surgeon experience can overcome the technical and ergonomic challenges, and specialized instruments and platforms have been developed to help ascend the learning curve[17,62].

The technology was also advocated for surgeons experienced with laparoscopy and minimally invasive techniques, and results described in the published literature are achieved by skilled laparoscopic surgeons beyond the learning curve performing the procedures[32,34,69,70]. In addition, published experience has centered on non-obese patients[23,66,70-74]. To increase utilization of this minimally invasive technique, its feasibility in different patient populations must be explored[72]. The learning curve to achieve competence with this technology has been defined, and there are no increased complications or negative outcomes reported during the early phases of the learning curve[56,72]. Therefore, increasing use of SILS for patient benefits and increased overall use of minimally invasive colorectal surgery is encouraged.

CONCLUSION

As the field of colorectal surgery has emphasized moving towards less invasive techniques, single incision laparoscopic surgery, a hybrid between traditional multiport laparoscopy and NOTES, is the natural evolution in minimally invasive surgery. SILS offers distinct benefits over traditional multiport laparoscopy, but widespread use has been limited from technical, ergonomic, and patient selection challenges. With experience demonstrating the safety and feasibility, and the learning curve for competence defined, increased use of SILS in colorectal surgery is encouraged.

REFERENCES

1 **Clinical Outcomes of Surgical Therapy Study Group**. A comparison of laparoscopically assisted and open colectomy for colon cancer. *N Engl J Med* 2004; **350**: 2050-2059 [PMID: 15141043 DOI: 10.1056/NEJMoa032651]

2 **Lacy AM**, García-Valdecasas JC, Delgado S, Castells A, Taurá P, Piqué JM, Visa J. Laparoscopy-assisted colectomy versus open colectomy for treatment of non-metastatic colon cancer: a randomised trial. *Lancet* 2002; **359**: 2224-2229 [PMID: 12103285 DOI: 10.1016/S0140-6736(02)09290-5]

3 **Delaney CP**, Kiran RP, Senagore AJ, Brady K, Fazio VW. Case-matched comparison of clinical and financial outcome after laparoscopic or open colorectal surgery. *Ann Surg* 2003; **238**: 67-72 [PMID: 12832967 DOI: 10.1097/00000658-200308000-00010]

4 **Schwenk W**, Haase O, Neudecker J, Müller JM. Short term benefits for laparoscopic colorectal resection. *Cochrane Database Syst Rev* 2005; (**3**): CD003145 [PMID: 16034888 DOI: 10.1002/14651858.cd003145.pub2]

5 **Guillou PJ**, Quirke P, Thorpe H, Walker J, Jayne DG, Smith AM, Heath RM, Brown JM; MRC CLASICC trial group. Short-term endpoints of conventional versus laparoscopic-assisted surgery in patients with colorectal cancer (MRC CLASICC trial): multicentre, randomised controlled trial. *Lancet* 2005; **365**: 1718-1726 [PMID: 15894098 DOI: 10.1016/S0140-6736(05)66545-2]

6 **Fleshman J**, Sargent DJ, Green E, Anvari M, Stryker SJ, Beart RW, Hellinger M, Flanagan R, Peters W, Nelson H. Laparoscopic colectomy for cancer is not inferior to open surgery based on 5-year data from the COST Study Group trial. *Ann Surg* 2007; **246**: 655-662; discussion 662-664 [PMID: 17893502 DOI: 10.1097/SLA.0b013e318155a762]

7 **Bonjer HJ**, Hop WC, Nelson H, Sargent DJ, Lacy AM, Castells A, Guillou PJ, Thorpe H, Brown J, Delgado S, Kuhrij E, Haglind E, Påhlman L. Laparoscopically assisted vs open colectomy for colon cancer: a meta-analysis. *Arch Surg* 2007; **142**: 298-303 [PMID: 17372057 DOI: 10.1001/archsurg.142.3.298]

8 **Jayne DG**, Guillou PJ, Thorpe H, Quirke P, Copeland J, Smith AM, Heath RM, Brown JM. Randomized trial of laparoscopic-assisted resection of colorectal carcinoma: 3-year results of the UK MRC CLASICC Trial Group. *J Clin Oncol* 2007; **25**: 3061-3068 [PMID: 17634484 DOI: 10.1200/JCO.2006.09.7758]

9 **Veldkamp R**, Kuhry E, Hop WC, Jeekel J, Kazemier G, Bonjer HJ, Haglind E, Påhlman L, Cuesta MA, Msika S, Morino M, Lacy AM. Laparoscopic surgery versus open surgery for colon cancer: short-term outcomes of a randomised trial. *Lancet Oncol* 2005; **6**: 477-484 [PMID: 15992696 DOI: 10.1016/S1470-2045(05)70221-7]

10 **Delaney CP**, Chang E, Senagore AJ, Broder M. Clinical outcomes and resource utilization associated with laparoscopic and open colectomy using a large national database. *Ann Surg* 2008; **247**: 819-824 [PMID: 18438119 DOI: 10.1097/SLA.0b013e31816d950e]

11 **Delaney CP**, Marcello PW, Sonoda T, Wise P, Bauer J, Techner L. Gastrointestinal recovery after laparoscopic colectomy: results of a prospective, observational, multicenter study. *Surg Endosc* 2010; **24**: 653-661 [PMID: 19688390 DOI: 10.1007/s00464-009-0652-7]

12 **Jayne DG**, Thorpe HC, Copeland J, Quirke P, Brown JM, Guillou PJ. Five-year follow-up of the Medical Research Council CLASICC trial of laparoscopically assisted versus open surgery for colorectal cancer. *Br J Surg* 2010; **97**: 1638-1645 [PMID: 20629110 DOI: 10.1002/bjs.7160]

13 **Green BL**, Marshall HC, Collinson F, Quirke P, Guillou P, Jayne DG, Brown JM. Long-term follow-up of the Medical Research Council CLASICC trial of conventional versus laparoscopically assisted resection in colorectal cancer. *Br J Surg* 2013; **100**: 75-82 [PMID: 23132548 DOI: 10.1002/bjs.8945]

14 **van der Pas MH**, Haglind E, Cuesta MA, Fürst A, Lacy AM, Hop WC, Bonjer HJ. Laparoscopic versus open surgery for rectal cancer (COLOR II): short-term outcomes of a randomised, phase 3 trial. *Lancet Oncol* 2013; **14**: 210-218 [PMID: 23395398 DOI: 10.1016/S1470-2045(13)70016-0]

15 **Carmichael JC**, Masoomi H, Mills S, Stamos MJ, Nguyen NT. Utilization of laparoscopy in colorectal surgery for cancer at academic medical centers: does site of surgery affect rate of laparoscopy? *Am Surg* 2011; **77**: 1300-1304 [PMID: 22127074]

16 **Kwon S**, Billingham R, Farrokhi E, Florence M, Herzig D, Horvath K, Rogers T, Steele S, Symons R, Thirlby R, Whiteford M, Flum DR. Adoption of laparoscopy for elective colorectal resection: a report from the Surgical Care and Outcomes Assessment Program. *J Am Coll Surg* 2012; **214**: 909-18.e1 [PMID: 22533998 DOI: 10.1016/j.jamcollsurg.2012.03.010]

17 **Cianchi F**, Staderini F, Badii B. Single-incision laparoscopic

colorectal surgery for cancer: state of art. *World J Gastroenterol* 2014; **20**: 6073-6080 [PMID: 24876729 DOI: 10.3748/wjg.v20.i20.6073]
18 **Curcillo PG**, Podolsky ER, King SA. The road to reduced port surgery: from single big incisions to single small incisions, and beyond. *World J Surg* 2011; **35**: 1526-1531 [PMID: 21523502 DOI: 10.1007/s00268-011-1099-2]
19 **Remzi FH**, Kirat HT, Kaouk JH, Geisler DP. Single-port laparoscopy in colorectal surgery. *Colorectal Dis* 2008; **10**: 823-826 [PMID: 18684153 DOI: 10.1111/j.1463-1318.2008.01660.x]
20 **Bucher P**, Pugin F, Morel P. Single port access laparoscopic right hemicolectomy. *Int J Colorectal Dis* 2008; **23**: 1013-1016 [PMID: 18607608 DOI: 10.1007/s00384-008-0519-8]
21 **Boni L**, Dionigi G, Cassinotti E, Di Giuseppe M, Diurni M, Rausei S, Cantore F, Dionigi R. Single incision laparoscopic right colectomy. *Surg Endosc* 2010; **24**: 3233-3236 [PMID: 20464415 DOI: 10.1007/s00464-010-1100-4]
22 **Adair J**, Gromski MA, Lim RB, Nagle D. Single-incision laparoscopic right colectomy: experience with 17 consecutive cases and comparison with multiport laparoscopic right colectomy. *Dis Colon Rectum* 2010; **53**: 1549-1554 [PMID: 20940605 DOI: 10.1007/DCR.0b013e3181e85875]
23 **Geisler DP**, Condon ET, Remzi FH. Single incision laparoscopic total proctocolectomy with ileopouch anal anastomosis. *Colorectal Dis* 2010; **12**: 941-943 [PMID: 19895601 DOI: 10.1111/j.1463-1318.2009.02115.x]
24 **Law WL**, Fan JK, Poon JT. Single-incision laparoscopic colectomy: early experience. *Dis Colon Rectum* 2010; **53**: 284-288 [PMID: 20173474 DOI: 10.1007/DCR.0b013e3181c959ba]
25 **Merchant AM**, Lin E. Single-incision laparoscopic right hemicolectomy for a colon mass. *Dis Colon Rectum* 2009; **52**: 1021-1024 [PMID: 19502875 DOI: 10.1007/DCR.0b013e3181a4fabe]
26 **Choi SI**, Lee KY, Park SJ, Lee SH. Single port laparoscopic right hemicolectomy with D3 dissection for advanced colon cancer. *World J Gastroenterol* 2010; **16**: 275-278 [PMID: 20066750 DOI: 10.3748/wjg.v16.i2.275]
27 **Ramos-Valadez DI**, Patel CB, Ragupathi M, Bartley Pickron T, Haas EM. Single-incision laparoscopic right hemicolectomy: safety and feasibility in a series of consecutive cases. *Surg Endosc* 2010; **24**: 2613-2616 [PMID: 20364353 DOI: 10.1007/s00464-010-1017-y]
28 **Papaconstantinou HT**, Sharp N, Thomas JS. Single-incision laparoscopic right colectomy: a case-matched comparison with standard laparoscopic and hand-assisted laparoscopic techniques. *J Am Coll Surg* 2011; **213**: 72-80; discussion 80-2 [PMID: 21420878 DOI: 10.1016/j.jamcollsurg.2011.02.010]
29 **Kim SJ**, Ryu GO, Choi BJ, Kim JG, Lee KJ, Lee SC, Oh ST. The short-term outcomes of conventional and single-port laparoscopic surgery for colorectal cancer. *Ann Surg* 2011; **254**: 933-940 [PMID: 22107740 DOI: 10.1097/SLA.0b013e318237826b]
30 **Chen WT**, Chang SC, Chiang HC, Lo WY, Jeng LB, Wu C, Ke TW. Single-incision laparoscopic versus conventional laparoscopic right hemicolectomy: a comparison of short-term surgical results. *Surg Endosc* 2011; **25**: 1887-1892 [PMID: 21359907 DOI: 10.1007/s00464-010-1481-4]
31 **Rijcken E**, Mennigen R, Argyris I, Senninger N, Bruewer M. Single-incision laparoscopic surgery for ileocolic resection in Crohn's disease. *Dis Colon Rectum* 2012; **55**: 140-146 [PMID: 22228156 DOI: 10.1097/DCR.0b013e31823d0e0d]
32 **Champagne BJ**, Papaconstantinou HT, Parmar SS, Nagle DA, Young-Fadok TM, Lee EC, Delaney CP. Single-incision versus standard multiport laparoscopic colectomy: a multicenter, case-controlled comparison. *Ann Surg* 2012; **255**: 66-69 [PMID: 22104563 DOI: 10.1097/SLA.0b013e3182378442]
33 **Huscher CG**, Mingoli A, Sgarzini G, Mereu A, Binda B, Brachini G, Trombetta S. Standard laparoscopic versus single-incision laparoscopic colectomy for cancer: early results of a randomized prospective study. *Am J Surg* 2012; **204**: 115-120 [PMID: 22178484 DOI: 10.1016/j.amjsurg.2011.09.005]
34 **Makino T**, Milsom JW, Lee SW. Feasibility and safety of single-incision laparoscopic colectomy: a systematic review. *Ann Surg* 2012; **255**: 667-676 [PMID: 22258065 DOI: 10.1097/SLA.0b013e31823fbae7]
35 **Moftah M**, Nazour F, Cunningham M, Cahill RA. Single port laparoscopic surgery for patients with complex and recurrent Crohn's disease. *J Crohns Colitis* 2014; **8**: 1055-1061 [PMID: 24589026 DOI: 10.1016/j.crohns.2014.02.003]
36 **Keller DS**, Ragupathi M, Haas EM. Single Incision Laparoscopic Colon and Rectal Surgery. *Clin Colon Rectal Surg* 2015; In press
37 **Atallah SB**, Debeche-Adams T. Incisionless laparoscopic stoma construction using a 12-mm Hassan trocar. *Am Surg* 2012; **78**: E495-E497 [PMID: 23089432]
38 **Lopez NE**, Peterson CY, Ramamoorthy SL, McLemore EC, Sedrak MF, Lowy AM, Horgan S, Talamini MA, Sicklick JK. Single-incision laparoscopic surgery through an ostomy site: a natural approach by an unnatural orifice. *Surg Laparosc Endosc Percutan Tech* 2015; **25**: 74-78 [PMID: 24743670]
39 **Vasilakis V**, Clark CE, Liasis L, Papaconstantinou HT. Noncosmetic benefits of single-incision laparoscopic sigmoid colectomy for diverticular disease: a case-matched comparison with multiport laparoscopic technique. *J Surg Res* 2013; **180**: 201-207 [PMID: 22626560 DOI: 10.1016/j.jss.2011.11.510]
40 **Chambers WM**, Bicsak M, Lamparelli M, Dixon AR. Single-incision laparoscopic surgery (SILS) in complex colorectal surgery: a technique offering potential and not just cosmesis. *Colorectal Dis* 2011; **13**: 393-398 [PMID: 20002691 DOI: 10.1111/j.1463-1318.2009.02158.x]
41 **Poon JT**, Cheung CW, Fan JK, Lo OS, Law WL. Single-incision versus conventional laparoscopic colectomy for colonic neoplasm: a randomized, controlled trial. *Surg Endosc* 2012; **26**: 2729-2734 [PMID: 22538676 DOI: 10.1007/s00464-012-2262-z]
42 **Horiuchi T**, Tanishima H, Tamagawa K, Matsuura I, Nakai H, Shouno Y, Tsubakihara H, Inoue M, Tabuse K. Randomized, controlled investigation of the anti-infective properties of the Alexis retractor/protector of incision sites. *J Trauma* 2007; **62**: 212-215 [PMID: 17215757 DOI: 10.1097/01.ta.0000196704.78785.ae]
43 **Reid K**, Pockney P, Draganic B, Smith SR. Barrier wound protection decreases surgical site infection in open elective colorectal surgery: a randomized clinical trial. *Dis Colon Rectum* 2010; **53**: 1374-1380 [PMID: 20847618 DOI: 10.1007/DCR.0b013e3181ed3f7e]
44 **Day W**, Lau P. Novel "glove" access port for single port surgery in right hemicolectomy: a pilot study. *Surg Laparosc Endosc Percutan Tech* 2011; **21**: e145-e147 [PMID: 21654290 DOI: 10.1097/SLE.0b013e31821aa97e]
45 **Livraghi L**, Berselli M, Bianchi V, Latham L, Farassino L, Cocozza E. Glove technique in single-port access laparoscopic surgery: results of an initial experience. *Minim Invasive Surg* 2012; **2012**: 415430 [PMID: 22567226 DOI: 10.1155/2012/415430]
46 **Rodicio Miravalles JL**, Rodríguez García JI, Llaneza Folgueras A, Avilés García P, González González JJ. [Single port laparoscopic colostomy using the glove technique]. *Medicina* (B Aires) 2014; **74**: 201-204 [PMID: 24918667]
47 **Sirikurnpiboon S**. Single-access laparoscopic rectal cancer surgery using the glove technique. *Asian J Endosc Surg* 2014; **7**: 206-213 [PMID: 24661727 DOI: 10.1111/ases.12099]
48 **Rao PP**, Rao PP, Bhagwat S. Single-incision laparoscopic surgery - current status and controversies. *J Minim Access Surg* 2011; **7**: 6-16 [PMID: 21197236]
49 **Saber AA**, El-Ghazaly TH. Single-incision transumbilical laparoscopic right hemicolectomy using SILS Port. *Am Surg* 2011; **77**: 252-253 [PMID: 21337901]
50 **Trakarnsanga A**, Akaraviputh T, Wathanaoran P, Phalanusitthepha C, Methasate A, Chinswangwattanakul V. Single-incision laparoscopic colectomy without using special articulating instruments: an initial experience. *World J Surg Oncol* 2011; **9**: 162 [PMID: 22151649 DOI: 10.1186/1477-7819-9-162]
51 **Zhou YM**, Wu LP, Zhao YF, Xu DH, Li B. Single-incision versus conventional laparoscopy for colorectal disease: a meta-analysis. *Dig Dis Sci* 2012; **57**: 2103-2112 [PMID: 22466079 DOI: 10.1007/

s10620-012-2145-0]
52 **Daher R**, Chouillard E, Panis Y. New trends in colorectal surgery: single port and natural orifice techniques. *World J Gastroenterol* 2014; **20**: 18104-18120 [PMID: 25561780 DOI: 10.3748/wjg.v20.i48.18104]
53 **Chew MH**, Chang MH, Tan WS, Wong MT, Tang CL. Conventional laparoscopic versus single-incision laparoscopic right hemicolectomy: a case cohort comparison of short-term outcomes in 144 consecutive cases. *Surg Endosc* 2013; **27**: 471-477 [PMID: 22806522 DOI: 10.1007/s00464-012-2460-8]
54 **Fujii S**, Watanabe K, Ota M, Watanabe J, Ichikawa Y, Yamagishi S, Tatsumi K, Suwa H, Kunisaki C, Taguri M, Morita S, Endo I. Single-incision laparoscopic surgery using colon-lifting technique for colorectal cancer: a matched case-control comparison with standard multiport laparoscopic surgery in terms of short-term results and access instrument cost. *Surg Endosc* 2012; **26**: 1403-1411 [PMID: 22101420 DOI: 10.1007/s00464-011-2047-9]
55 **Gaujoux S**, Bretagnol F, Ferron M, Panis Y. Single-incision laparoscopic colonic surgery. *Colorectal Dis* 2011; **13**: 1066-1071 [PMID: 21848732 DOI: 10.1111/j.1463-1318.2010.02404.x]
56 **Haas EM**, Nieto J, Ragupathi M, Aminian A, Patel CB. Critical appraisal of learning curve for single incision laparoscopic right colectomy. *Surg Endosc* 2013; **27**: 4499-4503 [PMID: 23877765 DOI: 10.1007/s00464-013-3096-z]
57 **Bulut O**, Nielsen CB, Jespersen N. Single-port access laparoscopic surgery for rectal cancer: initial experience with 10 cases. *Dis Colon Rectum* 2011; **54**: 803-809 [PMID: 21654246 DOI: 10.1007/DCR.0b013e3182147b4d]
58 **Fung AK**, Aly EH. Systematic review of single-incision laparoscopic colonic surgery. *Br J Surg* 2012; **99**: 1353-1364 [PMID: 22961513 DOI: 10.1002/bjs.8834]
59 **Pucher PH**, Sodergren MH, Singh P, Darzi A, Parakseva P. Have we learned from lessons of the past? A systematic review of training for single incision laparoscopic surgery. *Surg Endosc* 2013; **27**: 1478-1484 [PMID: 23073688]
60 **Islam A**, Castellvi AO, Tesfay ST, Castellvi AD, Wright AS, Scott DJ. Early surgeon impressions and technical difficulty associated with laparoendoscopic single-site surgery: a Society of American Gastrointestinal and Endoscopic Surgeons Learning Center study. *Surg Endosc* 2011; **25**: 2597-2603 [PMID: 21359887 DOI: 10.1007/s00464-011-1594-4]
61 **Chew MH**, Wong MT, Lim BY, Ng KH, Eu KW. Evaluation of current devices in single-incision laparoscopic colorectal surgery: a preliminary experience in 32 consecutive cases. *World J Surg* 2011; **35**: 873-880 [PMID: 21318430 DOI: 10.1007/s00268-011-0989-7]
62 **Dhumane PW**, Diana M, Leroy J, Marescaux J. Minimally invasive single-site surgery for the digestive system: A technological review. *J Minim Access Surg* 2011; **7**: 40-51 [PMID: 21197242]
63 **Gandhi DP**, Ragupathi M, Patel CB, Ramos-Valadez DI, Pickron TB, Haas EM. Single-incision versus hand-assisted laparoscopic colectomy: a case-matched series. *J Gastrointest Surg* 2010; **14**: 1875-1880 [PMID: 20922576 DOI: 10.1007/s11605-010-1355-z]
64 **Merchant AM**, Cook MW, White BC, Davis SS, Sweeney JF, Lin E. Transumbilical Gelport access technique for performing single incision laparoscopic surgery (SILS). *J Gastrointest Surg* 2009; **13**: 159-162 [PMID: 18972166 DOI: 10.1007/s11605-008-0737-y]
65 **Ragupathi M**, Ramos-Valadez DI, Yaakovian MD, Haas EM. Single-incision laparoscopic colectomy: a novel approach through a Pfannenstiel incision. *Tech Coloproctol* 2011; **15**: 61-65 [PMID: 21287224 DOI: 10.1007/s10151-010-0663-3]
66 **Rieger NA**, Lam FF. Single-incision laparoscopically assisted colectomy using standard laparoscopic instrumentation. *Surg Endosc* 2010; **24**: 888-890 [PMID: 19760335 DOI: 10.1007/s00464-009-0683-0]
67 **Waters JA**, Chihara R, Moreno J, Robb BW, Wiebke EA, George VV. Laparoscopic colectomy: does the learning curve extend beyond colorectal surgery fellowship? *JSLS* 2010; **14**: 325-331 [PMID: 21333183 DOI: 10.4293/108680810X12924466006800]
68 **Champagne BJ**, Lee EC, Leblanc F, Stein SL, Delaney CP. Single-incision vs straight laparoscopic segmental colectomy: a case-controlled study. *Dis Colon Rectum* 2011; **54**: 183-186 [PMID: 21228666 DOI: 10.1007/DCR.0b013e3181fd48af]
69 **Bemelman WA**, van Hogezand RA, Meijerink WJ, Griffioen G, Ringers J. Laparoscopic-assisted bowel resections in inflammatory bowel disease: state of the art. *Neth J Med* 1998; **53**: S39-S46 [PMID: 9883013 DOI: 10.1016/S0300-2977(98)00122-3]
70 **Geisler D**, Garrett T. Single incision laparoscopic colorectal surgery: a single surgeon experience of 102 consecutive cases. *Tech Coloproctol* 2011; **15**: 397-401 [PMID: 21887555 DOI: 10.1007/s10151-011-0756-7]
71 **Bucher P**, Pugin F, Morel P. Transumbilical single incision laparoscopic sigmoidectomy for benign disease. *Colorectal Dis* 2010; **12**: 61-65 [PMID: 19320667 DOI: 10.1111/j.1463-1318.2009.01825.x]
72 **Miller S**, Causey MW, Damle A, Maykel J, Steele S. Single-incision laparoscopic colectomy: training the next generation. *Surg Endosc* 2013; **27**: 1784-1790 [PMID: 23389059 DOI: 10.1007/s00464-012-2684-7]
73 **Remzi FH**, Kirat HT, Geisler DP. Laparoscopic single-port colectomy for sigmoid cancer. *Tech Coloproctol* 2010; **14**: 253-255 [PMID: 19953288 DOI: 10.1007/s10151-009-0545-8]
74 **Ross H**, Steele S, Whiteford M, Lee S, Albert M, Mutch M, Rivadeneira D, Marcello P. Early multi-institution experience with single-incision laparoscopic colectomy. *Dis Colon Rectum* 2011; **54**: 187-192 [PMID: 21228667 DOI: 10.1007/DCR.0b013e3181f8d972]

P- Reviewer: Bandyopadhyay SK, Karabicak I, McArdle A
S- Editor: Yu J **L- Editor**: A **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10830

World J Gastroenterol 2015 October 14; 21(38): 10830-10839
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Laparoscopic Surgery

Data analyses and perspectives on laparoscopic surgery for esophageal achalasia

Kazuto Tsuboi, Nobuo Omura, Fumiaki Yano, Masato Hoshino, Se-Ryung Yamamoto, Shunsuke Akimoto, Takahiro Masuda, Hideyuki Kashiwagi, Katsuhiko Yanaga

Kazuto Tsuboi, Nobuo Omura, Fumiaki Yano, Masato Hoshino, Se-Ryung Yamamoto, Shunsuke Akimoto, Takahiro Masuda, Hideyuki Kashiwagi, Katsuhiko Yanaga, Department of Surgery, The Jikei University School of Medicine, Tokyo 105-8461, Japan

Author contributions: Tsuboi K, Omura N, Yano F, Hoshino M, Yamamoto SR, Akimoto S, Masuda T and Kashiwagi H analyzed data; Tsuboi K, Omura N and Yanaga K wrote the paper.

Conflict-of-interest statement: Drs. Tsuboi, Omura, Yano, Hoshino, Yamamoto, Akimoto, Masuda, Kashiwagi and Yanaga have no conflicts of interest or financial ties to disclose.

Correspondence to: Kazuto Tsuboi, MD, Department of Surgery, The Jikei University School of Medicine, 3-25-8, Nishishimbashi, Minato-ku, Tokyo 105-8461, Japan. kazuto@jikei.ac.jp
Telephone: +81-3-34331111
Fax: +81-3-54724140

Received: April 9, 2015
Peer-review started: April 9, 2015
First decision: May 18, 2015
Revised: May 29, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 14, 2015

Abstract

In general, the treatment methods for esophageal achalasia are largely classified into four groups, including drug therapy using nitrite or a calcium channel blocker, botulinum toxin injection, endoscopic therapy such as endoscopic balloon dilation, and surgery. Various studies have suggested that the most effective treatment of esophageal achalasia is surgical therapy. The basic concept of this surgical therapy has not changed since Heller proposed esophageal myotomy for the purpose of resolution of lower esophageal obstruction for the first time in 1913, but the most common approach has changed from open-chest surgery to laparoscopic surgery. Currently, the laparoscopic surgery has been the procedure of choice for the treatment of esophageal achalasia. During the process of the transition from open-chest surgery to laparotomy, to thoracoscopic surgery, and to laparoscopic surgery, the necessity of combining antireflux surgery has been recognized. There is some debate as to which type of antireflux surgery should be selected. The Toupet fundoplication may be the most effective in prevention of postoperative antireflux, but many medical institutions have selected the Dor fundoplication which covers the mucosal surface exposed by myotomy. Recently, a new endoscopic approach, peroral endoscopic myotomy (POEM), has received attention. Future studies should examine the long-term outcomes and whether POEM becomes the gold standard for the treatment of esophageal achalasia.

Key words: Esophageal achalasia; Surgery; Treatment; Review; Laparoscopy

Core tip: Esophageal achalasia is the most common primary esophageal motility disorder and the major symptoms are dysphagia, vomiting, and chest pain. Various studies have suggested that the most effective

treatment of esophageal achalasia is surgical therapy and the basic concept of surgical therapy has not changed since Heller proposed esophageal myotomy for the purpose of resolution of lower esophageal obstruction. However, the most common approach has changed from open-chest surgery to laparoscopic surgery. This article reviews the outcomes of surgical procedures for esophageal achalasia from various view points and discusses the problems and prospects of laparoscopic surgery for esophageal achalasia.

Tsuboi K, Omura N, Yano F, Hoshino M, Yamamoto SR, Akimoto S, Masuda T, Kashiwagi H, Yanaga K. Data analyses and perspectives on laparoscopic surgery for esophageal achalasia. *World J Gastroenterol* 2015; 21(38): 10830-10839 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10830.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10830

INTRODUCTION

Esophageal achalasia is the most common primary esophageal motility disorder and is histologically characterized by lack of the Auerbach's nerve plexus[1]. It causes loss of esophageal peristalsis and/or a lack of lower esophageal sphincter relaxation during swallowing, which results in esophageal obstruction[2-5]. The major symptoms are dysphagia, vomiting, and chest pain[5]. There are neither racial nor gender differences in its incidence, according to epidemiologic studies. The disease is quite rare with a frequency of approximately 1 in 0.1 million[6,7]. The pathogenesis of esophageal achalasia has not been elucidated, and the pathogenic mechanism is not understood. At the moment, the goal of treatment is not complete resolution of the disease, but rather symptomatic improvement that may significantly affect the patients' quality of life (QOL). The treatment methods are largely classified into four groups, drug therapy using nitrite or a calcium channel blocker, botulinum toxin injection, endoscopic balloon dilation, and surgery[8]. Esophageal myotomy was the first surgery that was proposed by Heller in 1913. Thereafter, a combination with cardioplasty was adapted to prevent reflux esophagitis which was sometimes induced by the myotomy[9,10]. Currently, laparoscopic myotomy has been widely performed as a standard treatment approach in many institutions, because it is minimally invasive, achieves esthetic results, and has a defined surgical area[7,8,11-14]. The objective response rate by surgery is good, ranging from 88% to 97%[15-20]. There are some reports on the long-term outcome[21-23], which have demonstrated the safety and effectiveness of the procedure.

This article reviews the outcomes of surgical procedures for esophageal achalasia from various view points and discusses the problems and prospects of laparoscopic surgery for esophageal achalasia.

CHANGES IN SURGICAL PROCEDURES FOR ESOPHAGEAL ACHALASIA OVER TIME

Open surgery

Lower esophageal myotomy *via* left thoracotomy for the purpose of improvement of esophageal clearance was first reported by Heller. Heller's approach involved two incisions of approximately 8-cm made on the anterior and posterior esophageal walls to relieve the esophageal obstruction. The approach was modified by Williams *et al*[24] and the currently used long myotomy with anterior fundoplication was established. While the open-chest surgery was widely used, myotomy *via* laparotomy was also performed in order to avoid the complicated surgical procedures and postoperative wound pain of open-chest surgery. Abir *et al*[25] who studied 18 articles regarding Heller myotomy reported that the response rate in 2680 patients was 83% and concluded that the procedure was very effective. However, since Heller myotomy required myotomy of the gastroesophageal junction to prevent antireflux, there was a risk of postoperative gastroesophageal reflux disease (GERD). They reported that the overall incidence of postoperative GERD was 12.3%, but 11 of the 18 articles on surgical treatment of esophageal achalasia reported combination of myotomy with laparoscopic antireflux surgery (LARS). The occurrence of postoperative GERD in patients with antireflux surgery was lower than those in patients without antireflux surgery (10% *vs* 16%). These findings suggested that laparotomic myotomy should be performed along with antireflux surgery.

Minimally invasive surgery

Successful laparoscopic cholecystectomy was first reported in 1987. Since then, the surgical procedures for various pathologies have changed from open-chest surgery to laparoscopic surgery. This trend was also observed in the treatment of esophageal achalasia. Laparoscopic procedures have been rapidly adopted due to its minimally invasive nature and better visualization of the surgical area, compared with open-chest surgery and laparotomy. The procedure was performed using the left thoracic approach similar to that of open-chest surgery. Pellegrini *et al*[26] reported that 14 of 17 patients (82%) were satisfied with the surgical outcome that was comparable with that obtained after thoracotomy. The major advantage of thoracoscopic surgery is that long myotomy can be applied to the cranial esophagus. This approach does not require mobilization of the paraesophageal membrane, but it is difficult to reach the anterior wall of the gastric cardia, and an incision cannot reach the oblique muscle due to this lack of sufficient myotomy onto the cardia. Therefore, this approach will not damage the antireflux mechanism and be less likely

to cause postoperative reflux esophagitis. However, there are still risks of persistence or relapse of the obstruction sensation of the esophagus[26-28]. Thus, thoracoscopic surgery cases are limited in number whereas laparoscopic surgery is widely used.

Laparoscopic surgery was first reported by Shimi *et al*[29] in 1991. Since then, there are many successful reports not only on the short-term results but also on the long-term surgical outcomes[30,31]. The operating time and duration of postoperative hospital stay are shorter, and the symptomatic improvement is greater by laparoscopic surgery, when compared with thoracoscopic surgery[32].

COMPARISON OF SURGICAL RESULTS BY APPROACH

Open method vs minimally invasive surgery

Transthoracic approaches include open-chest surgery and thoracoscopic surgery. A retrospective study conducted by Kesler *et al*[33] which compared thoracoscopic myotomy and open-chest myotomy reported that the operating time and intraoperative blood loss were significantly decreased in the thoracoscopic myotomy group ($P < 0.05$, respectively), In addition, the patients in thoracoscopic myotomy was experiencing a shorter hospitalization, which was due to earlier resumption of oral nutrition, earlier removal of chest tube, and less requirement of postoperative analgesic agents. Those results indicated that intra- and post-operative results were better in the patients undergoing thoracoscopic surgery than that in those undergoing open-chest surgery. Furthermore, a study on the long-term outcomes by Pellegrini *et al*[27] showed relief of dysphagia seen in 88% of patients and 66% of patients had regained their original weight. The open-chest surgery was replaced with thoracoscopic surgery due to the minimally invasive closed-chest approach.

The transabdominal approach was also converted to laparoscopic surgery over time in the institutions which performed laparotomic myotomy. Ancona *et al*[34] performed a retrospective study on 17 patients undergoing the laparoscopic Heller-Dor procedure and 17 background-matched patients undergoing laparotomic surgery, and reported that the operating time was longer in the laparoscopic surgery group than that in the other group (178 min *vs* 125 min). In addition, the duration of the postoperative hospital stay and time to reintegrate into society were significantly decreased with the laparoscopic surgery ($P < 0.0001$, respectively). However this study were consisted with short-term examination, and the analysis in long-term were expected. The above-mentioned findings suggested that laparoscopic surgery was more effective than the transabdominal approach as a treatment for esophageal achalasia. Currently, laparoscopic surgery is a gold standard for treatment of esophageal achalasia. And we are not able to find any shortcoming in laparoscopic surgery for esophageal achalasia without lengthening of operating time if the patients have no contraindication of laparoscopic surgery.

Thoracoscopic surgery vs laparoscopic surgery

Since laparoscopic surgery involves minimal invasiveness and yields a similar response rate as compared to that of the open-chest method, minimally invasive surgery has been used as the first-line therapy. Ramacciato *et al*[32] performed a case controlled study to compare the results of thoracoscopic myotomy with laparoscopic myotomy in 16 patients with thoracoscopic surgery group and in 17 patients with laparoscopic surgery (with Dor) group. The results indicated that the operating time and duration of postoperative hospital stay were significantly decreased in the laparoscopic surgery group ($P = 0.0001$ in both), and the frequency of persistence and relapse of a sensation of postoperative obstruction in the esophagus was significantly greater in the thoracoscopic surgery group (38% *vs* 6%, $P = 0.04$). However this study was not enough for analyzing postoperative GERD including pH-metry, and further examination was expected. Patti *et al*[30] investigated 8-year experience for minimally invasive surgery for esophageal achalasia and they indicated that the laparoscopic surgery is the better choice for this disease.

PROBLEMS ASSOCIATED WITH LARS AFTER ESOPHAGEAL MYOTOMY

Laparoscopic myotomy only vs laparoscopic myotomy with fundoplication

The majority of the previous studies reached the consensus that laparoscopic surgery was the most suitable surgical procedure for esophageal achalasia[35]. One of the advantages of the transabdominal approach is that myotomy can be sufficiently extended to the gastric side[32], but the approach may induce postoperative gastroesophageal reflux. Several studies have been performed to validate whether laparoscopic myotomy alone or in combination therapy with antireflux surgery should be performed (Table 1)[10,36-39]. The observation period ranged from 6 to 96 mo but the postoperative improvement in the loss of the obstructive sensation was good, ranging from 70% to 100%. Thus, all of the studies showed no significant difference in symptomatic improvement between the groups. Nevertheless, the incidence of postoperative GERD symptoms tended to be higher when laparoscopic myotomy was performed alone, which suggested that cardioplasty was essential for prevention of gastroesophageal reflux.

Table 1 Comparison of surgical outcomes between laparoscopic Heller myotomy *vs* laparoscopic myotomy with fundoplication

Author	Year	Study design	Samples	Procedure	Follow up (mo)	Success	Postop-GERD
Campos *et al*[36]	2009	Meta-analysis	579	myotomy only	NA	90%	31%
			2507	myotomy + fundoplication	NA	90%	9%
Falkenback *et al*[37]	2003	RCT	10	myotomy only	96	70%	13%
			10	myotomy + fundoplication	96	70%	0.1%
Richards *et al*[10]	2004	RCT	21	myotomy only	6	100%	47.6%
			22	myotomy + fundoplication	6	95.5%	9.1%
Simić *et al*[38]	2010	RCT	22	myotomy only	36	100%	9.1%
			62	myotomy + fundoplication	36	91.7%	9.7%
Finley *et al*[39]	2007	Retrospective	24	myotomy only	12	100%	NA
			71	myotomy + fundoplication	12	98.6%	NA

NA: Not available; GERD: Gastroesophageal reflux disease; RCT: Retrospective cohort study.

Laparoscopic myotomy with Dor fundoplication vs laparoscopic myotomy with other types of fundoplication

A combination of laparoscopic myotomy and antireflux surgery may be desirable for treatment of esophageal achalasia, but it is still controversial which type of antireflux surgery should be performed. Many institutions in the world seem to select the Dor fundoplication, but the Nissen fundoplication and the Toupet fundoplication have also been performed. Previous studies mainly compared the Dor fundoplication with other types of fundoplications (Table 2)[40-43]. All types of antireflux surgery showed similar symptomatic improvements and a high response rate, but the incidence rate of postoperative GERD was slightly greater in the patients undergoing the Dor fundoplication. However, there was no statistically significant difference.

The 2012 guidelines of the Society of American Gastrointestinal and Endoscopic Surgeons[44] also strongly recommended a combination of laparoscopic myotomy with antireflux surgery. However, the guidelines declined to say which type of antireflux surgery should be performed but they did state that circumferential fundoplication should be avoided because of the risk of persistence and relapse of the postoperative obstructive sensation of the esophagus. In general, the previous studies indicated that the Toupet fundoplication had a slightly higher antireflux result, but there was no definite conclusion. There was a report that diverticula developed in the esophageal myotomy site when esophageal myotomy using the Toupet fundoplication was not sufficiently extended to the gastric side[45], which suggested that the Dor fundoplication which completely wraps the exposed mucosa should be performed.

COMPARISON BETWEEN SURGICAL PROCEDURES AND OTHER FUNDOPLICATION APPROACHES

Esophageal myotomy vs PD

Endoscopic balloon dilation is an effective treatment for esophageal achalasia[46,47]. The procedure was proposed by Vantrappen *et al*[48] in 1971 for the first time, and the response rate is currently 80%-90%[49]. However, a long-term follow-up study indicated that 60% of the patients had relapse and needed radiation[50]. Furthermore, the incidence of esophageal perforation after the procedure was not high, ranging from 0.5% to 5%, but 50% or more of these patients with perforations required emergency surgery. These patients are at the greatest disadvantages for this procedure[50,51]. In cases where patients had a previous history of balloon dilation, scar formation and other tissue injury that possibly occurred during the recovery process from submucosal hemorrhage may cause detachment and myotomy of the esophagus in the abdomen[52-54]. We studied whether a history of dilation influences surgical outcome and reported that there was no difference between those with and without previous Parkinson's disease (PD) in the operating time, perioperative blood loss, incidence rate of mucosal perforation during myotomy, and rate of postoperative symptomatic improvement[55].

There are many studies comparing the therapeutic effects of PD and laparoscopic myotomy, and Table 3 shows representative studies[56-59]. The duration of observation ranged from 3 mo to 5 years, and the rate of symptomatic improvement was greater with laparoscopic surgery, which indicated that laparoscopic surgery was strongly recommended to the patients with esophageal achalasia.

Esophageal myotomy vs botulinum toxin injection

The botulinum toxin inhibits release of acetylcholine from nerve terminals. Injection of botulinum toxin into the lower esophageal sphincter of patients with esophageal achalasia enables muscle relaxation and eases the passage of food into the esophagus. Since a significant symptomatic improvement was observed in patients with esophageal achalasia on short-term outcome in a placebo-controlled trial for one week, the therapy in now an effective treatment for esophageal achalasia[60]. However, the persistence of the therapeutic effect varied depending on the patient. A long-term study indicated that the response

Table 2 Comparison of surgical outcomes between laparoscopic Heller myotomy with Dor fundoplication *vs* laparoscopic myotomy with other fundoplication

Author	Year	Study design	Samples	Fundoplication	Follow up (mo)	Success	Postop-GERD
Rebecchi *et al*[40]	2008	RCT	71	Dor	125	97%	3%
			67	Nissen	125	85%	0%
Rawlings *et al*[41]	2012	RCT	36	Dor	6	91.7%	27.8%
			24	Toupet	6	95.8%	16.7%
Wright *et al*[42]	2007	Retrospective	52	Dor	46	82.7%	NA
			63	Toupet	45	95.2%	NA
Di Martino *et al*[43]	2011	Retrospective	30	Dor	24	93.4%	13.3%
			26	Nissen	24	92.3%	0%

NA: Not available; GERD: Gastroesophageal reflux disease; RCT: Retrospective cohort study.

Table 3 Comparison of surgical outcomes between laparoscopic Heller myotomy with fundoplication *vs* pneumatic dilation

Author	Year	Study design	Samples	Approach	Procedure	Follow up (mo)	Success	postop-GERD
Kostic *et al*[56]	2007	RCT	25	laparoscopy	myotomy + fundoplication	12	NA	NA
			26	endoscopy	pneumatic dilation	12	NA	NA
Novais *et al*[57]	2010	RCT	47	laparoscopy	myotomy + fundoplication	3	88.3%	4.7%
			47	endoscopy	pneumatic dilation	3	73.8%	31%
Boeckxstaens *et al*[58]	2011	RCT	106	laparoscopy	myotomy + fundoplication	43	90%	23%
			95	endoscopy	pneumatic dilation	43	86%	15%
Persson *et al*[59]	2014	RCT	25	laparoscopy	myotomy + fundoplication	60	92%	NA
			28	endoscopy	pneumatic dilation	60	64%	NA

NA: Not available; GERD: Gastroesophageal reflux disease; RCT: Retrospective cohort study.

rate of patients undergoing botulinum toxin injection was approximately 65%. The relatively greater effect was observed in elderly patients and in patients with an advanced type of esophageal achalasia, and the effect lasted approximately 1.3 years[61]. Zaninotto *et al*[62] performed a randomized controlled trial in 80 patients with esophageal achalasia to compare the therapeutic effects of laparoscopic myotomy and botulinum toxin injection. The results demonstrated that laparoscopic myotomy was safer, and the 6-mo post-treatment evaluation showed that the response rate was greater in laparoscopic myotomy compared with that in botulinum toxin injection (82% *vs* 66%, $P < 0.05$). Another evaluation after 2 years showed that the symptomatic improvement effect was seen in 87.5% of the laparoscopic myotomy group and in 34% of the botulinum toxin injection group. The long-term outcome of botulinum toxin injection was not successful enough, similar to dilation therapy, but the injection therapy may be effective in treating the patients who are not a candidate for either dilation or surgical procedures due to reduced activities of daily living (ADL).

Esophageal myotomy vs per oral endoscopic myotomy

Recently, a new treatment for esophageal achalasia, per oral endoscopic myotomy (POEM), has been introduced[63] and has received attention. The method is an adaptation of the natural orifice transluminal endoscopic surgery (NOTES) in the mediastinum, and has come to use in a small number of medical institutions[64]. This method is based on a lower esophageal sphincter (LES) myotomy in a porcine survival model which was performed for the first time in 2007[65]. POEM is quite novel and only a few comparative studies on the short-term outcome have been completed. Table 4 shows representative results[66,67]. There was no difference in the operating time and in the incidence of perforation between the two groups, but the incidence of postoperative GERD symptoms was slightly greater in the POEM group. Furthermore, Teitelbaum *et al*[68] performed the gastroesophageal junction distensibility measurements with a functional lumen imaging probe during laparoscopic myotomy with either cardioplasty or POEM on 25 patients with esophageal achalasia, and obtained similar results.

The long-term outcome of the new treatment method should be evaluated, but the short-term outcome was excellent, and the procedure was less invasive than laparoscopic surgery. Also in terms of a better esthetic outcome, the new procedure may have a higher potential. Future studies should evaluate the incidence of postoperative GERD and the therapeutic effects.

PROBLEMS AND PROSPECTS OF LAPAROSCOPIC SURGERY

Laparoscopic surgery for esophageal achalasia provides greater symptomatic improvement but some patients have a poor outcome. Such poor response

Table 4 Comparison of surgical outcomes between laparoscopic Heller myotomy vs peroral endoscopic myotomy *n* (%)

Author	Year	Study design	Samples	Procedure	Follow up (d)	OP time (min)	Major complication (perforation)	Postop-GERD
Hungness *et al*[66]	2013	Retrospective	55	myotomy	42	125	1 (2)	NA
			18	POEM	42	113	1 (6)	7 (39)
Ujiki *et al*[67]	2013	Case control	21	myotomy	164	154.5	1 (4.8)	4 (19)
			18	POEM	116	155.8	1 (5.6)	5 (27.8)

Symptomatic GERD or PPI required. NA: Not available; GERD: Gastroesophageal reflux disease; POEM: Peroral endoscopic myotomy; OP: Operation.

Table 5 Surgical outcomes of reoperation for patients with achalasia

Author	Year	Samples	Approach (primary)	Approach (re-do)	Mucosal injury	Re-revision	Satisfaction
Iqbal *et al*[71]	2006	15	10: Laparoscopy 3: Thoracoscopy 1: Laparotomy 1: Thoracotomy	15: Laparoscopy (2: Conversion to laparotomy)	30%	20%	40%-89%
Rakita *et al*[73]	2007	12		11: Laparoscopy 1: Thoracoscopy	NA	25%	82%
Grotenhuis *et al*[74]	2007	19	13: Abdominal 6: Thoracic	13: Abdominal 6: Thoracic	15.8%	10.5%	50%
Loviscek *et al*[75]	2013	43	20: Abdominal 23: Thoracic	26: Laparoscopy	4.7%	9.3%	79%
Omura *et al*[76]	2012	10	7: Laparoscopy 3: Thoracoscopy	5: Laparoscopy 2: Thoracoscopy 1: Laparotomy 1: Laparotomy + thoracotomy	30%	NA	90%

NA: Not available.

to laparoscopic surgery may mainly be caused by the surgeon's lack of technical skills, including insufficient esophageal myotomy, an overtight wrap, a loose wrap, and development of postoperative reflux esophagitis due to absence of cardioplasty[69,70]. Some studies have been performed to detect potential factors relating to the patients' background and pathology. Iqbal *et al*[71] compared 67 patients whose symptoms were improved and 15 patients whose symptom were not improved, and reported that the effect of surgery was more beneficial in patients with a short disease duration and in patients without a previous history of botox injection. Torquati *et al*[72] performed a comparison study in 200 patients undergoing laparoscopic Heller myotomy, including 170 responders and 30 non-responders. The preoperative manometry showed that the LES pressure was significantly greater in the responders than that in the non-responders, and the patients with a LES pressure > 35 mmHg had more than 21 times the likelihood to achieve excellent dysphagia relief after myotomy as compared with those with a LES pressure ⩽ 35 mmHg when the cutoff value was set at 35 mmHg for the LES pressure.

Patients who did not go into complete remission after the primary surgery or those who suffered from a relapse during the follow-up received the redo surgery at some medical institutions (Table 5)[71,73-76]. The rate of mucosal perforation was relatively high, ranging from 4.7% to 30%, but the patient satisfaction was 40% to 90%, which suggested that the redo surgery might need to be performed in highly experienced medical institutions for the purpose of QOL improvement.

High resolution manometry (HRM), which was recently introduced, is equipped with strong diagnostic capabilities in esophageal dysmotility. The pathology of esophageal achalasia is classified into Types Ⅰ to Ⅲ based on the level of esophageal motility. Several studies evaluated the treatment outcome by Type, and the analysis found that Type Ⅱ had the greatest therapeutic response, followed by Type Ⅰ and then Type Ⅲ[77,78]. Salvador *et al*[79] reported that the response of myotomy to esophageal achalasia Type Ⅰ, Ⅱ, and Ⅲ was 85.4%, 95.3%, and 69.4%, respectively. According to the above-mentioned findings, treatment of Type Ⅲ esophageal achalasia should be further studied and include a new treatment approach.

As to laparoscopic surgery for esophageal achalasia, a single incision and reduced port surgery focusing on the esthetic aspect[80] has widely been used, especially in high volume centers (Table 6)[81,82]. According to the report by Ross *et al*[83], many studies

Table 6 Surgical outcomes of reduced port surgery for patients with achalasia

Author	Year	Samples	Procedure	Follow up (mo)	OP time (min)	Mucosal injury	Conversion to laparotomy	Satisfaction
Barry *et al*[81]	2011	66	Myotomy + anterior fundoplication	NA	117	3%	0%	94%
Omura *et al*[82]	Epub	24	Myotomy + anterior fundoplication	8	230	13%	0%	90%

NA: Not available; OP: Operation.

reported that the learning curve of the laparoscopic surgery was 20 operations, but it might be easier to master the technique if the surgeons have had prior experience with conventional laparoscopic Heller myotomy (Lap-Heller). According to reports by two high volume centers that treat esophageal achalasia, the symptomatic improvement rate was similar to that by conventional methods, and no patients were converted to lap-Heller, which suggested that the outcome was sufficiently acceptable. In the future, younger patients and female patients with esophageal achalasia may prefer laparoscopic surgery, and such a surgical procedure will likely be widely used.

In general, esophageal achalasia is considered as a premalignant condition for esophageal squamous cell carcinoma, and the risk of developing the cancer might be 140-times greater than that in the general population[84]. Esophageal achalasia might be caused by either saburra in the esophagus due to decreased clearance of the lower esophagus or chronic exposure of saliva to the esophageal mucosa[84,85]. Some patients developed esophageal squamous cell carcinoma during the long follow-up after surgery[86,87]. Therefore, even after the symptoms are improved by surgery, follow-up by periodic upper gastrointestinal endoscopy is required.

CONCLUSION

Since laparoscopic Heller myotomy was reported by Heller as the first-line surgical therapy for esophageal achalasia, myotomy has been modified in various ways for the last 100 years. At the moment, there is no room for doubt that laparoscopic myotomy has become the gold standard for treatment of achalasia throughout the world. However, the current surgical therapy does not provide a complete resolution of esophageal achalasia. The therapy achieves successful symptomatic relief but there is still a need for more improvement. It is expected that measures against the technical problems that have been pointed out by the previous studies lead to the development of a new approach. Additionally, further studies might provide guidelines for treatment based on various factors.

REFERENCES

1 **O'Neill OM**, Johnston BT, Coleman HG. Achalasia: a review of clinical diagnosis, epidemiology, treatment and outcomes. *World J Gastroenterol* 2013; **19**: 5806-5812 [PMID: 24124325 DOI: 10.3748/wjg.v19.i35.5806]

2 **Achkar E**. Achalasia. *Gastroenterologist* 1995; **3**: 273-288 [PMID: 8775090]

3 **Uribe P**, Csendes A, Larrain A, Ayala M. Motility studies in fifty patients with achalasia of the esophagus. *Am J Gastroenterol* 1974; **62**: 333-336 [PMID: 4432846]

4 **Vaezi MF**, Richter JE. Diagnosis and management of achalasia. American College of Gastroenterology Practice Parameter Committee. *Am J Gastroenterol* 1999; **94**: 3406-3412 [PMID: 10606295 DOI: 10.1111/j.1572-0241.1999.01639.x]

5 **Reynolds JC**, Parkman HP. Achalasia. *Gastroenterol Clin North Am* 1989; **18**: 223-255 [PMID: 2668168]

6 **Mayberry JF**. Epidemiology and demographics of achalasia. *Gastrointest Endosc Clin N Am* 2001; **11**: 235-248, v [PMID: 11319059]

7 **Podas T**, Eaden J, Mayberry M, Mayberry J. Achalasia: a critical review of epidemiological studies. *Am J Gastroenterol* 1998; **93**: 2345-2347 [PMID: 9860390 DOI: 10.1111/j.1572-0241.1998.00686.x]

8 **Spiess AE**, Kahrilas PJ. Treating achalasia: from whalebone to laparoscope. *JAMA* 1998; **280**: 638-642 [PMID: 9718057 DOI: 10.1001/jama.280.7.638]

9 **Bonavina L**, Nosadini A, Bardini R, Baessato M, Peracchia A. Primary treatment of esophageal achalasia. Long-term results of myotomy and Dor fundoplication. *Arch Surg* 1992; **127**: 222-226; discussion 227 [PMID: 1540102 DOI: 10.1001/archsurg.1992.01420020112016]

10 **Richards WO**, Torquati A, Holzman MD, Khaitan L, Byrne D, Lutfi R, Sharp KW. Heller myotomy versus Heller myotomy with Dor fundoplication for achalasia: a prospective randomized double-blind clinical trial. *Ann Surg* 2004; **240**: 405-412; discussion 412-415 [PMID: 15319712 DOI: 10.1097/01.sla.0000136940.32255.51]

11 **Patient Care Committee, Society for Surgery of the Alimentary Tract**. Esophageal achalasia. SSAT patient care guidelines. *J Gastrointest Surg* 2004; **8**: 367-368 [PMID: 15115006]

12 **Pechlivanides G**, Chrysos E, Athanasakis E, Tsiaoussis J, Vassilakis JS, Xynos E. Laparoscopic Heller cardiomyotomy and Dor fundoplication for esophageal achalasia: possible factors predicting outcome. *Arch Surg* 2001; **136**: 1240-1243 [PMID: 11695966 DOI: 10.1001/archsurg.136.11.1240]

13 **Ancona E**, Peracchia A, Zaninotto G, Rossi M, Bonavina L, Segalin A. Heller laparoscopic cardiomyotomy with antireflux anterior fundoplication (Dor) in the treatment of esophageal achalasia. *Surg Endosc* 1993; **7**: 459-461 [PMID: 8211631]

14 **Tsuboi K**, Omura N, Yano F, Kashiwagi H, Yanaga K. Results after laparoscopic Heller-Dor operation for esophageal achalasia in 100 consecutive patients. *Dis Esophagus* 2009; **22**: 169-176 [PMID: 19018850 DOI: 10.1111/j.1442-2050.2008.00891.x]

15 **Csendes A**, Braghetto I, Burdiles P, Korn O, Csendes P, Henríquez A. Very late results of esophagomyotomy for patients with achalasia: clinical, endoscopic, histologic, manometric, and acid reflux studies in 67 patients for a mean follow-up of 190 months. *Ann Surg* 2006; **243**: 196-203 [PMID: 16432352 DOI: 10.1097/01.sla.0000197469.12632.e0]

16 **Fernández AF**, Martínez MA, Ruiz J, Torres R, Faife B, Torres

JR, Escoto CM. Six years of experience in laparoscopic surgery of esophageal achalasia. *Surg Endosc* 2003; **17**: 153-156 [PMID: 12399873 DOI: 10.1007/s00464-002-8576-5]
17 **Frantzides CT**, Moore RE, Carlson MA, Madan AK, Zografakis JG, Keshavarzian A, Smith C. Minimally invasive surgery for achalasia: a 10-year experience. *J Gastrointest Surg* 2004; **8**: 18-23 [PMID: 14746831]
18 **Hunter JG**, Richardson WS. Surgical management of achalasia. *Surg Clin North Am* 1997; **77**: 993-1015 [PMID: 9347828 DOI: 10.1016/S0039-6109(05)70602-2]
19 **Payne WS**. Heller's contribution to the surgical treatment of achalasia of the esophagus. 1914. *Ann Thorac Surg* 1989; **48**: 876-881 [PMID: 2688583]
20 **Rosemurgy A**, Villadolid D, Thometz D, Kalipersad C, Rakita S, Albrink M, Johnson M, Boyce W. Laparoscopic Heller myotomy provides durable relief from achalasia and salvages failures after botox or dilation. *Ann Surg* 2005; **241**: 725-33; discussion 733-5 [PMID: 15849508 DOI: 10.1097/01.sla.0000160702.31452.d5]
21 **Bessell JR**, Lally CJ, Schloithe A, Jamieson GG, Devitt PG, Watson DI. Laparoscopic cardiomyotomy for achalasia: long-term outcomes. *ANZ J Surg* 2006; **76**: 558-562 [PMID: 16813618 DOI: 10.1111/j.1445-2197.2006.03784.x]
22 **Ferulano GP**, Dilillo S, D'Ambra M, Lionetti R, Brunaccino R, Fico D, Pelaggi D. Short and long term results of the laparoscopic Heller-Dor myotomy. The influence of age and previous conservative therapies. *Surg Endosc* 2007; **21**: 2017-2023 [PMID: 17705085 DOI: 10.1007/s00464-007-9506-3]
23 **Jeansonne LO**, White BC, Pilger KE, Shane MD, Zagorski S, Davis SS, Hunter JG, Lin E, Smith CD. Ten-year follow-up of laparoscopic Heller myotomy for achalasia shows durability. *Surg Endosc* 2007; **21**: 1498-1502 [PMID: 17623235 DOI: 10.1007/s00464-007-9500-9]
24 **Williams VA**, Peters JH. Achalasia of the esophagus: a surgical disease. *J Am Coll Surg* 2009; **208**: 151-162 [PMID: 19228517 DOI: 10.1016/j.jamcollsurg.2008.08.027]
25 **Abir F**, Modlin I, Kidd M, Bell R. Surgical treatment of achalasia: current status and controversies. *Dig Surg* 2004; **21**: 165-176 [PMID: 15218230 DOI: 10.1159/000079341]
26 **Pellegrini C**, Wetter LA, Patti M, Leichter R, Mussan G, Mori T, Bernstein G, Way L. Thoracoscopic esophagomyotomy. Initial experience with a new approach for the treatment of achalasia. *Ann Surg* 1992; **216**: 291-296; discussion 296-299 [PMID: 1417178]
27 **Pellegrini CA**, Leichter R, Patti M, Somberg K, Ostroff JW, Way L. Thoracoscopic esophageal myotomy in the treatment of achalasia. *Ann Thorac Surg* 1993; **56**: 680-682 [PMID: 8379770]
28 **Maher JW**, Conklin J, Heitshusen DS. Thoracoscopic esophagomyotomy for achalasia: preoperative patterns of acid reflux and long-term follow-up. *Surgery* 2001; **130**: 570-576; discussion 576-577 [PMID: 11602886 DOI: 10.1067/msy.2001.116681]
29 **Shimi S**, Nathanson LK, Cuschieri A. Laparoscopic cardiomyotomy for achalasia. *J R Coll Surg Edinb* 1991; **36**: 152-154 [PMID: 1833541]
30 **Patti MG**, Pellegrini CA, Horgan S, Arcerito M, Omelanczuk P, Tamburini A, Diener U, Eubanks TR, Way LW. Minimally invasive surgery for achalasia: an 8-year experience with 168 patients. *Ann Surg* 1999; **230**: 587-593; discussion 593-594 [PMID: 10522728]
31 **Stewart KC**, Finley RJ, Clifton JC, Graham AJ, Storseth C, Inculet R. Thoracoscopic versus laparoscopic modified Heller Myotomy for achalasia: efficacy and safety in 87 patients. *J Am Coll Surg* 1999; **189**: 164-199; discussion 169-170 [PMID: 10437838 DOI: 10.1016/S1072-7515(99)00094-0]
32 **Ramacciato G**, Mercantini P, Amodio PM, Corigliano N, Barreca M, Stipa F, Ziparo V. The laparoscopic approach with antireflux surgery is superior to the thoracoscopic approach for the treatment of esophageal achalasia. Experience of a single surgical unit. *Surg Endosc* 2002; **16**: 1431-1437 [PMID: 12072992 DOI: 10.1007/s00464-001-9215-2]
33 **Kesler KA**, Tarvin SE, Brooks JA, Rieger KM, Lehman GA, Brown JW. Thoracoscopy-assisted Heller myotomy for the treatment of achalasia: results of a minimally invasive technique. *Ann Thorac Surg* 2004; **77**: 385-391; discussion 391-392 [PMID: 14759402 DOI: 10.1016/j.athoracsur.2003.06.018]
34 **Ancona E**, Anselmino M, Zaninotto G, Costantini M, Rossi M, Bonavina L, Boccu C, Buin F, Peracchia A. Esophageal achalasia: laparoscopic versus conventional open Heller-Dor operation. *Am J Surg* 1995; **170**: 265-270 [PMID: 7661295]
35 **Bello B**, Herbella FA, Allaix ME, Patti MG. Impact of minimally invasive surgery on the treatment of benign esophageal disorders. *World J Gastroenterol* 2012; **18**: 6764-6770 [PMID: 23239914 DOI: 10.3748/wjg.v18.i46.6764]
36 **Campos GM**, Vittinghoff E, Rabl C, Takata M, Gadenstätter M, Lin F, Ciovica R. Endoscopic and surgical treatments for achalasia: a systematic review and meta-analysis. *Ann Surg* 2009; **249**: 45-57 [PMID: 19106675 DOI: 10.1097/SLA.0b013e31818e43ab]
37 **Falkenback D**, Johansson J, Oberg S, Kjellin A, Wenner J, Zilling T, Johnsson F, Von Holstein CS, Walther B. Heller's esophagomyotomy with or without a 360 degrees floppy Nissen fundoplication for achalasia. Long-term results from a prospective randomized study. *Dis Esophagus* 2003; **16**: 284-290 [PMID: 14641290 DOI: 10.1111/j.1442-2050.2003.00348.x]
38 **Simić AP**, Radovanović NS, Skrobić OM, Raznatović ZJ, Pesko PM. Significance of limited hiatal dissection in surgery for achalasia. *J Gastrointest Surg* 2010; **14**: 587-593 [PMID: 20033338 DOI: 10.1007/s11605-009-1135-9]
39 **Finley C**, Clifton J, Yee J, Finley RJ. Anterior fundoplication decreases esophageal clearance in patients undergoing Heller myotomy for achalasia. *Surg Endosc* 2007; **21**: 2178-2182 [PMID: 17514394 DOI: 10.1007/s00464-007-9327-4]
40 **Rebecchi F**, Giaccone C, Farinella E, Campaci R, Morino M. Randomized controlled trial of laparoscopic Heller myotomy plus Dor fundoplication versus Nissen fundoplication for achalasia: long-term results. *Ann Surg* 2008; **248**: 1023-1030 [PMID: 19092347 DOI: 10.1097/SLA.0b013e318190a776]
41 **Rawlings A**, Soper NJ, Oelschlager B, Swanstrom L, Matthews BD, Pellegrini C, Pierce RA, Pryor A, Martin V, Frisella MM, Cassera M, Brunt LM. Laparoscopic Dor versus Toupet fundoplication following Heller myotomy for achalasia: results of a multicenter, prospective, randomized-controlled trial. *Surg Endosc* 2012; **26**: 18-26 [PMID: 21789646 DOI: 10.1007/s00464-011-1822-y]
42 **Wright AS**, Williams CW, Pellegrini CA, Oelschlager BK. Long-term outcomes confirm the superior efficacy of extended Heller myotomy with Toupet fundoplication for achalasia. *Surg Endosc* 2007; **21**: 713-718 [PMID: 17332964 DOI: 10.1007/s00464-006-9165-9]
43 **Di Martino N**, Brillantino A, Monaco L, Marano L, Schettino M, Porfidia R, Izzo G, Cosenza A. Laparoscopic calibrated total vs partial fundoplication following Heller myotomy for oesophageal achalasia. *World J Gastroenterol* 2011; **17**: 3431-3440 [PMID: 21876635 DOI: 10.3748/wjg.v17.i29.3431]
44 **Stefanidis D**, Richardson W, Farrell TM, Kohn GP, Augenstein V, Fanelli RD. SAGES guidelines for the surgical treatment of esophageal achalasia. *Surg Endosc* 2012; **26**: 296-311 [PMID: 22044977 DOI: 10.1007/s00464-011-2017-2]
45 **Katada N**, Sakuramoto S, Yamashita K, Shibata T, Moriya H, Kikuchi S, Watanabe M. Recent trends in the management of achalasia. *Ann Thorac Cardiovasc Surg* 2012; **18**: 420-428 [PMID: 23099422 DOI: 10.5761/atcs.ra.12.01949]
46 **Abid S**, Champion G, Richter JE, McElvein R, Slaughter RL, Koehler RE. Treatment of achalasia: the best of both worlds. *Am J Gastroenterol* 1994; **89**: 979-985 [PMID: 8017394]
47 **Katsinelos P**, Kountouras J, Paroutoglou G, Beltsis A, Zavos C, Papaziogas B, Mimidis K. Long-term results of pneumatic dilation for achalasia: a 15 years' experience. *World J Gastroenterol* 2005; **11**: 5701-5705 [PMID: 16237769 DOI: 10.3748/wjg.v11.i36.5587]
48 **Vantrappen G**, Hellemans J, Deloof W, Valembois P, Vandenbroucke J. Treatment of achalasia with pneumatic dilatations. *Gut* 1971; **12**: 268-275 [PMID: 5574797]
49 **Vaezi MF**, Richter JE. Current therapies for achalasia: comparison and efficacy. *J Clin Gastroenterol* 1998; **27**: 21-35 [PMID:

9706766]
50 **Karamanolis G**, Sgouros S, Karatzias G, Papadopoulou E, Vasiliadis K, Stefanidis G, Mantides A. Long-term outcome of pneumatic dilation in the treatment of achalasia. *Am J Gastroenterol* 2005; **100**: 270-274 [PMID: 15667481 DOI: 10.1111/j.1572-0241.2005.40093.x]
51 **West RL**, Hirsch DP, Bartelsman JF, de Borst J, Ferwerda G, Tytgat GN, Boeckxstaens GE. Long term results of pneumatic dilation in achalasia followed for more than 5 years. *Am J Gastroenterol* 2002; **97**: 1346-1351 [PMID: 12094848 DOI: 10.1111/j.1572-0241.2002.05771.x]
52 **Beckingham IJ**, Callanan M, Louw JA, Bornman PC. Laparoscopic cardiomyotomy for achalasia after failed balloon dilatation. *Surg Endosc* 1999; **13**: 493-496 [PMID: 10227950]
53 **Ponce J**, Juan M, Garrigues V, Pascual S, Berenguer J. Efficacy and safety of cardiomyotomy in patients with achalasia after failure of pneumatic dilatation. *Dig Dis Sci* 1999; **44**: 2277-2282 [PMID: 10573374]
54 **Gockel I**, Junginger T, Bernhard G, Eckardt VF. Heller myotomy for failed pneumatic dilation in achalasia: how effective is it? *Ann Surg* 2004; **239**: 371-377 [PMID: 15075654 DOI: 10.1097/01.sla.0000114228.34809.01]
55 **Tsuboi K**, Omura N, Yano F, Kashiwagi H, Kawasaki N, Suzuki Y, Yanaga K. Preoperative dilatation does not affect the surgical outcome of laparoscopic Heller myotomy and Dor fundoplication for esophageal achalasia. *Surg Laparosc Endosc Percutan Tech* 2009; **19**: 98-100 [PMID: 19390272 DOI: 10.1097/SLE.0b013e31819cb127]
56 **Kostic S**, Kjellin A, Ruth M, Lönroth H, Johnsson E, Andersson M, Lundell L. Pneumatic dilatation or laparoscopic cardiomyotomy in the management of newly diagnosed idiopathic achalasia. Results of a randomized controlled trial. *World J Surg* 2007; **31**: 470-478 [PMID: 17308851 DOI: 10.1007/s00268-006-0600-9]
57 **Novais PA**, Lemme EM. 24-h pH monitoring patterns and clinical response after achalasia treatment with pneumatic dilation or laparoscopic Heller myotomy. *Aliment Pharmacol Ther* 2010; **32**: 1257-1265 [PMID: 20955445 DOI: 10.1111/j.1365-2036.2010.04461.x]
58 **Boeckxstaens GE**, Annese V, des Varannes SB, Chaussade S, Costantini M, Cuttitta A, Elizalde JI, Fumagalli U, Gaudric M, Rohof WO, Smout AJ, Tack J, Zwinderman AH, Zaninotto G, Busch OR. Pneumatic dilation versus laparoscopic Heller's myotomy for idiopathic achalasia. *N Engl J Med* 2011; **364**: 1807-1816 [PMID: 21561346 DOI: 10.1056/NEJMoa1010502]
59 **Persson J**, Johnsson E, Kostic S, Lundell L, Smedh U. Treatment of achalasia with laparoscopic myotomy or pneumatic dilatation: long-term results of a prospective, randomized study. *World J Surg* 2015; **39**: 713-720 [PMID: 25409838 DOI: 10.1007/s00268-014-2869-4]
60 **Pasricha PJ**, Ravich WJ, Hendrix TR, Sostre S, Jones B, Kalloo AN. Intrasphincteric botulinum toxin for the treatment of achalasia. *N Engl J Med* 1995; **332**: 774-778 [PMID: 7862180 DOI: 10.1056/NEJM199503233321203]
61 **Pasricha PJ**, Rai R, Ravich WJ, Hendrix TR, Kalloo AN. Botulinum toxin for achalasia: long-term outcome and predictors of response. *Gastroenterology* 1996; **110**: 1410-1415 [PMID: 8613045 DOI: 10.1053/gast.1996.v110.pm8613045]
62 **Zaninotto G**, Annese V, Costantini M, Del Genio A, Costantino M, Epifani M, Gatto G, D'onofrio V, Benini L, Contini S, Molena D, Battaglia G, Tardio B, Andriulli A, Ancona E. Randomized controlled trial of botulinum toxin versus laparoscopic heller myotomy for esophageal achalasia. *Ann Surg* 2004; **239**: 364-370 [PMID: 15075653 DOI: 10.1097/01.sla.0000114217.52941.c5]
63 **Inoue H**, Minami H, Kobayashi Y, Sato Y, Kaga M, Suzuki M, Satodate H, Odaka N, Itoh H, Kudo S. Peroral endoscopic myotomy (POEM) for esophageal achalasia. *Endoscopy* 2010; **42**: 265-271 [PMID: 20354937 DOI: 10.1055/s-0029-1244080]
64 **Friedel D**, Modayil R, Stavropoulos SN. Per-oral endoscopic myotomy: major advance in achalasia treatment and in endoscopic surgery. *World J Gastroenterol* 2014; **20**: 17746-17755 [PMID: 25548473 DOI: 10.3748/wjg.v20.i47.17746]
65 **Pasricha PJ**, Hawari R, Ahmed I, Chen J, Cotton PB, Hawes RH, Kalloo AN, Kantsevoy SV, Gostout CJ. Submucosal endoscopic esophageal myotomy: a novel experimental approach for the treatment of achalasia. *Endoscopy* 2007; **39**: 761-764 [PMID: 17703382 DOI: 10.1055/s-2007-966764]
66 **Hungness ES**, Teitelbaum EN, Santos BF, Arafat FO, Pandolfino JE, Kahrilas PJ, Soper NJ. Comparison of perioperative outcomes between peroral esophageal myotomy (POEM) and laparoscopic Heller myotomy. *J Gastrointest Surg* 2013; **17**: 228-235 [PMID: 23054897 DOI: 10.1007/s11605-012-2030-3]
67 **Ujiki MB**, Yetasook AK, Zapf M, Linn JG, Carbray JM, Denham W. Peroral endoscopic myotomy: A short-term comparison with the standard laparoscopic approach. *Surgery* 2013; **154**: 893-897; discussion 897-900 [PMID: 24074429 DOI: 10.1016/j.surg.2013.04.042]
68 **Teitelbaum EN**, Boris L, Arafat FO, Nicodème F, Lin Z, Kahrilas PJ, Pandolfino JE, Soper NJ, Hungness ES. Comparison of esophagogastric junction distensibility changes during POEM and Heller myotomy using intraoperative FLIP. *Surg Endosc* 2013; **27**: 4547-4555 [PMID: 24043641 DOI: 10.1007/s00464-013-3121-2]
69 **Ellis FH**. Failure after esophagomyotomy for esophageal motor disorders. Causes, prevention, and management. *Chest Surg Clin N Am* 1997; **7**: 477-487; discussion 488 [PMID: 9246398]
70 **Duffy PE**, Awad ZT, Filipi CJ. The laparoscopic reoperation of failed Heller myotomy. *Surg Endosc* 2003; **17**: 1046-1049 [PMID: 12730729 DOI: 10.1007/s00464-002-8570-y]
71 **Iqbal A**, Tierney B, Haider M, Salinas VK, Karu A, Turaga KK, Mittal SK, Filipi CJ. Laparoscopic re-operation for failed Heller myotomy. *Dis Esophagus* 2006; **19**: 193-199 [PMID: 16722998 DOI: 10.1111/j.1442-2050.2006.00564.x]
72 **Torquati A**, Richards WO, Holzman MD, Sharp KW. Laparoscopic myotomy for achalasia: predictors of successful outcome after 200 cases. *Ann Surg* 2006; **243**: 587-591; discussion 591-593 [PMID: 16632992 DOI: 10.1097/01.sla.0000216782.10502.47]
73 **Rakita S**, Villadolid D, Kalipersad C, Thometz D, Rosemurgy A. Outcomes promote reoperative Heller myotomy for symptoms of achalasia. *Surg Endosc* 2007; **21**: 1709-1714 [PMID: 17440784 DOI: 10.1007/s00464-007-9226-8]
74 **Grotenhuis BA**, Wijnhoven BP, Myers JC, Jamieson GG, Devitt PG, Watson DI. Reoperation for dysphagia after cardiomyotomy for achalasia. *Am J Surg* 2007; **194**: 678-682 [PMID: 17936434 DOI: 10.1016/j.amjsurg.2007.01.035]
75 **Loviscek MF**, Wright AS, Hinojosa MW, Petersen R, Pajitnov D, Oelschlager BK, Pellegrini CA. Recurrent dysphagia after Heller myotomy: is esophagectomy always the answer? *J Am Coll Surg* 2013; **216**: 736-743; discussion 743-744 [PMID: 23415553 DOI: 10.1016/j.jamcollsurg.2012.12.008]
76 **Omura N**, Kashiwagi H, Yano F, Tsuboi K, Yanaga K. Reoperations for esophageal achalasia. *Surg Today* 2012; **42**: 1078-1081 [PMID: 22790707 DOI: 10.1007/s00595-012-0204-y]
77 **Pandolfino JE**, Kwiatek MA, Nealis T, Bulsiewicz W, Post J, Kahrilas PJ. Achalasia: a new clinically relevant classification by high-resolution manometry. *Gastroenterology* 2008; **135**: 1526-1533 [PMID: 18722376 DOI: 10.1053/j.gastro.2008.07.022]
78 **Pratap N**, Kalapala R, Darisetty S, Joshi N, Ramchandani M, Banerjee R, Lakhtakia S, Gupta R, Tandan M, Rao GV, Reddy DN. Achalasia cardia subtyping by high-resolution manometry predicts the therapeutic outcome of pneumatic balloon dilatation. *J Neurogastroenterol Motil* 2011; **17**: 48-53 [PMID: 21369491 DOI: 10.5056/jnm.2011.17.1.48]
79 **Salvador R**, Costantini M, Zaninotto G, Morbin T, Rizzetto C, Zanatta L, Ceolin M, Finotti E, Nicoletti L, Da Dalt G, Cavallin F, Ancona E. The preoperative manometric pattern predicts the outcome of surgical treatment for esophageal achalasia. *J Gastrointest Surg* 2010; **14**: 1635-1645 [PMID: 20830530 DOI: 10.1007/s11605-010-1318-4]
80 **Yano F**, Omura N, Tsuboi K, Hoshino M, Yamamoto SR, Kashiwagi H, Yanaga K. Single-incision laparoscopic Heller myotomy and Dor fundoplication for achalasia: report of a case.

Surg Today 2012; **42**: 299-302 [PMID: 22218875 DOI: 10.1007/s00595-011-0089-1]

81 **Barry L**, Ross S, Dahal S, Morton C, Okpaleke C, Rosas M, Rosemurgy AS. Laparoendoscopic single-site Heller myotomy with anterior fundoplication for achalasia. *Surg Endosc* 2011; **25**: 1766-1774 [PMID: 21487889 DOI: 10.1007/s00464-010-1454-7]

82 **Omura N**, Yano F, Tsuboi K, Hoshino M, Yamamoto SR, Akimoto S, Ishibashi Y, Kashiwagi H, Yanaga K. Short-term surgical outcomes of reduced port surgery for esophageal achalasia. *Surg Today* 2015; **45**: 1139-1143 [PMID: 25563589 DOI: 10.1007/s00595-014-1109-8]

83 **Ross SB**, Luberice K, Kurian TJ, Paul H, Rosemurgy AS. Defining the learning curve of laparoendoscopic single-site Heller myotomy. *Am Surg* 2013; **79**: 837-844 [PMID: 23896255]

84 **Shaligram A**, Unnirevi J, Simorov A, Kothari VM, Oleynikov D. How does the robot affect outcomes? A retrospective review of open, laparoscopic, and robotic Heller myotomy for achalasia. *Surg Endosc* 2012; **26**: 1047-1050 [PMID: 22038167 DOI: 10.1007/s00464-011-1994-5]

85 **Brücher BL**, Stein HJ, Bartels H, Feussner H, Siewert JR. Achalasia and esophageal cancer: incidence, prevalence, and prognosis. *World J Surg* 2001; **25**: 745-749 [PMID: 11376410]

86 **Sandler RS**, Nyrén O, Ekbom A, Eisen GM, Yuen J, Josefsson S. The risk of esophageal cancer in patients with achalasia. A population-based study. *JAMA* 1995; **274**: 1359-1362 [PMID: 7563560 DOI: 10.1001/jama.1995.03530170039029]

87 **Zaninotto G**, Rizzetto C, Zambon P, Guzzinati S, Finotti E, Costantini M. Long-term outcome and risk of oesophageal cancer after surgery for achalasia. *Br J Surg* 2008; **95**: 1488-1494 [PMID: 18991316 DOI: 10.1002/bjs.6413]

P- Reviewer: Chen JQ, Sharma SS, Zhu YL **S- Editor**: Yu J
L- Editor: A **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10840

World J Gastroenterol 2015 October 14; 21(38): 10840-10852
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Aberrant expression of peroxiredoxin 1 and its clinical implications in liver cancer

Yu-Lin Sun, Jian-Qiang Cai, Fang Liu, Xin-Yu Bi, Lan-Ping Zhou, Xiao-Hang Zhao

Yu-Lin Sun, Fang Liu, Lan-Ping Zhou, Xiao-Hang Zhao, State Key Laboratory of Molecular Oncology, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Jian-Qiang Cai, Xin-Yu Bi, Department of Abdominal Surgery, Cancer Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100021, China

Author contributions: Sun YL performed the majority of the work, data analysis, and wrote the manuscript; Liu F and Zhou LP assisted in the experiments; Bi XY collected the clinical specimens; Zhao XH and Cai JQ supervised this work; Sun YL, Cai JQ, and Zhao XH designed the study.

Supported by State Key Project for Infectious Diseases, No. 2013ZX10002009 and No. 2012ZX10002-017; State Key Project for Basic Research, No. 2014CBA02001 and No. 2014CBA02002; National High-tech R and D Program, No. 2012AA020206; and Natural Science Foundation of China, No. 81071789 and No. 81321091.

Institutional review board statement: All routine colonic biopsy specimens and blood samples from the patients were taken after informed consent and ethical permission was obtained for participation in the study.

Conflict-of-interest statement: To the best of our knowledge, no conflict of interest exists.

Correspondence to: Xiao-Hang Zhao, MD, PhD, Professor, State Key Laboratory of Molecular Oncology, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Panjiayuan Nanli 17, Chaoyang District, Beijing 100021, China. zhaoxh@cicams.ac.cn
Telephone: +86-10-67709015
Fax: +86-10-87778360

Received: April 21, 2015
Peer-review started: April 22, 2015
First decision: June 19, 2015
Revised: July 2, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 14, 2015

Abstract

AIM: To investigate the expression characteristics of peroxiredoxin 1 (PRDX1) mRNA and protein in liver cancer cell lines and tissues.

METHODS: The RNA sequencing data from 374 patients with liver cancer were obtained from The Cancer Genome Atlas. The expression and clinical characteristics of *PRDX1* mRNA were analyzed in this dataset. The Kaplan-Meier and Cox regression survival analysis was performed to determine the relationship between *PRDX1* levels and patient survival. Subcellular fractionation and Western blotting were used to demonstrate the expression of PRDX1 protein in six liver cancer cell lines and 29 paired fresh tissue specimens. After bioinformatics prediction, a putative post-translational modification form of PRDX1 was observed using immunofluorescence under confocal microscopy and immunoprecipitation analysis in liver cancer cells.

RESULTS: The mRNA of *PRDX1* gene was upregulated about 1.3-fold in tumor tissue compared with the adjacent non-tumor control (P = 0.005). Its abundance was significantly higher in men than women (P < 0.001). High levels of *PRDX1* mRNA were associated with a shorter overall survival time (P =

0.04) but not with recurrence-free survival. The Cox regression analysis demonstrated that patients with high *PRDX1* mRNA showed about 1.9-fold increase of risk for death (P = 0.03). In liver cancer cells, PRDX1 protein was strongly expressed with multiple different bands. PRDX1 in the cytosol fraction existed near the theoretical molecular weight, whereas two higher molecular weight bands were present in the membrane/organelle and nuclear fractions. Importantly, the theoretical PRDX1 band was increased, whereas the high molecular weight form was decreased in tumor tissues. Subsequent experiments revealed that the high molecular weight bands of PRDX1 might result from the post-translational modification by small ubiquitin-like modifier-1 (SUMO1).

CONCLUSION: PRDX1 was overexpressed in the tumor tissues of liver cancer and served as an independent poor prognostic factor for overall survival. PRDX1 can be modified by SUMO to play specific roles in hepatocarcinogenesis.

Key words: Peroxiredoxin 1; Liver cancer; Prognostic factor; Post-translational modification; SUMOylation

Core tip: Peroxiredoxin 1 (PRDX1) is an antioxidant enzyme, and, therefore, it is considered a tumor suppressor gene. However, only recently has various data revealed that PRDX1 not only functions in peroxide detoxification but also in tumorigenesis. Here, we found that PRDX1 was overexpressed in liver cancer at the transcriptional level, and it was an independent unfavorable prognostic factor for overall survival. In liver cancer cells, PRDX1 is post-translationally modified by small ubiquitin-like modifier. The downregulation of sumoylated PRDX1 in tumors might participate in hepatocarcinogenesis. PRDX1 represents both a prognostic biomarker and therapeutic target for liver cancer.

Sun YL, Cai JQ, Liu F, Bi XY, Zhou LP, Zhao XH. Aberrant expression of peroxiredoxin 1 and its clinical implications in liver cancer. *World J Gastroenterol* 2015; 21(38): 10840-10852 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10840.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10840

INTRODUCTION

Liver cancer is the second leading cause of cancer death worldwide, accounting for about 9.1% of total cancer deaths. It was estimated that 782500 new cases and 745500 deaths occurred globally in 2012, and China alone accounted for about 50% of the total number of cases and deaths[1]. Overwhelmingly, chronic infection with hepatitis B or C virus, alcoholic liver disease, and nonalcoholic fatty liver disease are the major risk factors of liver cancer[2]. The exposures of these factors generally contribute to the multistep development of liver cancer by promoting extensive oxidative stress, liver inflammation, and immune response[3]. Among them, reactive oxygen species (ROS) can promote many aspects of tumor development and progression *via* oxidative DNA and protein damage, lipid peroxidation, damage to tumor suppressor genes, and enhanced expression of proto-oncogenes *etc*.[4].

Under normal physiological conditions, the intracellular ROS are detoxified by non-enzymatic molecules (*i.e.*, glutathione, flavonoids, and vitamins A, C, and E) or antioxidant enzymes. There are at least five families of antioxidant enzymes with specifically scavenging capacity, including superoxide dismutases, catalases, peroxiredoxins (PRDXs), thioredoxins, and glutathione system *etc*.[4]. Among them, PRDXs use thioredoxin as the electron donor to catalyze the reduction of hydrogen peroxide, organic hydroperoxides, and peroxynitrite. Mammalian cells express six isoforms of PRDXs, which are classified into three subfamilies based on the location or absence of the essential catalytic cysteine (Cys) residue, 2-Cys (PRDX1, 2, 3, 4), atypical 2-Cys (PRDX5), and 1-Cys (PRDX6). PRDX1 is the most abundant and ubiquitously distributed isoform.

Notably, recent evidence suggests that hydrogen peroxide may serve as an intracellular signaling messenger molecule in response to stimulation in many mammalian cell types[4-7]. It oxidizes the critical residues of its effectors, as exemplified by the inhibition of protein-tyrosine phosphatases and the tumor suppressor phosphatase and tensin homolog (PTEN)[8,9]. Actually, PRDX1 has been reported to act as an intermediate in cell signaling *via* oxidizing several signaling proteins[10-12] to regulate cell proliferation, differentiation, apoptosis, migration, angiogenesis, and senescence[12-15]. Therefore, PRDX1 has a dual function in tumorigenesis. On the one hand, it functions as a tumor suppressor gene. *Prdx1*$^{-/-}$ mice have a shortened lifespan due to severe hemolytic anemia and several malignant cancers, including liver cancer[16]. In *Prdx1*$^{-/-}$ fibroblasts and mammary epithelial cells, it was shown to act as a safeguard for the lipid phosphatase activity of PTEN to suppress H-Ras and ErbB-2-induced cell transformation[17]. On the other hand, PRDX1 can act in a manner independent of its peroxide detoxifying function. The high level of PRDX1 was associated with a high potential for recurrence in squamous cell carcinoma of the tongue[18] and diminished overall survival and disease-free survival in gallbladder cancer, ovarian serous carcinomas, lung cancer, and pancreatic cancer[19-22]. In addition, inhibition of PRDX1 increases radio- and chemosensitivity in glioma and lung cancer[23-26]. In prostate cancer, it enhances the

transactivation of androgen receptor[27].

In liver cancer, the overexpression of PRDX1 mRNA and protein has been observed in limited clinical specimens. Increased PRDX1 expression was associated with tumor angiogenesis, progression, and tumor necrosis factor alpha related apoptosis inducing ligand resistance and served as an independent poor prognosis factor[28,29]. Silencing PRDX1 in HepG2 cells partially reversed the tumor phenotype *via* the downregulation of proteins involved in cell proliferation and differentiation[30]. In this study, we investigated the expression and clinical significance of *PRDX1* mRNA in liver cancer using an RNA sequencing dataset from The Cancer Genome Atlas (TCGA) (n = 374). Meanwhile, according to the protein expression and subcellular localization of PRDX1, a novel post-translational modification form of PRDX1 was explored in liver cancer cells.

MATERIALS AND METHODS

Cell lines and cell cultures

The human liver cancer cell lines HepG2, Hep3B, and SK-HEP-1 were obtained from the American Type Culture Collection (Rockville, MD, United States). Bel-7402, Bel-7404, and SMMC-7721 liver cancer cells were purchased form Institute of Biochemistry and Cell Biology of Chinese Academy of Sciences (Shanghai, China); HLE cell was purchased from the Human Science Research Resources Bank (Osaka, Japan). They were maintained in recommended media at 37 ℃ with 5% CO_2.

Protein extraction from cells

For total proteins extraction, cells in the exponential phase of growth were harvested using a protein lysis buffer (pH 7.4) containing 50 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS), 10 mmol/L N-methylmaleylimide (Sigma-Aldrich, St. Louis, MO, United States) and protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). The protein content was determined by Coomassie Plus Protein Assay (Pierce, Rockford, IL, United States). In addition, subcellular protein extraction was performed using ProteoExtract™ Subcellular Proteome Extraction Kit (Calbiochem, Billerica, MA, United States) according to the manufacturer's guidelines.

Clinical specimen collection and preparation

Surgical tissues from liver cancer patients were collected after obtaining informed consent and approval from the Institutional Review Board of the Cancer Institute and Hospital of Chinese Academy of Medical Sciences (Beijing, China). All patients were diagnosed by two senior pathologists without chemo/radiotherapy before surgical operation. A total of 29 fresh tumor and paired adjacent non-tumor liver tissue samples were collected from patients (26 male, three female; median age, 54 ± 12; range 32-78 years) undergoing resection during the period from May 2006 to November 2007. Among them, 17 cases were α-fetoprotein (AFP)-normal, while 12 were AFP-positive. The tissue samples were collected and washed right after surgical resection. They were then snap-frozen in liquid nitrogen immediately and stored at -80 ℃. Fresh tissue samples were homogenized and the proteins were extracted using the protein lysis buffer described above.

Western blot analysis

Approximately 15 μg of total proteins or subcellular proteins were diluted in Laemmli buffer containing 10% β-mercaptoethanol and boiled at 95 ℃ for 10 min. Samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes. After blocking, the membranes were incubated with anti-PRDX1 (ab15571, Abcam, Cambridge, United Kingdom) and anti-β-actin (Sigma-Aldrich) antibodies. Following intensive washing, the membranes were developed with horseradish peroxidase conjugated second antibodies (Jackson Immunoresearch Lab., West Grove, PA, United States) and visualized using an enhanced chemiluminescence system (Santa Cruz Biotech., Dallas, TX, United States). The upregulation or downregulation of PRDX1 was defined as higher or lower relative band intensity in tumors compared with their paired adjacent normal liver tissues.

Immunofluorescence under confocal microscopy

HepG2, Hep3B, and SK-HEP-1 cells were grown in 0.01% poly-l-Lysine coated slides for 24 h. After fixed with 4% paraformaldehyde for 30 min at room temperature and washed three times with PBS (pH 7.4), the cells were blocked with 1% bovine serum albumin (BSA) and 0.1% Triton X-100 for 30 min at room temperature. Washed cells were incubated for 30 min with rabbit anti-PRDX1 and mouse anti-SUMO1 (Zymed Lab., San Francisco, CA, United States) antibodies. Then, the cells were incubated in the dark for 60 min with Alexa Fluor 488-conjugated goat anti-rabbit and Alexa Fluor 594-conjugated goat anti-mouse (Life Technologies, Carlsbad, CA, United States) secondary IgG. The fluorescence signals were captured under a TCS SP2 laser confocal microscope (Leica Microsystems, Wetzlar, Germany).

Immunoprecipitation

For co-immunoprecipitation analysis, 1.5 mg of whole cell lysate of HepG2 was precleared by incubating with protein G-agarose beads (Roche Diagnostics, Basel, Switzerland) at 4 ℃ for 1 h. The collected supernatant was incubated at 4 ℃ with 4 μg of rabbit anti-PRDX1 antibody, mouse anti-SUMO1 antibody, or nonimmune

rabbit/mouse IgG (Zhongshan Biotechnology, Beijing, China) overnight with rotation. The immune complex was precipitated by incubation with 50 μL of protein G-agarose for 3 h at 4 ℃. The agarose beads were pelleted by centrifugation and washed three times with lysis buffer. The beads were suspended in 2 × Laemmli sample buffer and boiled for 5 min. Protein G-agarose beads were removed from the complex by centrifugation at 10000 *g* for 5 min. The supernatant was loaded onto 10% SDS-PAGE for Western blot analysis with respective antibodies to PRDX1 and SUMO1.

TCGA RNA sequencing data mining and statistical analysis

The liver cancer transcriptome dataset was obtained from TCGA. The RNA sequencing data from 49 non-tumor liver tissues and 374 tumor tissues were available. The expression of *PRDX1* mRNA and its clinical significance was analyzed. Mann-Whitney *U* test was used to compare the Read per Million (RPM) between two groups. The Kaplan-Meier method was used to determine the relationship between the RPM of PRDX1 and patient survival, and log-rank analysis was performed to compare survival curves. Univariate and multivariate analyses were performed using the Cox regression model. In addition, the bioinformatic tool SUMOplot (http://www.abgent.com/sumoplot) was used to predict the putative SUMOylation sites of PRDX1. P values < 0.05 were considered significant. All analyses were performed using Graphpad prism 6.0 (GraphPad Software Inc., La Jolla, CA, United States).

RESULTS

Upregulation of PRDX1 mRNA in human liver cancer tissues

According to the RNA sequencing data from TCGA, *PRDX1* mRNA was upregulated approximately 1.3-fold in tumor tissues (n = 374) compared with adjacent non-tumor livers (n = 49) (Figure 1A, P = 0.005). Based on the median RPM value of *PRDX1* in tumor tissues, all 374 cases were divided into two groups, high level group and low level group. Kaplan-Meier survival analysis with a log-rank test showed a significant correlation between high *PRDX1* mRNA expression and shorter overall survival time (P = 0.04) in liver cancer patients (Figure 1B, left panel). The median survival times of high and low expression groups were 635 and 498 d, respectively. However, the expression of *PRDX1* mRNA was not associated with recurrence of the patients with liver cancer (Figure 1B, right panel). Even for patients who had received a curative resection (R0 resection, n = 310), high levels of *PRDX1* mRNA also was correlated with shorter overall survival (P = 0.05) but not with recurrence (Supplementary file).

These findings were further confirmed by both univariate and multivariate Cox regression analysis (Tables 1 and 2). In the univariate analysis, compared with the low *PRDX1* mRNA expression group, patients with the high *PRDX1* mRNA exhibited a 1.55-fold increase of relative risk (RR) for overall survival (P = 0.04). Other significant risk factors included Child-Pugh classification (RR = 2.49, P = 0.01) and tumor, node, metastases (TNM) staging (RR = 2.39, P < 0.001). As concluded by the multivariate analysis, TNM staging (RR = 2.36, P = 0.007), Child-Pugh classification (RR = 2.30, P = 0.03), and PRDX1 mRNA expression (RR = 1.89, P = 0.03) were the independent prognostic factors for death. For recurrence-free survival, in the univariate analysis, residual tumor (RR = 2.25, P = 0.05), vascular invasion (RR = 1.65, P = 0.05), and TNM staging (RR = 4.32, P < 0.001) were associated with increased risk of recurrence. Only TNM staging (RR = 5.04, P < 0.001) was considered as an independent recurrent factor.

The correlations between the clinicopathologic characteristics of liver cancer patients and the expression of *PRDX1* mRNA in their tumors were also compared (Figure 1C). The levels of *PRDX1* mRNA were higher in males than females (P < 0.001). The correlation between *PRDX1* mRNA and other features, such as age, differentiation degree, vascular invasion, Child-Pugh classification, TNM staging, hepatic fibrosis degree, serum AFP levels, and hepatic inflammation in adjacent liver tissue, was not observed.

Expression and localization of PRDX1 protein in liver cancer cell lines

In addition to whole cell lysates, cytosol, membrane/organelle, and nuclear protein fractions were extracted from liver cancer cells (HepG2, Hep3B, SK-HEP-1, Bel-7404, SMMC-7721, and HLE) to enhance the visibility of moderate- and low-abundance proteins. PRDX1 was expressed in the whole lysates of all six cells, represented by multiple different bands (Figure 2). Additionally, the subcellular protein analysis showed that PRDX1 in the cytosol fraction existed near the theoretical molecular weight form (22 kDa), whereas two higher molecular weight bands, approximately 35 and 50 kDa, were present in the membrane/organelle and nuclear fractions, especially the membrane/organelle fraction (Figure 2). Thus, PRDX1 protein was ubiquitously distributed in the liver cancer cells, and it existed as multiple forms in the membrane/organelle and nuclear fractions.

Expression of PRDX1 protein in human liver cancer samples

To confirm the observation in liver cancer cells, the expression of PRDX1 in liver cancer patients was analyzed using Western blotting. We found that PRDX1 was detected as two bands with different molecular weight, the theoretical 22 kDa band and the higher 50 kDa band. Compared with the non-tumorous corresponding tissues, the theoretical PRDX1 band was

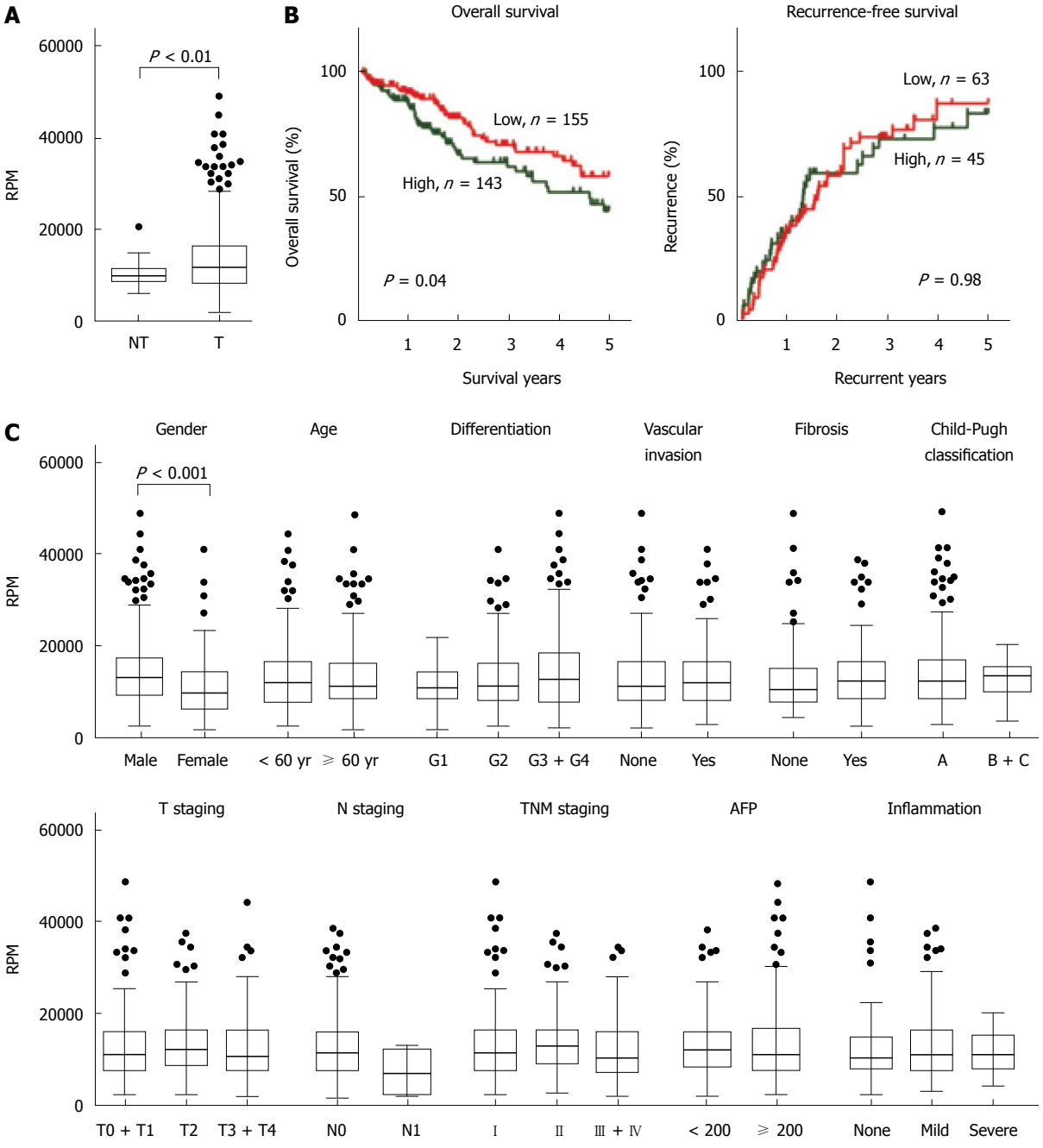


Figure 1 Expression and clinicopathological characteristics of peroxiredoxin 1 mRNA presented in The Cancer Genome Atlas liver cancer RNA sequencing dataset. A: Peroxiredoxin 1 (PRDX1) mRNA was significantly up-regulated in tumor tissues (n = 374) compared with the adjacent non-tumor tissues (n = 50); B: Kaplan-Meier curves of overall survival (left panel) and recurrence (right panel) according to the *PRDX1* levels in tumor samples. Log-rank test was performed; C: The clinicopathological characteristics analysis of *PRDX1* expression in 374 liver cancer cases. RPM: Read per Million; RPM: Read per Million; AFP: α-fetoprotein.

increased in 48.3% (14/29) of neoplastic liver tissues, whereas the higher 50 kDa form was downregulated in 69.0% (20/29) of tumor tissues (Figure 3). The total intensity of PRDX1 was downregulated in 18 out of 29 tumor tissues (62.1%, 18/29).

The clinical association trend for PRDX1 expression was observed in these 29 patients. We only found that downregulation of the 50 kDa band was greater in younger patients (< 60 years old). However, there was no correlation between PRDX1 protein and gender, tumor size, differentiation, TNM staging, or serum AFP levels.

Bioinformatic prediction of mechanisms involved in the formation of high molecular weight PRDX1

The theoretical molecular weight of PRDX1 is approximately 22 kDa. Our Western blot analysis, with reducing SDS-PAGE with 10% β-mercaptoethanol, revealed two extra high molecular weight bands near 35 and 50 kDa, especially in subcellular fractions. Therefore, we suspected that PRDX1 possessed some covalent modifications that were increasing the

Table 1 Univariate and multivariate survival analysis for overall survival and recurrence-free survival in The Cancer Genome Atlas patients with hepatocellular carcinoma

Variables	Overall survival		Recurrence-free survival	
	Relative risk (95%CI)	*P* value	Relative risk (95%CI)	*P* value
Univariate				
Age (> 60 *vs* ≤ 60 yr)	1.17 (0.76-1.80)	0.48	1.33 (0.83-2.14)	0.24
Gender (female *vs* male)	1.21 (0.78-1.86)	0.39	0.91 (0.72-1.15)	0.44
Differentiation (Poorly *vs* well and moderately)	1.27 (0.83-1.95)	0.28	0.76 (0.48-1.21)	0.25
Residual tumor (R1 + R2 *vs* R0)	1.95 (0.94-4.06)	0.07	2.25 (1.02-4.98)	0.05
Child-Pugh classification (grade B *vs* A)	2.49 (1.21-5.12)	0.01	2.22 (0.95-5.19)	0.07
Vascular invasion (macro + micro *vs* none)	1.52 (0.94-2.46)	0.08	1.65 (1.00-2.71)	0.05
TNM staging (Ⅲ + Ⅳ *vs* Ⅰ + Ⅱ)	2.39 (1.51-3.79)	< 0.001	4.32 (2.50-7.47)	< 0.001
Fibrosis (fibrosis + cirrhosis *vs* none)	0.98 (0.88-1.08)	0.63	0.81 (0.46-1.41)	0.45
AFP (≥ 200 ng/mL *vs* < 200 ng/mL)	1.15 (0.68-1.92)	0.61	0.85 (0.49-1.49)	0.58
PRDX1 expression (high *vs* low)	1.55 (1.01-2.36)	0.04	1.01 (0.63-1.60)	0.98
Inflammation in adjacent liver (severe + mild *vs* none)	1.20 (0.79-1.80)	0.39	1.30 (0.82-2.07)	0.26
Multivariate				
Residual tumor (R1 + R2 *vs* R0)	-	-	0. 89 (0.28-2.89)	0.77
Child-Pugh classification (grade B *vs* A)	2.30 (1.10-4.78)	0.03	-	-
Vascular invasion (macro + micro *vs* none)	-	-	1.42 (0.79-2.55)	0.23
TNM staging (Ⅲ + Ⅳ *vs* Ⅰ + Ⅱ)	2.36 (1.26-4.41)	0.007	5.04 (2.76-9.22)	< 0.001
PRDX1 expression (high *vs* low)	1.89 (1.07-3.36)	0.03	-	-

AFP: α-fetoprotein; PRDX1: Peroxiredoxin 1.

Table 2 Expression of peroxiredoxin 1 protein and its clinical significance

	22 kDa band		*P* value	50 kDa band		*P* value
	n	Up-regulated (%)		*n*	Down-regulated (%)	
Gender						
Male	26	45.2 (12/26)	1.00	26	69.2 (18/26)	1.00
Female	3	33.3 (1/3)		3	66.7 (2/3)	
Age						
≥ 60	10	30.0 (3/10)	0.43	10	40.0 (4/10)	0.03
< 60	19	52.6 (10/19)		19	65.5 (16/19)	
Tumor size						
≤ 3 cm	7	42.9 (3/7)	1.00	7	42.9 (3/7)	0.16
> 3 cm	22	45.5 (10/22)		22	77.3 (17/22)	
Differentiation						
Well	7	28.6 (2/7)	0.41	7	71.45 (5/7)	1.00
Moderately	18	55.6 (10/18)		18	66.7 (12/18)	
Poorly	4	25.0 (1/4)		4	75.0 (3/4)	
TNM staging						
Ⅰ-Ⅱ	20	35.0 (7/20)	0.23	20	70.0 (14/20)	1.00
Ⅲ-Ⅳ	9	66.7 (6/9)		9	66.7 (6/9)	
AFP						
≥ 200 ng/mL	7	42.9 (3/7)	1.00	7	71.4 (5/7)	1.00
< 200 ng/mL	22	45.5 (10/22)		22	68.2 (15/22)	

AFP: α-fetoprotein.

molecular weight. The prediction of SUMOplot tool identified two consensus sequence of SUMOylation with high probability and four sites with low probability in PRDX1 (Figure 4A). The molecular weight of SUMO1 is near 12 kDa, thus we supposed that the higher molecular weight bands of PRDX1 were due to its SUMOylation.

PRDX1 might be sumoylated in liver cancer cells

To investigate whether PRDX1 might be sumoylated, we determined the colocalization of PRDX1 and SUMO1 in three liver cancer cells, HepG2, Hep3B, and SK-HEP-1. We found that green endogenous PRDX1 was partially co-localized with the red SUMO1 molecules in the cytoplasm, according to immunofluorescence and confocal microscopy analysis (Figure 4B).

Furthermore, a co-immunoprecipitation assay was performed. HepG2 cells were lysed in the presence of N-ethylmaleimide, which inhibits SUMO-specific proteases, and were immunoprecipitated with an anti-PRDX1 or anti-SUMO1 antibody. As shown in Figure 4C, when the anti-SUMO1 antibody was used to precipitate SUMO1 interacting proteins, compared with non-immune IgG control, the theoretical and higher molecular weight bands of PRDX1 could be detected. Conversely, when the PRDX1 and its interacting

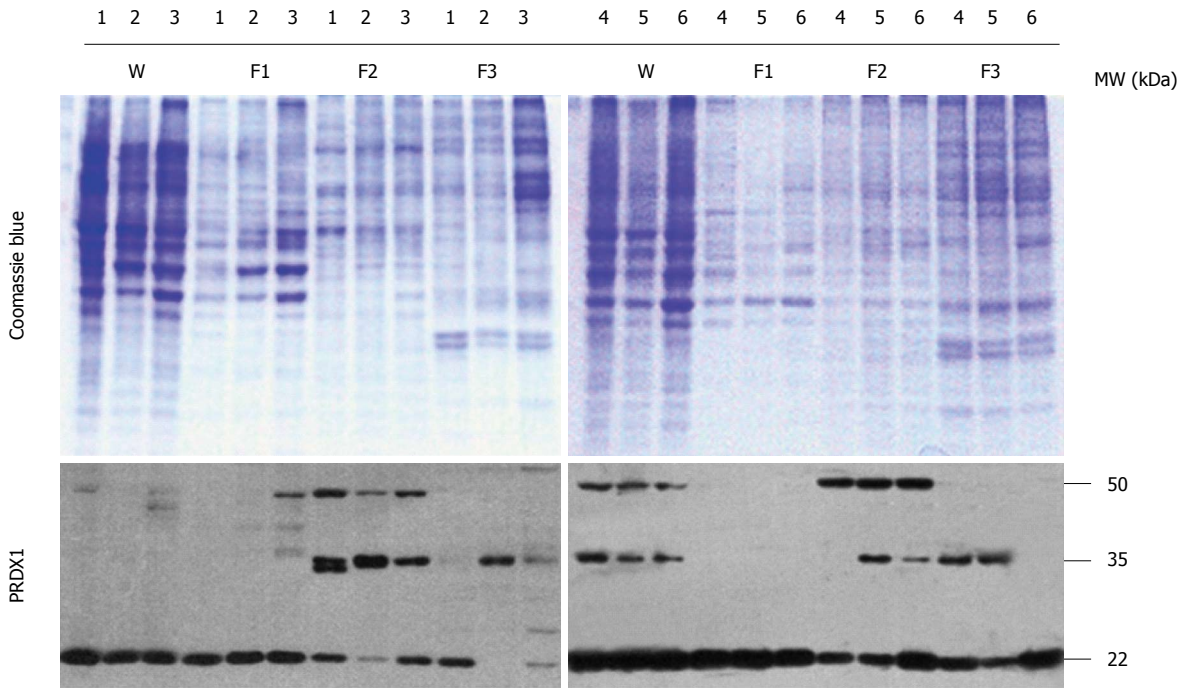


Figure 2 Western blotting analysis of peroxiredoxin 1 protein in liver cancer cells. The codes of liver cancer cells: 1: HepG2; 2: Hep3B; 3: SK-HEP-1; 4: Bel-7404; 5: SMMC-7721; 6: HLE. W: Whole lysates; F1: Cytosol fraction; F2: Membrane/organelle fraction; F3: Nucleus fraction. The upper panel is the Coomassie Blue stained SDS-PAGE gel, and the lower panel is the Western blotting of PRDX1. PRDX1: Peroxiredoxin 1.

proteins were enriched by anti-PRDX1 antibody, a weak band, about 50 kDa, was also recognized by anti-SUMO1 antibody (Figure 4D). Unfortunately, due to the low molecular weight characteristics of SUMO1, it was not detected in our Western blot system. These results indicated that PRDX1 and SUMO1 interacted with one another and suggested that PRDX1 might undergo SUMOylation in liver cancer cells.

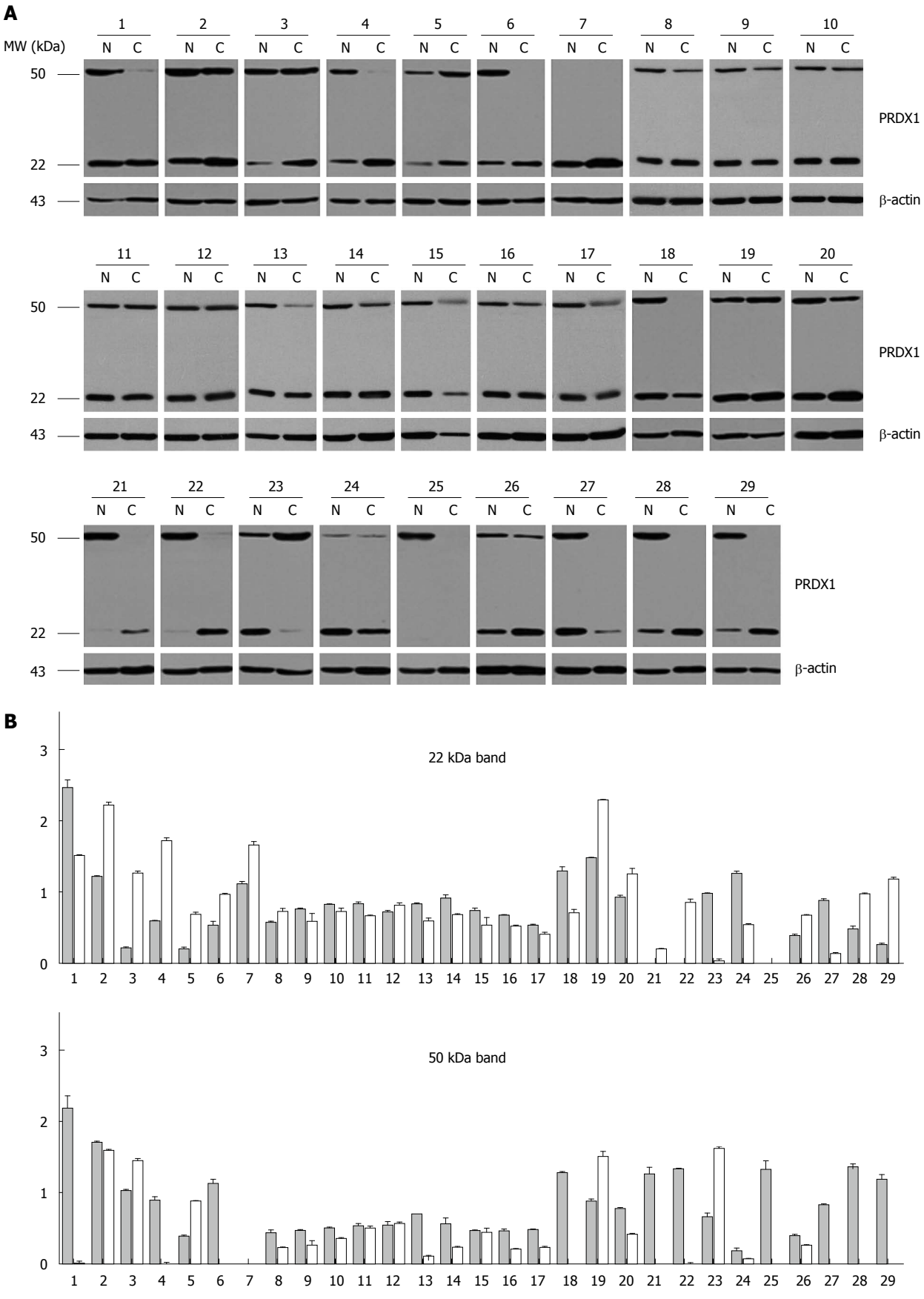
DISCUSSION

In the present study, we found *PRDX1* mRNA was upregulated in the tumor tissues of liver cancer in a large sample size. Increased *PRDX1* was associated with male gender and shorter overall survival. Western blotting revealed that PRDX1 had two bands on SDS-PAGE gel (about 35 and 50 kDa) that were higher molecular weight than the theoretical molecular weight (22 kDa). These higher molecular weight bands mainly existed in the membrane/organelle and nuclear fractions. Subsequent immunofluorescence and co-immunoprecipitation assays hinted that the higher molecular weight bands might be due to SUMOylation of PRDX1.

In addition to its peroxide detoxifying function, PRDX1 interacts and regulates the activity of several vital proteins. For example, PRDX1 can bind the SH3 domain of c-Abl, Myc Box Ⅱ domain of c-Myc, C2 domain of PTEN, androgen receptor, apoptosis signal-regulating kinase 1, mammalian ste20-like kinase 1 to modulate their activities[13,17,27,31-34]. Moreover, PRDX1 promotes tumor development and progression through Toll-like receptor 4 and mammalian target of rapamycin/p70S6K pathways and tumor growth factor β1-induced epithelial-mesenchymal transition[14,15,35]. Meanwhile, PRDX1 can act as a chaperone to enhance the transactivation potential of NF-κB in estrogen receptor negative breast cancer cells[36]. Recently, PRDX1 was found to bind RNA and serve as a transcription anti-terminator to enhance the survival of cells exposed to cold stress[37].

In tumors, hypoxia or ROS can induce the expression of PRDX1[38-41]. Although PRDX1 was shown to be overexpressed in most of tumors, because of its complex functions, its clinical significance was dependent on tumor type. For example, PRDX1 was a favorable prognostic factor in esophageal squamous cell carcinoma, breast cancer, bladder cancer, and cholangiocarcinoma[42-45], whereas an opposite role was attributed to PRDX1 in squamous cell carcinoma of the tongue, gallbladder cancer, ovarian serous carcinomas, lung cancer, pancreatic cancer, and liver cancer[18-22,29].

Our study confirmed that *PRDX1* mRNA was upregulated in liver cancer tumor tissues, and its high levels were associated with shorter overall survival time. However, according to our large sample size, we did not observe the correlation between *PRDX1* and the recurrence-free survival of patients, as reported previously[29]. Moreover, the expression of *PRDX1* was higher in male patients. A previous study also found that expression levels of *PRDX1* were relatively high in hepatitis C virus-related hepatocellular carcinoma samples from men[46], concordant with our results. It was known that liver cancer has a high male-to-


A
MW (kDa)
1 2 3 4 5 6 7 8 9 10
N C
50
22
43
PRDX1
β-actin
11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 27 28 29
B
22 kDa band
50 kDa band
0 1 2 3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

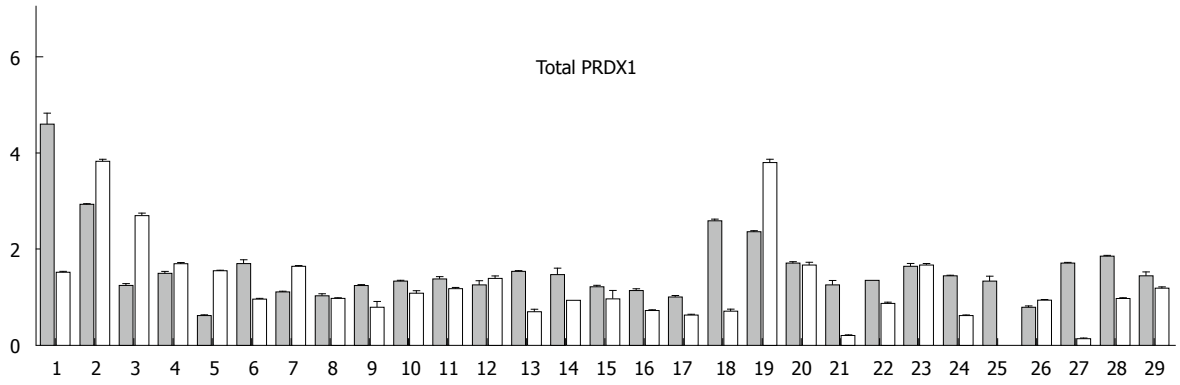


Figure 3 Expression of peroxiredoxin 1 in human liver cancer specimens. A: Western blot analysis of tumor (C) and matching adjacent non-tumor liver tissues (N) of 29 patients. β-actin protein levels are shown as a loading control. The patients were coded from 1 to 29; B: Densitometric analysis of 29 hepatocellular carcinoma cases. The black and gray bars represent the relative band intensity of peroxiredoxin 1 (PRDX1) in non-tumor or tumor tissues, showing the ratio between 22 kDa, 50 kDa or total PRDX1 and β-actin. Each data point represents the mean ± SD derived from three independent experiments.

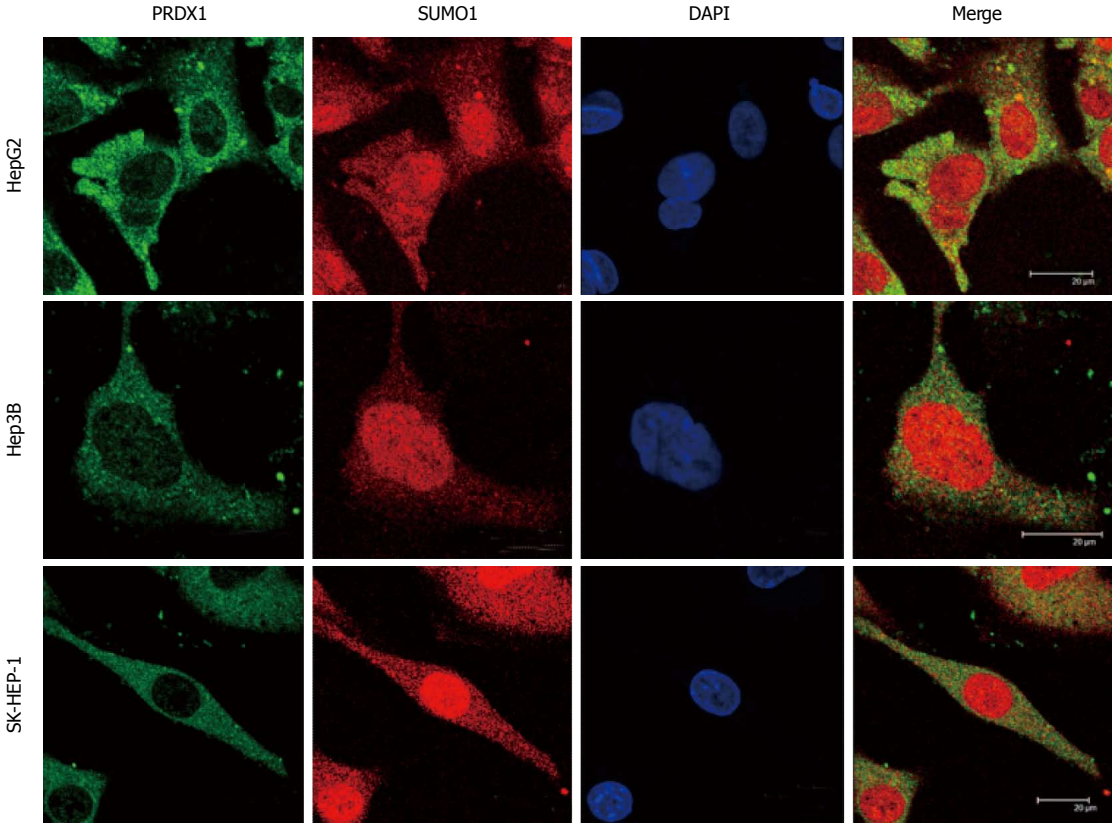
A

1 MSSGNAKIGH PAPNFKATAV MPDGQFKDIS LSDYKGKYVV FFFYPLDFTF
51 VCPTEIIAFS DRAEEFKKLN CQVIGASVDS HFCHLAWVNT PKKQGGLGPM
101 NIPLVSDPKR TIAQDYGVLK ADEGISFRGL FIIDDKGILR QITVNDLPVG
151 RSVDETLRLV QAFQFTDKHG EVCPAGWKPG SDTIKPDYQK SKEYFSKQK

Motifs with high probability
Motifs with low probability
Overlapping Motifs

No.	Pos.	Group	Score	No.	Pos.	Group	Score
1	K185	PGSDT **IKPD** VQKSK	0.94	4	K178	VCPAG **WKPG** SDTIK	0.47
2	K120	QDYGV **LKAD** EGISF	0.91	5	K168	AFQFT **DKHG** EVCPA	0.33
3	K7	MSSGN **AKIG** HPAPN	0.62	6	K93	WVNTP **KKQG** GLGPM	0.31

B



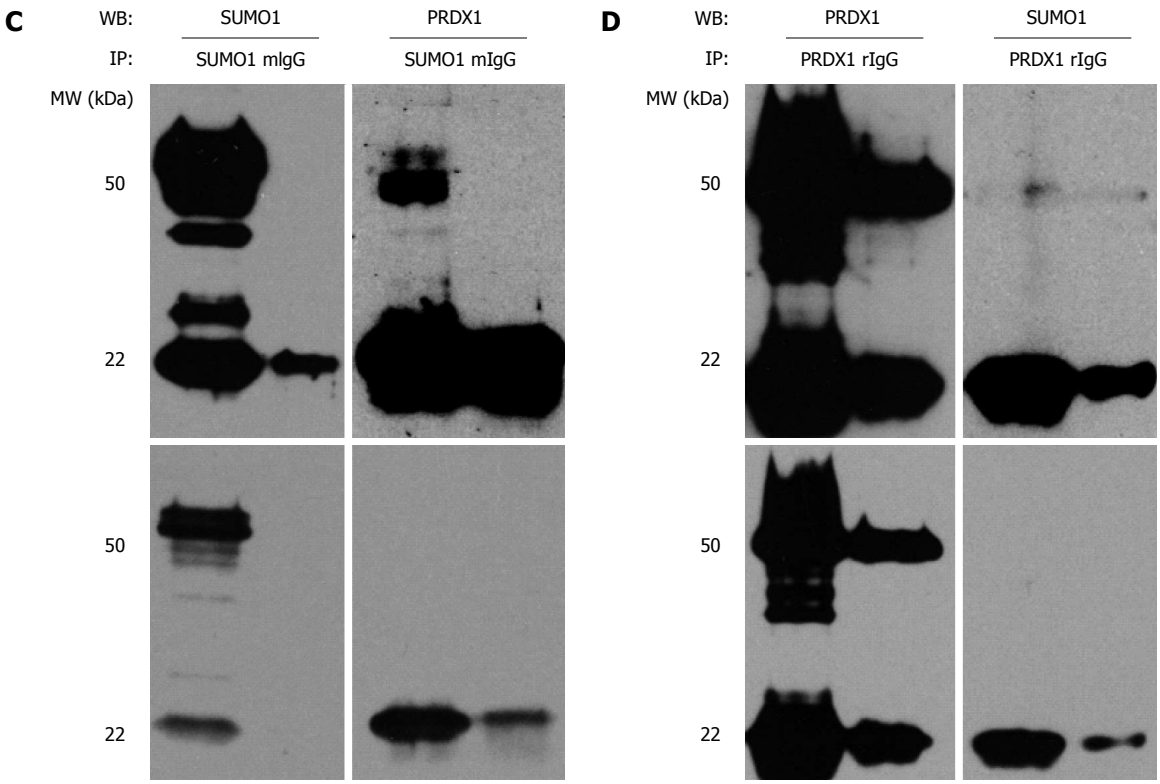


Figure 4 Peroxiredoxin 1 might be sumoylated in liver cancer cells. A: The bioinformatic prediction of PRDX1 using SUMOplot tool; B: Immunofluorescence staining visualized under a confocal microscope illustrating the co-localization of PRDX1 and SUMO1 proteins in the cytoplasm of three liver cancer cells. C, D: Co-immunoprecipitation of PRDX1 with SUMO1 in HepG2 cell extract; C: Lysates were subjected to immunoprecipitation (IP) with anti-SUMO1 antibody, followed by Western blotting (WB) with anti-PRDX1 and anti-SUMO1 to detect sumoylated PRDX1; D: Lysates were subjected to IP with anti-PRDX1 antibody, followed by WB with anti-SUMO1 and anti-PRDX1 to detect sumoylated PRDX1. The upper and lower panels are the results of dark and light exposure by Western blotting.

female incidence rate ratio of 2-4:1[1], and men with liver cancer tend to have a more invasive phenotype and shorter survival[47]. Thus, the prognostic value of *PRDX1* mRNA might relate to the gender-disparity of liver cancer.

At the protein level, we found that PRDX1 had three different molecular weight forms in liver cancer cells, and the higher molecular weight bands were mainly distributed in the cell organelles and nucleus. They were also detected in the tissue lysates. Meanwhile, the higher molecular weight bands were downregulated in tumor tissues, whereas the theoretical molecular weight band was upregulated. Our Western blot system is reducing SDS-PAGE including 10% β-mercaptoethanol; therefore, the dimer cannot explain the phenomenon. We predicted that the shift in molecular weight was due to a covalent modification rather than a disulfide bond for PRDX1 in liver cancer. To support this hypothesis, we performed the bioinformatic prediction, immunofluorescence, and co-immunoprecipitation assay, and the putative modification forms of PRDX1 by SUMO were confirmed.

SUMOs are ubiquitin-like polypeptides that covalently conjugate to proteins in an ATP-dependent enzymatic cascade that resembles ubiquitylation[48]. Hundreds of proteins can be modified by SUMOs, including oxidative stress-related proteins. Hydrogen peroxide enhances the global protein SUMO conjugation profile and induces a reversible blockade of SUMO proteases sentrin-specific protease 1[49,50]. ROS induces a rapid de-SUMOylation of transcription factors c-Fos and c-Jun, resulting in stimulation of their activity and activation of numerous anti-oxidant proteins[51,52]. Thus, it seems that SUMOylation is a fine sensor for ROS and participates in anti-oxidative responses and ROS-dependent cell death[51].

It is known that the activity of PRDX1 can be regulated by some post-translational modifications. For example, phosphorylation of PRDX1 on Thr90 or Ser32 reduced its peroxidase activity[53-55]. Acetylation of PRDX1 on Lys197 increased its reducing activity[56]. Glutathionylation of PRDX1 at Cys52, Cys83, and Cys173 inactivated its molecular chaperone function[57]. Similarly, consequences of SUMOylation can also modulate the functions and activities of target proteins. It is widely accepted that SUMOylation can mask the interaction surface, induce conformational changes, and create SUMO-dependent interaction with downstream effectors[48]. However, due to low steady-state levels of endogenous protein modification and isopeptidase activity in nondenaturing lysates, detection and analysis of SUMOylation are challenged. Therefore, the SUMOylation of PRDXs had not been reported yet. To increase the visibility of sumoylated PRDX1, we added 10 mmol/L N-ethylmaleimide to our cell lysate buffer to stable SUMO conjugates,

leading to the discovery of the higher molecular weight bands of PRDX1. It was known that PRDX1 exhibits both nuclear and cytoplasmic localization in cells[58]. However, PRDX1 has no nuclear localization signals (NLS) as predicted by PredictNLS (https://www.predictprotein.org/) and cNLS Mapper (http://nls-mapper.iab.keio.ac.jp/) bioinformatics tools. One of the roles of SUMOylation is as a molecular switch to control the nuclear localization[59]; therefore, the SUMOylation of PRDX1 might provide a possible mechanism to localize PRDX1 to the nucleus. Furthermore, the downregulation of sumoylated PRDX1 might be involved in hepatocarcinogenesis. The confirmation and functions of sumoylated PRDX1 are continuing to be explored in our laboratory.

In conclusion, our results demonstrated upregulation of *PRDX1* mRNA in liver cancer is an independent poor prognostic factor for overall survival. PRDX1 protein might modified by SUMO in liver cancer cells to form higher molecular weight bands. The sumoylated PRDX1 protein was downregulated in tumor tissues, suggesting its specific functions may be distinct from the un-modified forms in hepatocarcinogenesis. Overall, PRDX1 acts as an "oncogene" in liver cancer cells. It may be a useful prognostic marker and a promising molecular target for the therapeutic intervention of liver cancer.

COMMENTS

Background

Liver cancer is the second leading cause of cancer death worldwide. The exposures to common etiological factors generally lead to extensive oxidative stress and the promotion of hepatocarcinogenesis *via* oxidative DNA, protein, and lipid damage. In normal cells, several antioxidant enzymes and non-enzymatic molecules participate in the detoxification of intracellular reactive oxygen species. Among them, peroxiredoxin 1 (PRDX1) catalyzes the reduction of hydrogen peroxide, organic hydroperoxides, and peroxynitrite.

Research frontiers

As a major hydroperoxide scavenging enzyme in cytoplasm, PRDX1 is considered a tumor suppressor gene. However, PRDX1 recently was found to act in a manner independent of its anti-oxidative function. It also regulates cell proliferation, differentiation, apoptosis, migration, angiogenesis, and radio-chemosensitivity. Therefore, PRDX1 plays a dual role in tumorigenesis.

Innovations and breakthroughs

In liver cancer, the overexpression of PRDX1 had been observed in limited clinical specimens. In this study, the authors investigated the expression characteristics of PRDX1 mRNA and protein in liver cancer using RNA sequencing dataset and Western blotting. In addition, the subcellular distribution and a putative post-translational modification form of PRDX1 were explored.

Applications

PRDX1 was overexpressed in the tumor tissues of liver cancer and was shown to serve as an independent unfavorable prognostic factor for overall survival. PRDX1 protein might be modified by small ubiquitin-like modifier (SUMO) in liver cancer cells to form higher molecular weight isoforms. PRDX1 may be a useful prognostic marker for liver cancer, and it is a promising molecular target for the therapeutic intervention of liver cancer.

Terminology

PRDXs use thioredoxin as the electron donor to catalyze the reduction of hydrogen peroxide, organic hydroperoxides, and peroxynitrite. Mammalian cells express six isoforms of PRDXs, which are classified into three subfamilies based on the location or absence of the essential catalytic cysteine residue. PRDX1 is the most abundant and ubiquitously distributed isoform.

Peer-review

This manuscript is well written and suggests that PRDX1 overexpressed in the tumor tissue of liver cancer may be a poor prognostic factor for overall survival. In addition, it might be modified by SUMO to play specific roles in hepatocarcinogenesis. However, as the authors point out, SUMOylation of PRDX1 was not confirmed and its function in hepatocarcinogenesis remains unclear. Further studies are needed to confirm this idea.

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 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]

3 **Kumar M**, Zhao X, Wang XW. Molecular carcinogenesis of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: one step closer to personalized medicine? *Cell Biosci* 2011; **1**: 5 [PMID: 21711594 DOI: 10.1186/2045-3701-1-5]

4 **Liou GY**, Storz P. Reactive oxygen species in cancer. *Free Radic Res* 2010; **44**: 479-496 [PMID: 20370557 DOI: 10.3109/10715761003667554]

5 **Rhee SG**, Chae HZ, Kim K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* 2005; **38**: 1543-1552 [PMID: 15917183 DOI: 10.1016/j.freeradbiomed.2005.02.026]

6 **Rhee SG**. Cell signaling. H2O2, a necessary evil for cell signaling. *Science* 2006; **312**: 1882-1883 [PMID: 16809515 DOI: 10.1126/science.1130481]

7 **Rhee SG**, Woo HA, Kil IS, Bae SH. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. *J Biol Chem* 2012; **287**: 4403-4410 [PMID: 22147704 DOI: 10.1074/jbc.R111.283432]

8 **Tonks NK**. Redox redux: revisiting PTPs and the control of cell signaling. *Cell* 2005; **121**: 667-670 [PMID: 15935753 DOI: 10.1016/j.cell.2005.05.016]

9 **D'Autréaux B**, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 2007; **8**: 813-824 [PMID: 17848967 DOI: 10.1038/nrm2256]

10 **Neumann CA**, Cao J, Manevich Y. Peroxiredoxin 1 and its role in cell signaling. *Cell Cycle* 2009; **8**: 4072-4078 [PMID: 19923889]

11 **Jarvis RM**, Hughes SM, Ledgerwood EC. Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. *Free Radic Biol Med* 2012; **53**: 1522-1530 [PMID: 22902630 DOI: 10.1016/j.freeradbiomed.2012.08.001]

12 **Turner-Ivey B**, Manevich Y, Schulte J, Kistner-Griffin E, Jezierska-Drutel A, Liu Y, Neumann CA. Role for Prdx1 as a specific sensor in redox-regulated senescence in breast cancer. *Oncogene* 2013; **32**: 5302-5314 [PMID: 23334324 DOI: 10.1038/onc.2012.624]

13 **Morinaka A**, Funato Y, Uesugi K, Miki H. Oligomeric peroxiredoxin-I is an essential intermediate for p53 to activate MST1 kinase and apoptosis. *Oncogene* 2011; **30**: 4208-4218 [PMID: 21516123 DOI: 10.1038/onc.2011.139]

14 **Riddell JR**, Bshara W, Moser MT, Spernyak JA, Foster BA, Gollnick SO. Peroxiredoxin 1 controls prostate cancer growth through Toll-like receptor 4-dependent regulation of tumor vasculature. *Cancer Res* 2011; **71**: 1637-1646 [PMID: 21343392 DOI: 10.1158/0008-5472.CAN-10-3674]

15 **Gong F**, Hou G, Liu H, Zhang M. Peroxiredoxin 1 promotes tumorigenesis through regulating the activity of mTOR/p70S6K

pathway in esophageal squamous cell carcinoma. *Med Oncol* 2015; **32**: 455 [PMID: 25579166 DOI: 10.1007/s12032-014-0455-0]
16 **Neumann CA**, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH, Van Etten RA. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 2003; **424**: 561-565 [PMID: 12891360 DOI: 10.1038/nature01819nature01819]
17 **Cao J**, Schulte J, Knight A, Leslie NR, Zagozdzon A, Bronson R, Manevich Y, Beeson C, Neumann CA. Prdx1 inhibits tumorigenesis via regulating PTEN/AKT activity. *EMBO J* 2009; **28**: 1505-1517 [PMID: 19369943]
18 **Yanagawa T**, Omura K, Harada H, Ishii T, Uwayama J, Nakaso K, Iwasa S, Koyama Y, Onizawa K, Yusa H, Yoshida H. Peroxiredoxin I expression in tongue squamous cell carcinomas as involved in tumor recurrence. *Int J Oral Maxillofac Surg* 2005; **34**: 915-920 [PMID: 15955662]
19 **Cai CY**, Zhai LL, Wu Y, Tang ZG. Expression and clinical value of peroxiredoxin-1 in patients with pancreatic cancer. *Eur J Surg Oncol* 2015; **41**: 228-235 [PMID: 25434328 DOI: 10.1016/j.ejso.2014.11.037]
20 **Li J**, Yang ZL, Ren X, Zou Q, Yuan Y, Liang L, Chen M, Chen S. ILK and PRDX1 are prognostic markers in squamous cell/adenosquamous carcinomas and adenocarcinoma of gallbladder. *Tumour Biol* 2013; **34**: 359-368 [PMID: 23065574 DOI: 10.1007/s13277-012-0557-2]
21 **Chung KH**, Lee DH, Kim Y, Kim TH, Huh JH, Chung SG, Lee S, Lee C, Ko JJ, An HJ. Proteomic identification of overexpressed PRDX 1 and its clinical implications in ovarian carcinoma. *J Proteome Res* 2010; **9**: 451-457 [PMID: 19902980 DOI: 10.1021/pr900811x]
22 **Kim JH**, Bogner PN, Baek SH, Ramnath N, Liang P, Kim HR, Andrews C, Park YM. Up-regulation of peroxiredoxin 1 in lung cancer and its implication as a prognostic and therapeutic target. *Clin Cancer Res* 2008; **14**: 2326-2333 [PMID: 18413821 DOI: 10.1158/1078-0432.CCR-07-4457]
23 **Dittmann LM**, Danner A, Gronych J, Wolter M, Stühler K, Grzendowski M, Becker N, Bageritz J, Goidts V, Toedt G, Felsberg J, Sabel MC, Barbus S, Reifenberger G, Lichter P, Tews B. Downregulation of PRDX1 by promoter hypermethylation is frequent in 1p/19q-deleted oligodendroglial tumours and increases radio- and chemosensitivity of Hs683 glioma cells in vitro. *Oncogene* 2012; **31**: 3409-3418 [PMID: 22158042 DOI: 10.1038/onc.2011.513]
24 **Hwang KE**, Park DS, Kim YS, Kim BR, Park SN, Lee MK, Park SH, Yoon KH, Jeong ET, Kim HR. Prx1 modulates the chemosensitivity of lung cancer to docetaxel through suppression of FOXO1-induced apoptosis. *Int J Oncol* 2013; **43**: 72-78 [PMID: 23615915 DOI: 10.3892/ijo.2013.1918]
25 **Poschmann G**, Grzendowski M, Stefanski A, Bruns E, Meyer HE, Stühler K. Redox proteomics reveal stress responsive proteins linking peroxiredoxin-1 status in glioma to chemosensitivity and oxidative stress. *Biochim Biophys Acta* 2015; **1854**: 624-631 [PMID: 25484280 DOI: 10.1016/j.bbapap.2014.11.011]
26 **Li G**, Xie B, Li X, Chen Y, Xu Y, Xu-Welliver M, Zou L. Downregulation of peroxiredoxin-1 by β-elemene enhances the radiosensitivity of lung adenocarcinoma xenografts. *Oncol Rep* 2015; **33**: 1427-1433 [PMID: 25607351 DOI: 10.3892/or.2015.3732]
27 **Park SY**, Yu X, Ip C, Mohler JL, Bogner PN, Park YM. Peroxiredoxin 1 interacts with androgen receptor and enhances its transactivation. *Cancer Res* 2007; **67**: 9294-9303 [PMID: 17909037]
28 **Song IS**, Kim SU, Oh NS, Kim J, Yu DY, Huang SM, Kim JM, Lee DS, Kim NS. Peroxiredoxin I contributes to TRAIL resistance through suppression of redox-sensitive caspase activation in human hepatoma cells. *Carcinogenesis* 2009; **30**: 1106-1114 [PMID: 19406930 DOI: 10.1093/carcin/bgp104]
29 **Sun QK**, Zhu JY, Wang W, Lv Y, Zhou HC, Yu JH, Xu GL, Ma JL, Zhong W, Jia WD. Diagnostic and prognostic significance of peroxiredoxin 1 expression in human hepatocellular carcinoma. *Med Oncol* 2014; **31**: 786 [PMID: 24297309 DOI: 10.1007/s12032-013-0786-2]
30 **Aguilar-Melero P**, Prieto-Álamo MJ, Jurado J, Holmgren A, Pueyo C. Proteomics in HepG2 hepatocarcinoma cells with stably silenced expression of PRDX1. *J Proteomics* 2013; **79**: 161-171 [PMID: 23277276 DOI: 10.1016/j.jprot.2012.12.005]
31 **Jin DY**, Chae HZ, Rhee SG, Jeang KT. Regulatory role for a novel human thioredoxin peroxidase in NF-kappaB activation. *J Biol Chem* 1997; **272**: 30952-30961 [PMID: 9388242]
32 **Mu ZM**, Yin XY, Prochownik EV. Pag, a putative tumor suppressor, interacts with the Myc Box II domain of c-Myc and selectively alters its biological function and target gene expression. *J Biol Chem* 2002; **277**: 43175-43184 [PMID: 12196529 DOI: 10.1074/jbc.M206066200M206066200]
33 **Chhipa RR**, Lee KS, Onate S, Wu Y, Ip C. Prx1 enhances androgen receptor function in prostate cancer cells by increasing receptor affinity to dihydrotestosterone. *Mol Cancer Res* 2009; **7**: 1543-1552 [PMID: 19737972]
34 **Kim SY**, Kim TJ, Lee KY. A novel function of peroxiredoxin 1 (Prx-1) in apoptosis signal-regulating kinase 1 (ASK1)-mediated signaling pathway. *FEBS Lett* 2008; **582**: 1913-1918 [PMID: 18501712]
35 **Ha B**, Kim EK, Kim JH, Lee HN, Lee KO, Lee SY, Jang HH. Human peroxiredoxin 1 modulates TGF-β1-induced epithelial-mesenchymal transition through its peroxidase activity. *Biochem Biophys Res Commun* 2012; **421**: 33-37 [PMID: 22475482 DOI: 10.1016/j.bbrc.2012.03.103]
36 **Wang X**, He S, Sun JM, Delcuve GP, Davie JR. Selective association of peroxiredoxin 1 with genomic DNA and COX-2 upstream promoter elements in estrogen receptor negative breast cancer cells. *Mol Biol Cell* 2010; **21**: 2987-2995 [PMID: 20631257]
37 **Kim JH**, Lee JM, Lee HN, Kim EK, Ha B, Ahn SM, Jang HH, Lee SY. RNA-binding properties and RNA chaperone activity of human peroxiredoxin 1. *Biochem Biophys Res Commun* 2012; **425**: 730-734 [PMID: 22877757 DOI: 10.1016/j.bbrc.2012.07.142]
38 **Kim HJ**, Chae HZ, Kim YJ, Kim YH, Hwangs TS, Park EM, Park YM. Preferential elevation of Prx I and Trx expression in lung cancer cells following hypoxia and in human lung cancer tissues. *Cell Biol Toxicol* 2003; **19**: 285-298 [PMID: 14703116]
39 **Kim YJ**, Ahn JY, Liang P, Ip C, Zhang Y, Park YM. Human prx1 gene is a target of Nrf2 and is up-regulated by hypoxia/reoxygenation: implication to tumor biology. *Cancer Res* 2007; **67**: 546-554 [PMID: 17234762 DOI: 10.1158/0008-5472.CAN-06-2401]
40 **Zhang M**, Hou M, Ge L, Miao C, Zhang J, Jing X, Shi N, Chen T, Tang X. Induction of peroxiredoxin 1 by hypoxia regulates heme oxygenase-1 via NF-κB in oral cancer. *PLoS One* 2014; **9**: e105994 [PMID: 25162226 DOI: 10.1371/journal.pone.0105994]
41 **Shiota M**, Izumi H, Miyamoto N, Onitsuka T, Kashiwagi E, Kidani A, Hirano G, Takahashi M, Ono M, Kuwano M, Naito S, Sasaguri Y, Kohno K. Ets regulates peroxiredoxin1 and 5 expressions through their interaction with the high-mobility group protein B1. *Cancer Sci* 2008; **99**: 1950-1959 [PMID: 19016754]
42 **Hoshino I**, Matsubara H, Akutsu Y, Nishimori T, Yoneyama Y, Murakami K, Sakata H, Matsushita K, Ochiai T. Tumor suppressor Prdx1 is a prognostic factor in esophageal squamous cell carcinoma patients. *Oncol Rep* 2007; **18**: 867-871 [PMID: 17786348]
43 **O'Leary PC**, Terrile M, Bajor M, Gaj P, Hennessy BT, Mills GB, Zagozdzon A, O'Connor DP, Brennan DJ, Connor K, Li J, Gonzalez-Angulo AM, Sun HD, Pu JX, Pontén F, Uhlén M, Jirström K, Nowis DA, Crown JP, Zagozdzon R, Gallagher WM. Peroxiredoxin-1 protects estrogen receptor α from oxidative stress-induced suppression and is a protein biomarker of favorable prognosis in breast cancer. *Breast Cancer Res* 2014; **16**: R79 [PMID: 25011585 DOI: 10.1186/bcr3691]
44 **Quan C**, Cha EJ, Lee HL, Han KH, Lee KM, Kim WJ. Enhanced expression of peroxiredoxin I and VI correlates with development, recurrence and progression of human bladder cancer. *J Urol* 2006; **175**: 1512-1516 [PMID: 16516038 DOI: 10.1016/S0022-5347(05)00659-2]
45 **Yonglitthipagon P**, Pairojkul C, Chamgramol Y, Loukas A, Mulvenna J, Bethony J, Bhudhisawasdi V, Sripa B. Prognostic significance of peroxiredoxin 1 and ezrin-radixin-moesin-

binding phosphoprotein 50 in cholangiocarcinoma. *Hum Pathol* 2012; **43**: 1719-1730 [PMID: 22446018 DOI: 10.1016/j.humpath.2011.11.021]
46 **Takemoto N**, Iizuka N, Yamada-Okabe H, Hamada K, Tamesa T, Okada T, Hashimoto K, Sakamoto K, Takashima M, Miyamoto T, Uchimura S, Hamamoto Y, Oka M. Sex-based molecular profiling of hepatitis C virus-related hepatocellular carcinoma. *Int J Oncol* 2005; **26**: 673-678 [PMID: 15703822]
47 **Dohmen K**, Shigematsu H, Irie K, Ishibashi H. Longer survival in female than male with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2003; **18**: 267-272 [PMID: 12603526]
48 **Flotho A**, Melchior F. Sumoylation: a regulatory protein modification in health and disease. *Annu Rev Biochem* 2013; **82**: 357-385 [PMID: 23746258 DOI: 10.1146/annurev-biochem-061909-093311]
49 **Xu Z**, Lam LS, Lam LH, Chau SF, Ng TB, Au SW. Molecular basis of the redox regulation of SUMO proteases: a protective mechanism of intermolecular disulfide linkage against irreversible sulfhydryl oxidation. *FASEB J* 2008; **22**: 127-137 [PMID: 17704192 DOI: 10.1096/fj.06-7871com]
50 **Bossis G**, Melchior F. Regulation of SUMOylation by reversible oxidation of SUMO conjugating enzymes. *Mol Cell* 2006; **21**: 349-357 [PMID: 16455490 DOI: 10.1016/j.molcel.2005.12.019]
51 **Feligioni M**, Nisticò R. SUMO: a (oxidative) stressed protein. *Neuromolecular Med* 2013; **15**: 707-719 [PMID: 24052421 DOI: 10.1007/s12017-013-8266-6]
52 **Bossis G**, Malnou CE, Farras R, Andermarcher E, Hipskind R, Rodriguez M, Schmidt D, Muller S, Jariel-Encontre I, Piechaczyk M. Down-regulation of c-Fos/c-Jun AP-1 dimer activity by sumoylation. *Mol Cell Biol* 2005; **25**: 6964-6979 [PMID: 16055710 DOI: 10.1128/MCB.25.16.6964-6979.2005]
53 **Chang TS**, Jeong W, Choi SY, Yu S, Kang SW, Rhee SG. Regulation of peroxiredoxin I activity by Cdc2-mediated phosphorylation. *J Biol Chem* 2002; **277**: 25370-25376 [PMID: 11986303 DOI: 10.1074/jbc.M110432200M110432200]
54 **Zykova TA**, Zhu F, Vakorina TI, Zhang J, Higgins LA, Urusova DV, Bode AM, Dong Z. T-LAK cell-originated protein kinase (TOPK) phosphorylation of Prx1 at Ser-32 prevents UVB-induced apoptosis in RPMI7951 melanoma cells through the regulation of Prx1 peroxidase activity. *J Biol Chem* 2010; **285**: 29138-29146 [PMID: 20647304]
55 **Rawat SJ**, Creasy CL, Peterson JR, Chernoff J. The tumor suppressor Mst1 promotes changes in the cellular redox state by phosphorylation and inactivation of peroxiredoxin-1 protein. *J Biol Chem* 2013; **288**: 8762-8771 [PMID: 23386615 DOI: 10.1074/jbc.M112.414524]
56 **Parmigiani RB**, Xu WS, Venta-Perez G, Erdjument-Bromage H, Yaneva M, Tempst P, Marks PA. HDAC6 is a specific deacetylase of peroxiredoxins and is involved in redox regulation. *Proc Natl Acad Sci USA* 2008; **105**: 9633-9638 [PMID: 18606987]
57 **Park JW**, Piszczek G, Rhee SG, Chock PB. Glutathionylation of peroxiredoxin I induces decamer to dimers dissociation with concomitant loss of chaperone activity. *Biochemistry* 2011; **50**: 3204-3210 [PMID: 21401077 DOI: 10.1021/bi101373h]
58 **Wen ST**, Van Etten RA. The PAG gene product, a stress-induced protein with antioxidant properties, is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity. *Genes Dev* 1997; **11**: 2456-2467 [PMID: 9334312]
59 **Liu GH**, Gerace L. Sumoylation regulates nuclear localization of lipin-1alpha in neuronal cells. *PLoS One* 2009; **4**: e7031 [PMID: 19753306 DOI: 10.1371/journal.pone.0007031]

P- Reviewer: Yu DY **S- Editor**: Ma YJ
L- Editor: Filipodia **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10853

World J Gastroenterol 2015 October 14; 21(38): 10853-10865
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Dimethyl sulfoxide inhibits zymosan-induced intestinal inflammation and barrier dysfunction

Yu-Meng Li, Hai-Bin Wang, Jin-Guang Zheng, Xiao-Dong Bai, Zeng-Kai Zhao, Jing-Yuan Li, Sen Hu

Yu-Meng Li, Jin-Guang Zheng, Zeng-Kai Zhao, Jing-Yuan Li, Sen Hu, Laboratory for Shock and Multiple Organ Dysfunction of Burns Institute, Key Research Laboratory of Tissue Repair and Regeneration of PLA, and Beijing Key Research Laboratory of Skin Injury and Repair Regeneration, the First Hospital Affiliated to the Chinese PLA General Hospital, Beijing 100048, China

Yu-Meng Li, Medical School of Chinese PLA, Beijing 100853, China

Hai-Bin Wang, Department of Clinical Laboratory, The First Hospital Affiliated to the Chinese PLA General Hospital, Beijing 100048, China

Jin-Guang Zheng, Xiao-Dong Bai, Department of Burn Surgery, the General Hospital of Armed Police Forces, Beijing 100039, China

Zeng-Kai Zhao, Beijing Tongzhou Hospital of Traditional Chinese Medicine, Beijing 101100, China

Author contributions: Li YM and Wang HB contributed equally to this study; Li YM, Zheng JG, Wang HB Bai XD and Hu S designed the research; Li YM, Zheng JG, Wang HB, Zhao ZK and Li JY performed the research; Li YM, Zheng JG and Hu S analyzed the data; Li YM, Wang HB, Bai XD and Hu S prepared and revised manuscript; all authors have read and approved the final manuscript.

Supported by National 11th Five-Year Plan of China for Military Medical Projects, No. 06Z055; and the National Natural Science Foundation of China, No. 81301607.

Institutional review board statement: The study was reviewed and approved by the First Hospital Affiliated to the Chinese PLA General Hospital Institutional Review Board.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the First Hospital Affiliated to the Chinese PLA General Hospital (IACUC protocol number: [11401500004432]). The experiments were conducted in compliance with the Guide for Care and Use of Laboratory Animals of the National Research Council, China.

Conflict-of-interest statement: All authors of this paper have read this manuscript; and no conflicts of interest exist in this study.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at [bs0425@163.com]. Participants gave informed consent for data sharing. Besides, no additional data are available.

Correspondence to: Sen Hu, MD, PhD, Professor, Laboratory for Shock and Multiple Organ Dysfunction of Burns Institute, Key Research Laboratory of Tissue Repair and Regeneration of PLA, and Beijing Key Research Laboratory of Skin Injury and Repair Regeneration, the First Hospital Affiliated to the Chinese PLA General Hospital, No. 51 Fu Cheng Road, Beijing 100048, China. bs0425@163.com
Telephone: +86-10-66867397
Fax: +86-10-68989139

Received: June 7, 2015
Peer-review started: June 8, 2015
First decision: July 10, 2015
Revised: July 25, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 14, 2015

Abstract

AIM: To investigate whether dimethyl sulfoxide (DMSO) inhibits gut inflammation and barrier dysfunction following zymosan-induced systemic inflammatory

response syndrome and multiple organ dysfunction syndrome.

METHODS: Sprague-Dawley rats were randomly divided into four groups: sham with administration of normal saline (SS group); sham with administration of DMSO (SD group); zymosan with administration of normal saline (ZS group); and zymosan with administration of DMSO (ZD group). Each group contained three subgroups according to 4 h, 8 h, and 24 h after surgery. At 4 h, 8 h, and 24 h after intraperitoneal injection of zymosan (750 mg/kg), the levels of intestinal inflammatory cytokines [tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-10] and oxides (myeloperoxidase, malonaldehyde, and superoxide dismutase) were examined. The levels of diamine oxidase (DAO) in plasma and intestinal mucosal blood flow (IMBF) were determined. Intestinal injury was also evaluated using an intestinal histological score and apoptosis of intestinal epithelial cells was determined by deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The intestinal epithelial tight junction protein, ZO-1, was observed by immunofluorescence.

RESULTS: DMSO decreased TNF-α and increased IL-10 levels in the intestine compared with the ZS group at the corresponding time points. The activity of intestinal myeloperoxidase in the ZS group was higher than that in the ZD group 24 h after zymosan administration ($P < 0.05$). DMSO decreased the content of malondialdehyde (MDA) and increased the activity of superoxide dehydrogenase (SOD) 24 h after zymosan administration. The IMBF was lowest at 24 h and was 49.34% and 58.26% in the ZS group and ZD group, respectively ($P < 0.05$). DMSO alleviated injury in intestinal villi, and the gut injury score was significantly lower than the ZS group (3.6 ± 0.2 *vs* 4.2 ± 0.3, $P < 0.05$). DMSO decreased the level of DAO in plasma compared with the ZS group (65.1 ± 4.7 U/L *vs* 81.1 ± 5.0 U/L, $P < 0.05$). DMSO significantly preserved ZO-1 protein expression and localization 24 h after zymosan administration. The TUNEL analysis indicated that the number of apoptotic intestinal cells in the ZS group was much higher than the ZD group ($P < 0.05$).

CONCLUSION: DMSO inhibited intestinal cytokines and protected against zymosan-induced gut barrier dysfunction.

Key words: Dimethyl sulfoxide; Zymosan; Inflammation; Intestinal barrier; Tight junction

Core tip: We examined whether the administration of dimethyl sulfoxide (DMSO) inhibited zymosan-induced intestinal inflammation and barrier dysfunction to provide an experimental basis for the use of DMSO in protecting intestinal barrier function. We found that DMSO can inhibit intestinal cytokines and protect against zymosan-induced gut barrier dysfunction.

Li YM, Wang HB, Zheng JG, Bai XD, Zhao ZK, Li JY, Hu S. Dimethyl sulfoxide inhibits zymosan-induced intestinal inflammation and barrier dysfunction. *World J Gastroenterol* 2015; 21(38): 10853-10865 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10853.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10853

INTRODUCTION

A large number of bacteria and viruses is found in the human intestine, and the intestinal mucosal barrier is the most important defense mechanism in the body. Intestinal mucosal barrier integrity can separate the luminal content from the body and prevent intestinal bacteria and endotoxin translocation. The intestinal mucosal barrier is composed of a mechanical barrier, immunological barrier, biological barrier, and chemical barrier. A decline in intestinal mucosal barrier function allows luminal bacteria, toxins, and other macromolecules, such as antigens, into the body, which is a key initiation factor in intestinal inflammation and deterioration. Increased permeability exposes the mucosal immune system in the intestinal lumen to foods and bacterial antigens, which stimulate the immune system and lead to the occurrence of gut inflammation.

A growing body of evidence indicates that intestinal ischemia plays a critical role in the development of excessive inflammatory-induced organ dysfunction[1,2]. When intestinal permeability and tight junction proteins are damaged, the gut becomes a source of pro-inflammatory mediators, which may amplify systemic inflammatory response syndrome (SIRS) and induce a septic state and distant organ failure. Moreover, it can lead to multiple organ dysfunction syndrome (MODS) and even death[3-5]. In the pathogenesis of MODS induced by an uncontrolled systemic inflammatory response, the intestine is the first organ to be affected and is one of the most easily damaged organs in the pathological process.

Research shows that when the intestine is ischemic, infected, or inflamed, bacteria and their toxins can rapidly activate originally static functions of intestinal innate macrophages to produce large amounts of pro-inflammatory cytokines. These pro-inflammatory factors cause further aggregation of monocytes and polymorphonuclear leukocytes in the intestinal microcirculation and intestinal tissue and release more inflammatory cytokines, oxygen free radicals, and inhibit gastrointestinal motility medium[1,6]. This response causes excessive inflammation of the intestine, mucosal edema, intestinal barrier dysfunction, and intestinal paralysis, triggering intestinal bacteria

and endotoxin translocation and gut-derived sepsis and MODS. Thus, effectively inhibiting the production of intestinal pro-inflammatory cytokines and reducing the production of inflammatory cytokines and oxygen free radicals to protect intestinal tissue from excessive inflammatory damage is significantly important. However, available drugs that protect gut barrier function due to excessive inflammatory response are limited.

Dimethyl sulfoxide (DMSO), a hydrophile-lipophile molecule, has anti-inflammatory, analgesic, diuretic, and vasodilatation activity, improves the microcirculation, and affects platelet aggregation hypertonicity[7]. Due to its anti-inflammatory properties, DMSO has also been evaluated in the treatment of inflammatory diseases such as cystitis and arthritis[8]. In addition, DMSO has been approved by the United States Food and Drug Administration for the treatment of interstitial cystitis by bladder instillation[9,10]. Therefore, this study aimed to determine whether the administration of DMSO inhibited zymosan-induced intestinal inflammation and barrier dysfunction and to provide an experimental basis for the use of DMSO in protecting intestinal barrier function.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (8-10 wk, 251.5 ± 8.7 g) were purchased from the Experimental Animal Center of Military Medical Sciences of the Chinese People's Liberation Army (PLA). The rats were housed in mesh cages in a room maintained at 25 ℃, illuminated by a 12:12 h light-dark cycle, and provided with standard rodent chow and water *ad libitum*. The rats were fasted overnight and allowed free access to water up to 4 h before surgery. The Committee of Scientific Research of the First Hospital Affiliated to the Chinese PLA General Hospital, China approved all the research protocols. The experiments were conducted in compliance with the Guide for Care and Use of Laboratory Animals of the National Research Council, China.

Animal model

After sterilization of the abdomen, an intraperitoneal injection of high-dose zymosan (750 mg/kg) was administered followed by a subcutaneous injection of DMSO (3 mL/kg, diluted in saline 1:2) or normal saline (3 mL/kg) 1 h after zymosan administration. The animals were allowed to breathe spontaneously under a nose cone scavenging system, using a veterinary anesthesia delivery system (Kent Scientific TOPO, Torrington, CT, United States). Rectal temperature was maintained at 37 ℃ with a heating pad and a heating lamp. Following the injection of zymosan, the animals developed acute peritonitis. The rats were very ill during the first day, as shown by ruffled fur, skin folds, lethargy, diarrhea, high body temperature, and decreased body weight[11].

Zymosan preparation

Zymosan (Sigma Chemical, St. Louis, MO, United States) was accurately weighed, and the appropriate volume of sterile saline was added to produce a zymosan suspension of 60 mg/mL. A high frequency magnetic stirrer was used to stir the suspension until blended. Disinfection was carried out in a 100 ℃ water bath for 80 min, and the suspension was then cooled to room temperature. The suspension was heated to 40 ℃, and high-frequency vibration blending was performed before use. The zymosan suspension was then injected intraperitoneally at the dose of 750 mg/kg.

Animal grouping and treatment

All the animals underwent the same procedure and were then randomly divided into four groups, weighed, and scored. In the ZS group and ZD group, an intraperitoneal injection of high-dose zymosan (750 mg/kg) was administered followed by a subcutaneous injection of DMSO (3 mL/kg, diluted in saline 1:2) in the ZD group and normal saline (3 mL/kg) in the ZS group 1 h after zymosan administration. In the SS group and SD group, an intraperitoneal injection of normal saline was administered in each group and then 1 h later a subcutaneous injection of DMSO (3 mL/kg, diluted in saline 1:2) was administered in the ZD group and normal saline (3 mL/kg) in the ZS group. The SS group and SD group were treated as the surgery and drug controls, respectively. Each group was divided into three subgroups, according to 4 h, 8 h, and 24 h after injury. In total, there were 12 subgroups (four groups with four time points each), with eight samples in each subgroup. Because the model has a 50% mortality rate at 24 h, each subgroup consisted of 16-20 rats. From the surviving rats, eight animals from each subgroup were randomly selected for the final analysis.

Blood and intestine samples

The rats were anesthetized by inhalation of 3% isoflurane (Yeeran Technology Limited, Beijing, China), and the aorta was punctured and exsanguinated at 4 h, 8 h, and 24 h after surgery. For diamine oxidase (DAO), blood was collected, and plasma was obtained by centrifuging the blood at 10000 *g* for 10 min at 4 ℃. The animals were sacrificed, and the distal small intestine was harvested. Segments of distal small intestine were fixed in 4% paraformaldehyde for histologic evaluation, deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis, and immunofluorescent staining. Segments of the distal small intestine stored at -40 ℃ for enzyme linked immunosorbent assay (ELISA).

Determination of tumor necrosis factor alpha and interleukin-10 levels in intestine

Intestine tissue (100 mg) in 1 mL phosphate-buffered

saline (PBS) was homogenized at 4 ℃ with a Polytron homogenizer. After centrifugation at 10000 *g* at 4 ℃ for 10 min, the supernatants were collected. Tumor necrosis factor alpha (TNF-α) and interleukin (IL)-10 in the intestine supernatants were quantified with a commercial ELISA kit (Nanjing Jiancheng Corp., China) according to the manufacturer's instructions. Intestinal TNF-α and IL-10 levels were expressed as picograms per milligram of protein.

Determination of intestinal tissue myeloperoxidase activity

Intestinal tissue myeloperoxidase (MPO) activity was determined using a kit according to the manufacturer's instructions. The tissue homogenate and reagent were placed in a water bath at 60 ℃ for 10 min after being thoroughly mixed. The absorbance value of each tube was then determined at 460 nm immediately after removal from the water bath. The activity of MPO in the intestine was calculated according to the following formula: MPO (U/weight grams) = [determination optical density (OD) value - control OD value]/11.3 × sample volume (g).

Determination of malonaldehyde content in intestine

Intestinal malonaldehyde (MDA) was determined using a kit according to the manufacturer's instructions. The tissue homogenate and reagent were placed in a water bath at 95 ℃ for 40 min after being thoroughly mixed. After cooling, the mixture was centrifuged at 4000 r/m for 10 min. The absorbance value of each supernatant was then determined at 532 nm immediately after removal from the water bath. The content of MDA in the intestine was calculated according to the following formula: MDA (nmol/mgProt) = {[(determination tube absorbance - blank tube absorbance)/(standard tube absorbance - blank tube absorbance)] × standard concentrations}/protein content.

Determination of superoxide dismutase activity in intestine

Intestinal superoxide dismutase (SOD) activity was determined using a kit according to the manufacturer's instructions. The tissue homogenate and reagent were placed in a water bath at 37 ℃ for 40 min after being thoroughly mixed. After 10 min at room temperature, the absorbance value of each supernatant was determined at 550 nm. The activity of SOD in the intestine was calculated according to the following formula: SOD (U/mL) = [(control tube absorbance - determination tube absorbance)/control tube absorbance]/(50% × reaction system dilution multiple × sample dilution multiple).

Measurement of intestinal mucosal blood flow

A laser Doppler flowmeter (Perimed AB; Stockholm, Sweden) was used to monitor intestinal mucosal blood flow (IMBF) at 4 h, 8 h, and 24 h after surgery. The probe of the blood flow meter was aimed at the proximal jejunum, and the laser was focused on the mesentery. The flow signal was measured for 30 s, and a 10-s stable signal was selected to calculate the mean value expressed in the blood perfusion unit (BPU).

Histopathologic score

Segments of the distal ileum were fixed in 4% paraformaldehyde for 48 h, embedded in paraffin, and sectioned. Hematoxylin and eosin staining of the intestine was performed after deparaffinization and rehydration. Two pathologists, who were blinded to the experimental groups, viewed and evaluated the sections under a light microscope. Three randomly selected fields from each specimen were graded using a scoring system that characterized gut injury on a scale of 0 to 4, as developed by Chiu *et al*[12].

Intestinal epithelial permeability

Determination of DAO activity was performed to assess gut barrier function. The activity of DAO was evaluated using an assay kit (Jiancheng Biotech Ltd., Nanjing, China) according to the manufacturer's instructions.

Immunofluorescence

After deparaffinization, the intestine sections were rehydrated and incubated in citrate buffer (Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) for heat-induced antigen retrieval. After three washes with PBS, the sections were incubated with 3% bovine serum albumin (BSA) (Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) for 30 min to block nonspecific binding sites. The sections were then incubated with the ZO-1 antibody (1:100; Life Technologies, Gaithersburg, MD, United States) at 4 ℃ overnight. The following day, after washing with PBS three times, the sections were treated with Alexa Fluor 488 secondary goat anti-rabbit antibody in 1% BSA for 1 h at room temperature followed by three washes with PBS. Sections were mounted using Antifade Solution (Applygen Technologies Inc., Beijing, China). The negative control was incubated with PBS instead of the ZO-1 antibody, and the other steps were the same as above. Images were viewed using an Olympus fluorescence microscope (BX51-DP71, Center Valley, PA, United States) with exposure-matched settings.

TUNEL analysis

TUNEL analysis was performed using the In Situ Cell Death Detection kit (Roche Diagnostics GmbH., Penzberg, Germany) according to the manufacturer's instructions. Segments of the distal ileum were fixed in 4% paraformaldehyde for 48 h, embedded in paraffin, and sectioned. The sections were incubated with pepsin digestion liquid in a wet box for 60 min after deparaffinization and rehydration. After two washes, 100 L DNase 1 (1500 U/mL) was added to the positive

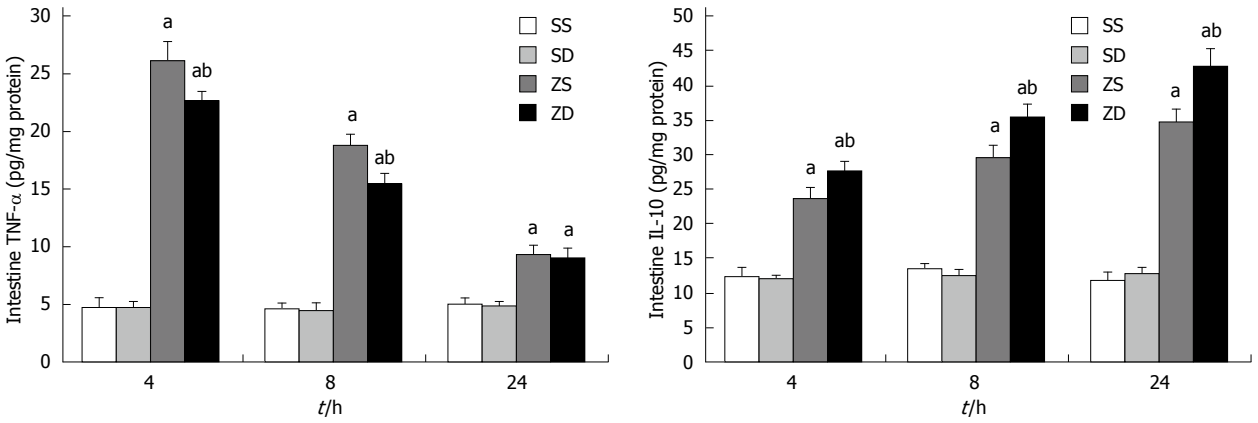


Figure 1 Tumor necrosis factor-α and interleukin-10 levels in rat intestine at 4, 8, and 24 h after intraperitoneal injection of zymosan. Intestine samples were obtained at 4, 8, and 24 h after intraperitoneal injection of zymosan. Data are expressed as mean ± SD (n = 8 per group at each time point). [a]$P < 0.05$ *vs* group SS and group SD; [b]$P < 0.05$ *vs* group ZS. TNF-α: Tumor necrosis factor-α; IL-10: Interleukin-10; DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

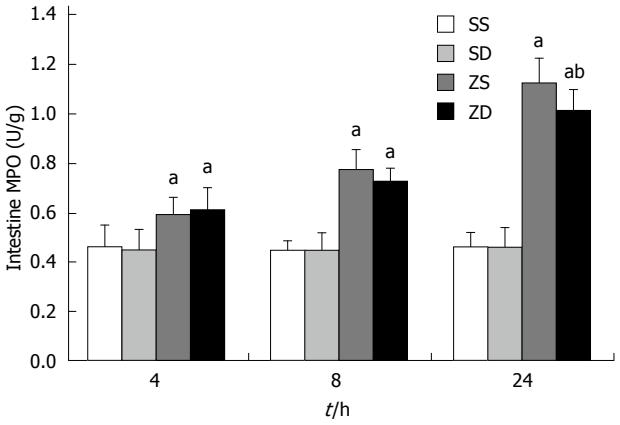


Figure 2 Activity of intestinal myeloperoxidase. Data are expressed as mean ± SD. [a]$P < 0.05$ *vs* group SS and group SD, [b]$P < 0.05$ *vs* group ZS (n = 8 per group at each time point). MPO: Myeloperoxidase; DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

control group and incubated in a wet box for 20 min. Fifty μL TUNEL reaction mixture solution (50 L enzyme solution + 450 L label solution) was added to the positive control group and the experimental group, and 50 L label solution was added to the negative control group. The sections were incubated in the dark in the wet box for 60 min. Differential interference contrast microscopy images were then obtained at 400 × magnification following the random selection of intestinal mucosa in five non-overlapping regions. The number of apoptotic intestinal mucosa cells and total intestinal mucosa cells were counted, and then the cell apoptosis rate was determined by the following equation: cell apoptosis rate = the number of apoptotic cells/total cells × 100%.

Statistical analysis

Data were analyzed using a commercial statistical software package (SPSS Statistics 17.0, Chicago, IL, United States). Continuous variables were expressed as mean ± SE. Statistically significant differences were determined using one way analysis of variance (ANOVA). Dunnett's test was used to compare within groups and SNK-q analysis was used to compare between groups. If variables were non-normally distributed, the Kruskal-Wallis H test was used. In all tests, a P value < 0.05 was considered statistically significant.

RESULTS

Effect of DMSO on intestinal cytokine levels

Figure 1 illustrates the effect of DMSO on TNF-α and IL-10 levels in rat intestine following intraperitoneal administration of zymosan. Zymosan induced increases in TNF-α and IL-10 in intestinal homogenates relative to saline treated controls, and DMSO reduced TNF-α levels and increased IL-10 levels in zymosan treated animals. The TNF-α and IL-10 levels in group ZS and group ZD were significantly higher than those in group SS and group SD ($P < 0.05$) following zymosan administration. The content of TNF-α in group ZD was significantly lower than that in group ZS at 4 h and 8 h ($P < 0.05$), while the IL-10 level in group ZD was higher than that in group ZS at all time points.

DMSO decreases intestinal MPO activity

The activity of MPO in group ZS and group ZD was significantly different from that in group SS and group SD. A significant decrease in MPO in group ZD compared with group ZS was observed at 24 h ($P < 0.05$). This indicated that DMSO reduced the accumulation of neutrophils in the gut (Figure 2).

DMSO lowers intestinal MDA content and SOD activity

The MDA content and SOD activity were significantly increased after intraperitoneal injection of zymosan. Both MDA content and SOD activity increased with time and were highest at 24 h. Furthermore, MDA

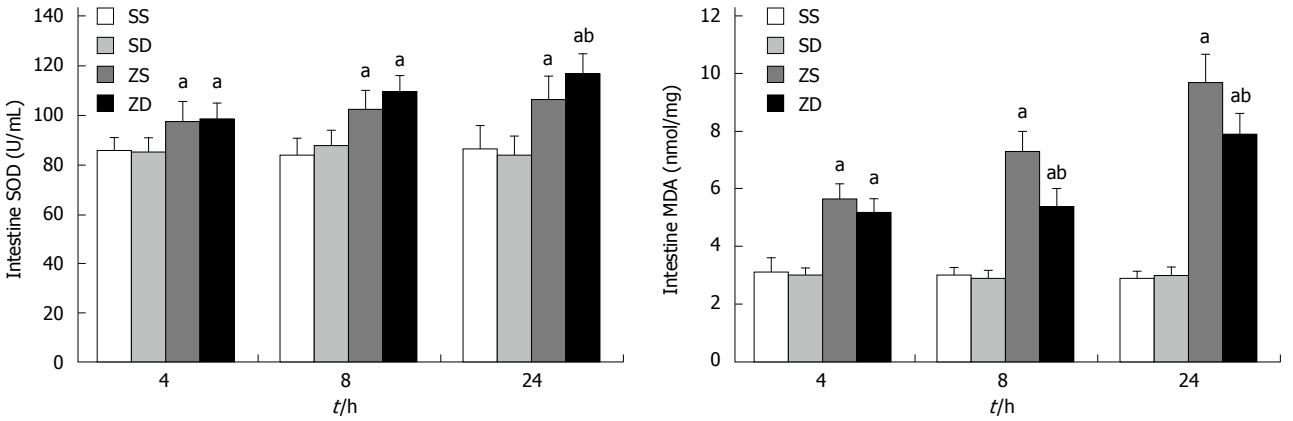


Figure 3 Activity of superoxide dismutase and the content of malonaldehyde in rat intestine. Data are expressed as mean ± SD. $^{a}P < 0.05$ *vs* group SS and group SD, $^{b}P < 0.05$ *vs* group ZS ($n = 8$ per group at each time point). SOD: Superoxide dismutase; MDA: Malonaldehyde; DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

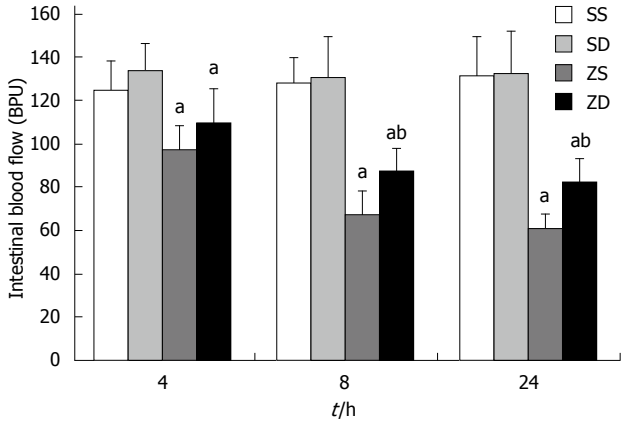


Figure 4 Effect of DMSO on intestinal mucosal blood flow. Data are expressed as mean ± SD ($n = 8$ per group at each time point). $^{a}P < 0.05$ *vs* group SS and group SD; $^{b}P < 0.05$ *vs* group ZS. IMBF: Intestinal mucosal blood flow; DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

content in group ZD was lower than that in group ZS, and SOD activity was highest at 24 h in group ZD ($P < 0.05$). This indicated that DMSO decreased MDA content, increased SOD activity, and reduced the damage caused by lipid peroxidation (Figure 3).

DMSO increases IMBF

IMBF in group SS and group SD was not significantly different at 4 h, 8 h, and 24 h. IMBF in group ZS and group ZD was significantly lower than that in group SS and group SD ($P < 0.05$) after zymosan administration. The lowest level in group ZS and group ZD, was 46.29% of that in group SS and 62.21% of that in group SD, respectively, at 24 h. The levels of IMBF in group ZD were significantly higher than those in group ZS at 8 h and 24 h ($P < 0.05$). These results indicated that DMSO improved IMBF and intestinal perfusion (Figure 4).

DMSO decreases intestinal injury

Histologic evaluation of intestinal mucosa was performed based on Chiu's grading system[12]. Histopathologic analysis of the sham group (sham + SS and sham + SD) showed a normal mucosal pattern. The villi were packed, tall, and intact. Compared with the sham group, intraperitoneal injection of zymosan caused significant mucosal damage. The intestinal villi became erosive, hyperemic, edematous, and atrophic. The villous stroma was full of inflammatory cells, and epithelial cell villi showed necrosis and exfoliation. These effects increased with time. No significant difference was observed between group ZS and group ZD at 4 h ($P > 0.05$). However, DMSO treatment significantly attenuated mucosal damage at 24 h ($P < 0.05$) (Figures 5 and 6).

DMSO lowers the release of DAO

The activity of DAO in group SS and group SD was not significantly different. The activity of DAO in group ZS and group ZD was significantly higher than that in group SS and group SD ($P < 0.05$) after zymosan administration. The activity was highest at 24 h in group ZS (81.10 ± 5.01 U/L) and group ZD (65.09 ± 4.74 U/L) and increased to 73.58% and 67.08% of group SS (21.43 ± 3.12 U/L) and group SD (21.43 ± 3.12 U/L), respectively. The activity of DAO in group ZD was significantly lower than that in group ZS ($P < 0.05$) at 8 h and 24 h. These results indicate that DMSO reduced the release of DAO into the bloodstream and protected intestinal structure and function (Figure 7).

DMSO prevents loss and redistribution of ZO-1

To assess the effects of DMSO on the expression of ZO-1, a tight junction protein, immunoflourescence was performed. Exposure-matched fluorescent intensity correlated with ZO-1 protein expression after immunostaining. In the sham group, ZO-1 was

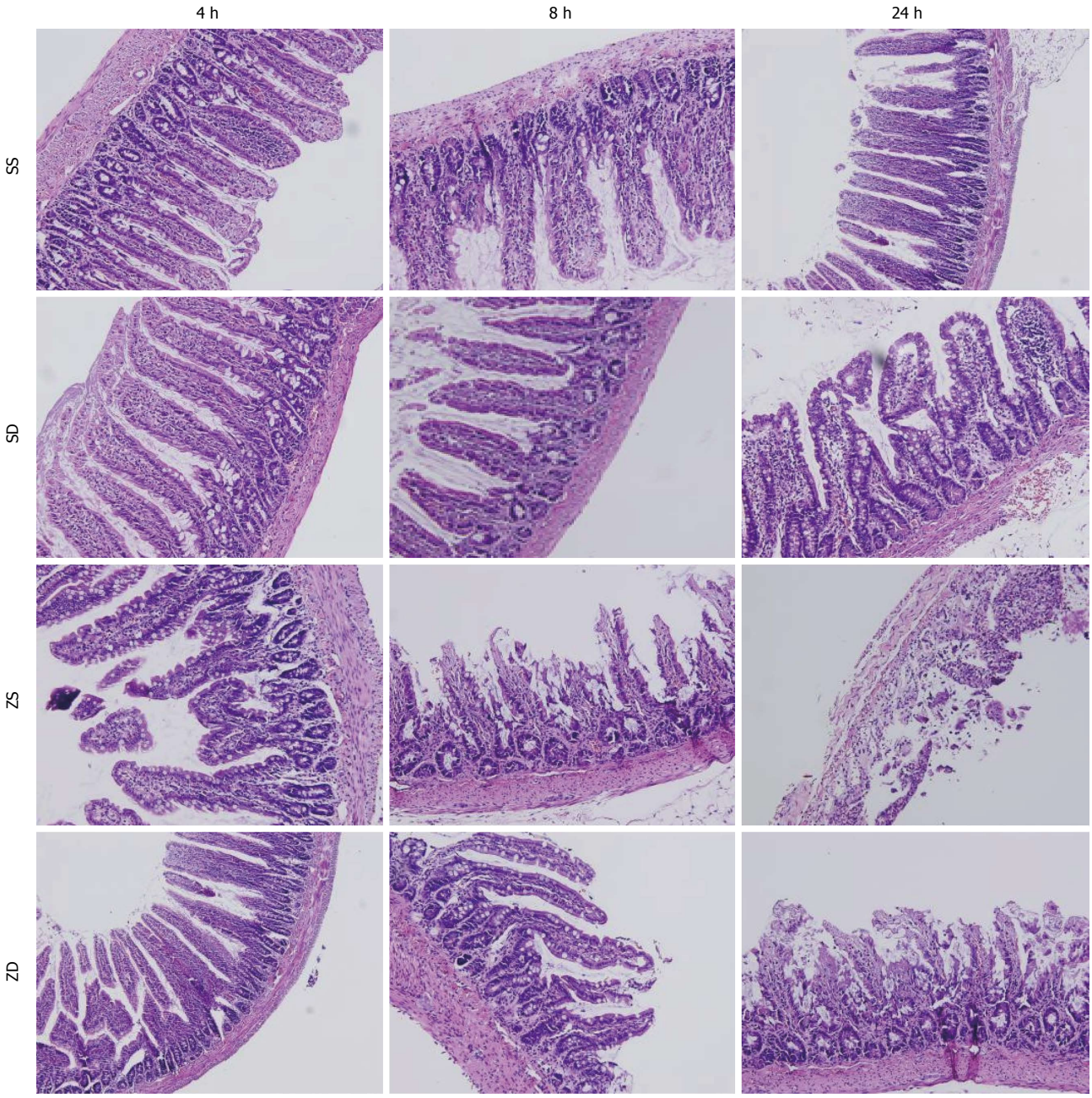


Figure 5 Intestinal histology. Dimethyl sulfoxide protected against intestinal injury following intraperitoneal injection of zymosan. Sections of the distal ileum were harvested at 4, 8, and 24 h after intraperitoneal injection of zymosan and stained with hematoxylin and eosin. All images were obtained at × 200 magnification with the black bar = 5 μm (n = 8 per group). DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

densely and continuously distributed along the apical membrane of epithelial cells (Figure 8). The expression pattern of ZO-1 was similar in group SS and group SD at all time points. Intraperitoneal administration of zymosan caused a loss of ZO-1 expression at 8 h (Figure 8), and zymosan-induced loss of ZO-1 was more pronounced at 24 h, resulting in a low expression of ZO-1 at the cell periphery (Figure 8). The pattern of ZO-1 expression in group ZS was lower than that in group SS at 8 h and 24 h. Following treatment with DMSO, the loss of ZO-1 was attenuated, and the level of ZO-1 continually improved 8 and 24 h after intraperitoneal administration of zymosan (Figure 8). Intraperitoneal zymosan (ZS group) resulted in a significant reduction in intestinal ZO-1 expression and DMSO treatment (ZD group) attenuated the degradation of ZO-1 at 8 and 24 h.

DMSO decreases apoptosis in the intestine

The rate of apoptosis in intestinal tissues in group SS and group SD were not significantly different, and the rate of apoptosis in intestinal cells in group ZS and group ZD was significantly higher than that in the sham control group (all $P < 0.05$). The rate

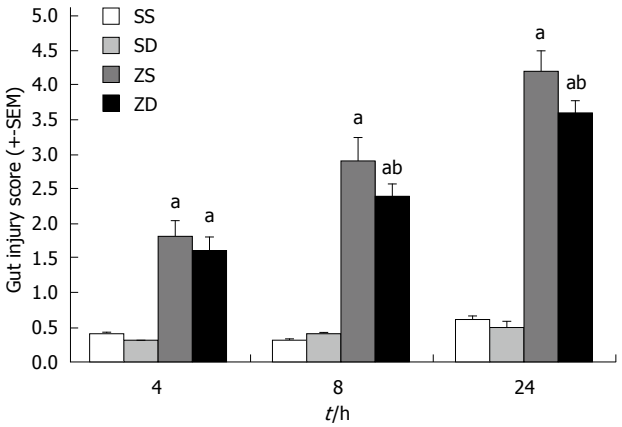


Figure 6 Gut injury scores. Gut injury was scored by a pathologist blinded to the experimental groups on a scale of 0-4, (as described in Materials and Methods). $^{a}P < 0.05$ *vs* group SS and group SD, $^{b}P < 0.05$ *vs* group ZS ($n = 8$ per group at 4, 8, and 24 h after intraperitoneal injection of zymosan). DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

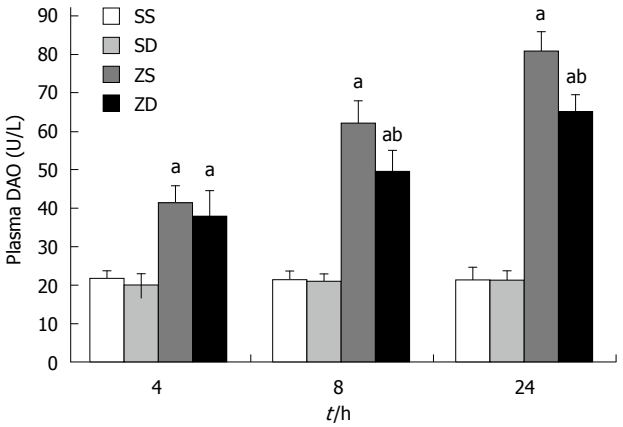


Figure 7 Effect of dimethyl sulfoxide on diamine oxidase in plasma. Blood samples and intestinal samples were obtained at 4, 8, and 24 h after intraperitoneal administration of zymosan. DMSO protected the intestine from an increase in permeability. $^{a}P < 0.05$ *vs* group SS and group SD, $^{b}P < 0.05$ *vs* group ZS. DAO: Diamine oxidase; DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

of apoptosis increased with time after zymosan administration. The rate of apoptosis in intestinal tissues in group ZD was significantly lower than that in group ZS ($P < 0.05$) at all time points. These results indicate that DMSO may inhibit intestinal cell apoptosis (Figure 9).

DISCUSSION

MODS refers to the clinical syndrome of simultaneous or sequential dysfunction of two or more organs leading to an unstable internal environment after severe trauma, shock, and infection[13]. Under physiological conditions, the body maintains a balance between pro-inflammatory and anti-inflammatory reactions, as a protective response against foreign invasion. Pro-inflammatory factors initiate and promote inflammation and injury to the body through the release of "aggressive" inflammatory mediators. Anti-inflammatory cytokines are released by pro-inflammatory cytokines and are involved in defense and promote anti-inflammatory reactions and tissue repair. However, when the pro-inflammatory or inflammatory reaction is too strong or too weak, the body is in a state of immune hyperfunction or immune suppression, the inflammatory response cannot be controlled, and homeostasis is disrupted. Infection, trauma, and ischemia reperfusion injury results in the excessive activation of inflammatory cells, such as macrophages, neutrophils, and endothelial cells, with the excessive release of inflammatory mediators. This causes the "waterfall effect", inducing a SIRS, and, if not treated, leads to multiple organ dysfunction[14-16]. Therefore, the balance between pro-inflammatory mediators and anti-inflammatory factors determines the prognosis of the disease, and the imbalance between the two types of cytokines is an important cause of further development of SIRS and MODS.

Zymosan is a substance derived from the cell wall of the yeast *Saccharomyces cerevisiae*. Intraperitoneal injection of zymosan in mice or rats leads to systemic inflammation and organ damage by inducing a wide range of inflammatory mediators of the complement system[17], prostaglandins and leukotrienes[18], platelet aggregation factor[19], oxygen radicals[20], and lysosomal enzymes[21]. In the mid-1980s, the zymosan-induced generalized inflammation (ZIGI) model was first introduced by Goris[22]. To date, the ZIGI model is recognized as the best model as it resembles human MODS and has been widely used to study systemic inflammation in relation to organ failure. Cuzzocera[23,24] administered intraperitoneal zymosan to animals, inducing acute peritonitis and multiple organ damage within 18 h. Inflammatory lesions play a role in the process of systemic inflammation and multiple organ damage induced by zymosan. Zymosan induces the excessive release of inflammatory mediators, damages vascular endothelial cells, and slows blood flow. Inflammatory cells and platelets adhere to the endothelium, and leukocytes migrate into the gap, releasing a variety of inflammatory transmitters, that damage the endothelial barrier and tissue.

After intraperitoneal injection of zymosan, pro-inflammatory factors and anti-inflammatory factors in the blood increase significantly, and the uncontrolled synthesis and release of these factors induce SIRS. In recent years, research has shown that DMSO can inhibit the activation of nuclear factor-kappa B (NF-κB) stimulated by lipopolysaccharide in mouse macrophages[25] and intestinal Caco-2 cells[26], lower mRNA expression of cytokines, and reduce the biological activity of TNF. DMSO inhibits the activation of rat NF-κB in sepsis, the expression of

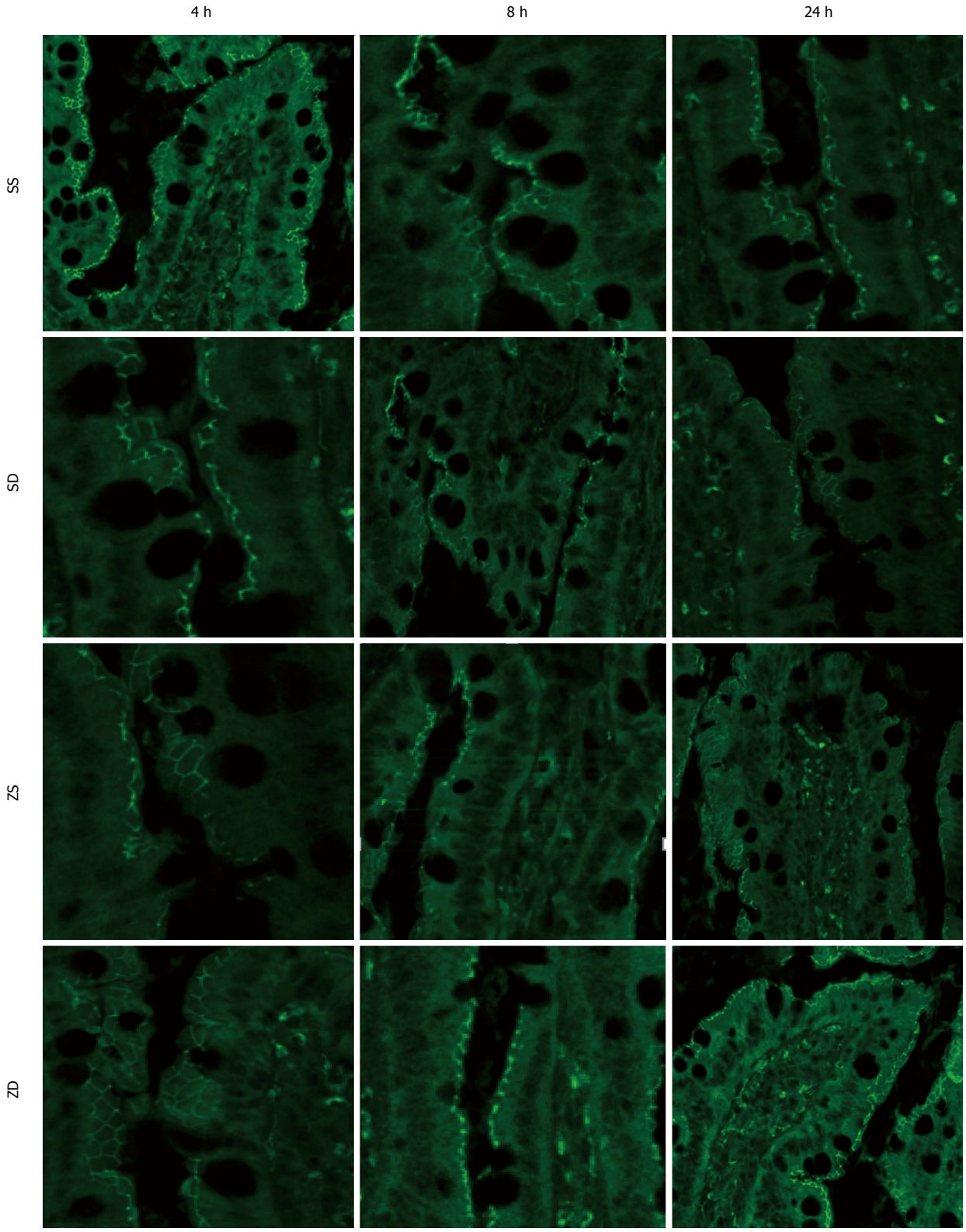
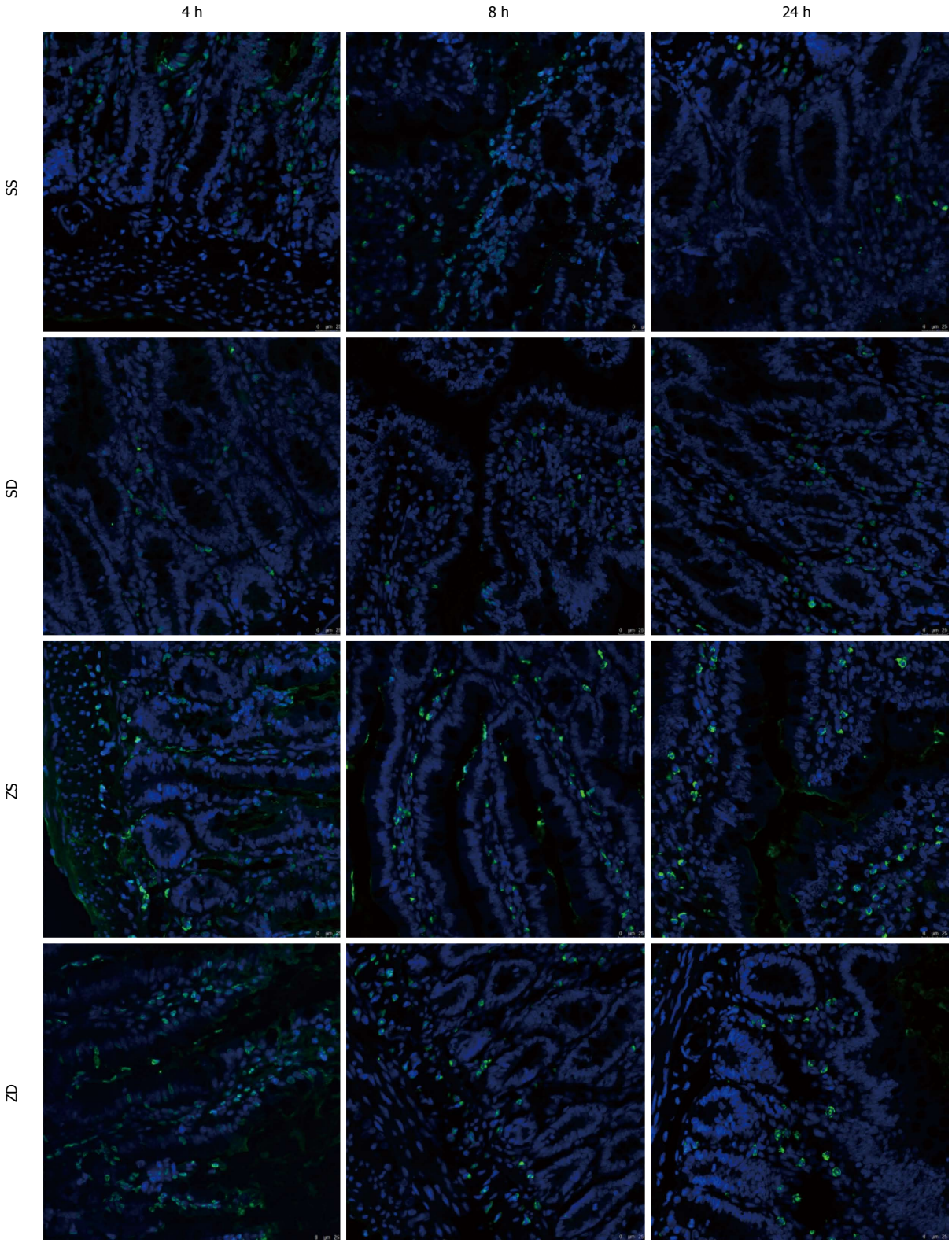


Figure 8 Intestinal ZO-1 immunofluorescent staining at 4 h, 8 h, and 24 h after injection of zymosan. Animals in group ZS showed low fluorescent intensity at the cell periphery after intraperitoneal injection of zymosan, and DMSO administration resulted in preservation of ZO-1 staining. All images were taken at × 400 magnification with the black bar = 5 μm (*n* = 5 per group). DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

the intracellular adhesion molecule-1 (ICAM-1) gene, and the expression of inflammatory factors, such as TNF-α[27]. Following selective inhibition of the nod-like receptor family pryin domain containing-3 (NLRP3) inflammatory complex, inhibition of mature IL-1, casP1, casP1 activity, and ASC pyroptosomes, DMSO


4 h
8 h
24 h
SS
SD
ZS
ZD

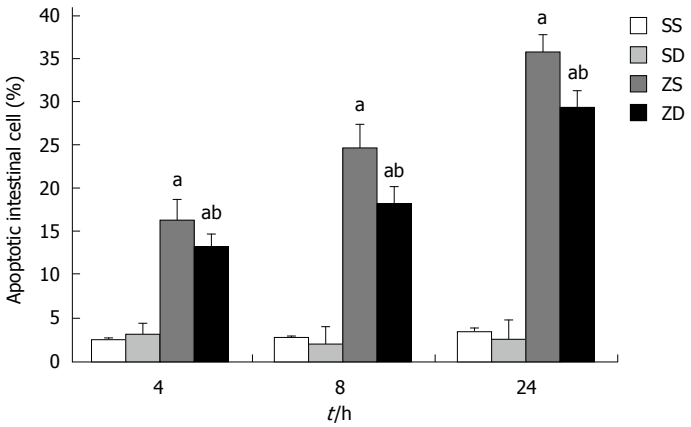


Figure 9 Effect of dimethyl sulfoxide on the percentage of apoptotic intestinal cells by the TUNEL assay. TUNEL stained paraffin sections from rats at 4, 8, and 24 h (original magnification, × 400). Dimethyl sulfoxide inhibited intestinal cell apoptosis. $^{a}P < 0.05$ *vs* group ZD, $^{b}P < 0.05$ *vs* group ZS. DMSO: Dimethyl sulfoxide; SS: Sham with administration of normal saline; SD: Sham with administration of DMSO; ZS: Zymosan with administration of normal saline; ZD: Zymosan with administration of DMSO.

was further confirmed to have anti-inflammatory effects in animal sepsis and inflammatory bowel disease models[28].

TNF-α is the primary initiator of the inflammatory cytokine cascade during SIRS, and IL-10 is considered to be the most important anti-inflammatory cytokine *in vivo*. The increase in TNF-α, a positive feedback response following activation of NF-κB, upregulates the expression and release of cytokines, such as IL-2 and ICAM-1, and plays a role in causing inflammatory damage to tissues. DMSO reduces the formation of inflammatory mediators, localizes the inflammatory response, and controls the systemic inflammatory response in the appropriate range, preventing the development of SIRS and even MODS. IL-10 inhibits the activity of NF-κB in at least two ways: (1) DMSO prevents the dissociation of NF-κB and protein IkappaB (Iκβ) by inhibiting the activity of Iκβ kinase; and (2) DMSO inhibits the combination of NF-κB with the DNA transcriptional regulatory region, thereby inhibiting the transcription of corresponding inflammatory factors[29,30].

In the present study, zymosan induced peritonitis, ascites leakage, and intestinal edema and significantly reduced intestinal blood flow, decreased the expression of intestinal tight junction protein ZO-1, increased intestinal permeability, which were correlated with the release of pro-inflammatory factors such as TNF-α. After the administration of DMSO, intestinal inflammatory factor TNF-α was significantly decreased, and IL-10, blood flow, and expression of ZO-1 were increased, indicating that DMSO decreased the synthesis and release of inflammatory factor TNF-α and increased the release of IL-10 to alleviate inflammatory damage caused by these factors. Previous studies have shown that macrophage Toll-like receptor 2 combined with zymosan leads to activation of NF-κB and generation of the pro-inflammatory factor TNF-α[31,32]. In this study, DMSO inhibited the synthesis and release of TNF-α, indicating that DMSO is likely to inhibit the activation of NF-κB and reduce the synthesis and release of TNF-α.

The activity of MPO in tissues reflects the aggregation of neutrophils at inflammatory sites. After intraperitoneal injection of zymosan, intestinal MPO activity significantly increased with time, as demonstrated by the aggregation of neutrophils in the intestine. However, the effect of DMSO on neutrophil activity and white blood cell count was not significant, with only some effects at 24 h. DMSO may inhibit the oxidative stress reaction mediated by neutrophils to alleviate injury caused by zymosan.

After intraperitoneal injection of zymosan, viscera microcirculation blood flow decreased, and the tissue was ischemic and anoxic and produced a large number of free radicals. Therefore, the animals continued to be in a state of oxidative stress, endogenous antioxidant enzyme activity was reduced, and the body's reduction/oxidation system balance was disrupted. MDA is the main metabolite in lipid peroxidation, and its content can reflect the degree of lipid peroxidation and indirectly reflect the degree of oxidative damage[33]. Previous research showed that DMSO can reduce MDA and NO levels, inhibit or increase the level of glutathione, and alleviate liver injury and ischemia reperfusion-induced transaminase release[34,35]. DMSO can also reduce renal damage caused by $HgCl_2$[36]. In addition, SOD is an important antioxidant enzyme in organisms and the primary enzyme involved in scavenging free radicals. Oxygen free radicals can activate NF-κB, thus a reduction in oxidative products and an improvement in antioxidant enzymes decrease the serum and tissue levels of pro-inflammatory cytokines and protect organ function[37]. In this study, intraperitoneal injection of zymosan increased MDA content and SOD activity in the intestine, while subcutaneous injection of DMSO suppressed the increase in MDA and SOD. These results demonstrate that DMSO can reduce the damage caused by visceral lipid peroxidation mediated by oxygen free radicals.

Cell apoptosis regulates body development and maintains a stable internal environment *via* a

series of genes that control the process of active cell death. Intestinal mucosal epithelial cell proliferation, differentiation, and apoptosis are processes of dynamic change. However, the balance is disrupted in inflammatory bowel disease, where the occurrence and scope of epithelial cell apoptosis is higher than that in normal tissue[38]. Epithelial cell apoptosis is mainly due to activation of the Fas/Fas ligand (FasL) signal transduction pathway and both Bcl-2 and Bax[39,40]. In this study, intestinal epithelial cell apoptosis was increased following intraperitoneal administration of zymosan and was reduced by DMSO, however, the specific mechanism involved in the effect of DMSO requires further study.

In conclusion, DMSO reduced intestinal tissue injury after intraperitoneal injection of zymosan, restored intestinal blood flow, and protected intestinal function. The mechanism likely involves regulation of the balance between pro-inflammatory and anti-inflammatory factors, inhibition of peroxidation in organs, oxygen free radical scavenging, reduction in intestinal epithelial cell apoptosis, and alleviation of intestinal function damage.

ACKNOWLEDGMENTS

The authors thank Dr. Jiang-Yang Lu, Yi-Duo Jin, Xiu-Li Ma, Hui-Zhen Ding, Yi Yang, and Dong-Mei Yang from the Department of Pathology, First Hospital Affiliated to the PLA General Hospital, for their technical assistance. The authors also thank Hui-Ping Zhang, Ming-Juan Gao, and Xiao-Na Zhang for their assistance with the experiments.

COMMENTS

Background

When severe injury occurs, the blood supply to the intestinal tract is sharply reduced, which results in gut barrier dysfunction. The incidence of serious complications is increased following dysfunction of the gut barrier. This promotes bacterial translocation and the local production of cytokines. Bacteria and their endotoxins move into the circulation and remote organs, contributing to subsequent local and systemic inflammation. This may lead to systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). Therefore, protecting the intestinal barrier function is important. Thus, interventions such as drugs to prevent excessive inflammation and the reduction/oxidation reaction are of great significance in controlling SIRS and MODS induced by zymosan.

Research frontiers

The current treatment options for SIRS and MODS are limited, therefore, it is important to identify alternative therapies. Dimethyl sulfoxide (DMSO) has been found to have anti-inflammatory, analgesic, diuretic, and vasodilatation activities, to improve the microcirculation, and to affect platelet aggregation hypertonicity. In addition, DMSO has been studied in the treatment of inflammatory diseases, such as cystitis and arthritis. DMSO can reduce malonaldehyde (MDA) and nitric oxide (NO) level, and alleviate liver injury. DMSO can also reduce renal damage caused by $HgCl_2$. However, whether DMSO can protect intestinal function in SIRS and MODS and its specific mechanism are unclear.

Innovations and breakthroughs

The most important novel findings in this study are that DMSO inhibits zymosan-induced intestinal inflammation and barrier dysfunction. Regulation of the balance between pro-inflammatory and anti-inflammatory reactions and inhibition of excessive oxidation are considered possible mechanisms underlying DMSO regulation of zymosan-induced intestinal barrier function.

Applications

These study results provide possible mechanisms of DMSO regulation of intestinal barrier function after zymosan-induced systemic inflammatory response syndrome and MODS.

Terminology

Intestinal barrier function: refers to the function of the intestine in preventing harmful substances, such as bacteria and toxins, from entering the intestinal mucosa, other organs, and the blood circulation. The normal intestinal mucosal barrier is composed of a mechanical barrier, chemical barrier, immunologic barrier, and biological barrier. Inflammation of the intestine: Intestinal ischemia, infection, and inflammation can activate intestinal inflammatory cells to release many cytokines and oxygen free radicals and inhibit gastrointestinal motility medium, resulting in excessive inflammation, mucosal edema, and intestinal barrier damage. DMSO is a hydrophile-lipophile molecule and is widely used as a solvent for biological compounds. It has anti-inflammatory, analgesic, diuretic, and vasodilatation activity, improves the microcirculation, and affects platelet aggregation hypertonicity. Zymosan: zymosan is a substance derived from the cell wall of the yeast *Saccharomyces cerevisiae*. It is composed of polysaccharide chains of various molecular weights and contains approximately 73% polysaccharides, 15% proteins, and 7% lipids and inorganic components. When injected into animals, it results in inflammation by inducing a wide range of inflammatory mediators.

Peer-review

This is a well written and set up study. The authors provide a sufficient overview of the study background and clearly present the hypothesis of the study. The aim of the study was fulfilled. The results are presented sufficiently well and discussed well. The nine figures clearly and correctly present the results.

REFERENCES

1 **Moore FA**. The role of the gastrointestinal tract in postinjury multiple organ failure. *Am J Surg* 1999; **178**: 449-453 [PMID: 10670850]
2 **Deitch EA**, Xu D, Franko L, Ayala A, Chaudry IH. Evidence favoring the role of the gut as a cytokine-generating organ in rats subjected to hemorrhagic shock. *Shock* 1994; **1**: 141-145 [PMID: 7749933]
3 **Wang W**, Smail N, Wang P, Chaudry IH. Increased gut permeability after hemorrhage is associated with upregulation of local and systemic IL-6. *J Surg Res* 1998; **79**: 39-46 [PMID: 9735238]
4 **Thuijls G**, de Haan JJ, Derikx JP, Daissormont I, Hadfoune M, Heineman E, Buurman WA. Intestinal cytoskeleton degradation precedes tight junction loss following hemorrhagic shock. *Shock* 2009; **31**: 164-169 [PMID: 18650780 DOI: 10.1097/SHK.0b013e31817fc310]
5 **Fink MP**, Delude RL. Epithelial barrier dysfunction: a unifying theme to explain the pathogenesis of multiple organ dysfunction at the cellular level. *Crit Care Clin* 2005; **21**: 177-196 [PMID: 15781156]
6 **Hassoun HT**, Kone BC, Mercer DW, Moody FG, Weisbrodt NW, Moore FA. Post-injury multiple organ failure: the role of the gut. *Shock* 2001; **15**: 1-10 [PMID: 11198350]
7 **Santos NC**, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. *Biochem Pharmacol* 2003; **65**: 1035-1041 [PMID: 12663039]
8 **Kloesch B**, Liszt M, Broell J, Steiner G. Dimethyl sulphoxide and dimethyl sulphone are potent inhibitors of IL-6 and IL-8 expression in the human chondrocyte cell line C-28/I2. *Life Sci* 2011; **89**: 473-478 [PMID: 21821055 DOI: 10.1016/j.lfs.2011.07.015]

9 **Sant GR**. Intravesical 50% dimethyl sulfoxide (Rimso-50) in treatment of interstitial cystitis. *Urology* 1987; **29**: 17-21 [PMID: 3551281]

10 **Parkin J**, Shea C, Sant GR. Intravesical dimethyl sulfoxide (DMSO) for interstitial cystitis--a practical approach. *Urology* 1997; **49**: 105-107 [PMID: 9146010]

11 **Jansen MJ**, Hendriks T, Verhofstad AA, Lange W, Geeraedts LM, Goris RJ. Gradual development of organ damage in the murine zymosan-induced multiple organ dysfunction syndrome. *Shock* 1997; **8**: 261-267 [PMID: 9329127]

12 **Chiu CJ**, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 1970; **101**: 478-483 [PMID: 5457245 DOI: 10.1001/archsurg.1970.01340280030009]

13 **Kramer N**, Meyer TJ, Meharg J, Cece RD, Hill NS. Randomized, prospective trial of noninvasive positive pressure ventilation in acute respiratory failure. *Am J Respir Crit Care Med* 1995; **151**: 1799-1806 [PMID: 7767523]

14 **Hotchkiss RS**, Karl IE. The pathophysiology and treatment of sepsis. *N Engl J Med* 2003; **348**: 138-150 [PMID: 12519925]

15 **Lee WL**, Slutsky AS. Sepsis and endothelial permeability. *N Engl J Med* 2010; **363**: 689-691 [PMID: 20818861 DOI: 10.1056/NEJMcibr1007320]

16 **Hotchkiss RS**, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. *Lancet Infect Dis* 2013; **13**: 260-268 [PMID: 23427891 DOI: 10.1016/S1473-3099(13)70001-X]

17 **Pillemer L**, Ecker EE. Anticomplementary factor in fresh yeast. *J Biol Chem* 1941; **137**: 139-142

18 **Humes JL**, Sadowski S, Galavage M, Goldenberg M, Subers E, Bonney RJ, Kuehl FA. Evidence for two sources of arachidonic acid for oxidative metabolism by mouse peritoneal macrophages. *J Biol Chem* 1982; **257**: 1591-1594 [PMID: 6799509]

19 **Roubin R**, Mencia-Huerta JM, Landes A, Benveniste J. Biosynthesis of platelet-activating factor (PAF-acether). IV. Impairment of acetyl-transferase activity in thioglycollate-elicited mouse macrophages. *J Immunol* 1982; **129**: 809-813 [PMID: 6806384]

20 **Nauseef WM**, Root RK, Newman SL, Malech HL. Inhibition of zymosan activation of human neutrophil oxidative metabolism by a mouse monoclonal antibody. *Blood* 1983; **62**: 635-644 [PMID: 6309281]

21 **Bonney RJ**, Wightman PD, Davies P, Sadowski SJ, Kuehl FA, Humes JL. Regulation of prostaglandin synthesis and of the selective release of lysosomal hydrolases by mouse peritoneal macrophages. *Biochem J* 1978; **176**: 433-442 [PMID: 743251]

22 **Goris RJ**, Boekholtz WK, van Bebber IP, Nuytinck JK, Schillings PH. Multiple-organ failure and sepsis without bacteria. An experimental model. *Arch Surg* 1986; **121**: 897-901 [PMID: 3729706]

23 **Imperatore F**, Cuzzocrea S, De Lucia D, Sessa M, Rinaldi B, Capuano A, Liguori G, Filippelli A, Rossi F. Hyperbaric oxygen therapy prevents coagulation disorders in an experimental model of multiple organ failure syndrome. *Intensive Care Med* 2006; **32**: 1881-1888 [PMID: 16977483]

24 **Impellizzeri D**, Mazzon E, Di Paola R, Paterniti I, Bramanti P, Cuzzocrea S. Effect of NADPH-oxidase inhibitors in the experimental model of zymosan-induced shock in mice. *Free Radic Res* 2011; **45**: 820-834 [PMID: 21623687 DOI: 10.3109/10715762.2011.581667]

25 **Kelly KA**, Hill MR, Youkhana K, Wanker F, Gimble JM. Dimethyl sulfoxide modulates NF-kappa B and cytokine activation in lipopolysaccharide-treated murine macrophages. *Infect Immun* 1994; **62**: 3122-3128 [PMID: 8039880]

26 **Hollebeeck S**, Raas T, Piront N, Schneider YJ, Toussaint O, Larondelle Y, During A. Dimethyl sulfoxide (DMSO) attenuates the inflammatory response in the in vitro intestinal Caco-2 cell model. *Toxicol Lett* 2011; **206**: 268-275 [PMID: 21878375 DOI: 10.1016/j.toxlet.2011.08.010]

27 **Chang CK**, Llanes S, Schumer W. Inhibitory effect of dimethyl sulfoxide on nuclear factor-kappa B activation and intercellular adhesion molecule 1 gene expression in septic rats. *J Surg Res* 1999; **82**: 294-299 [PMID: 10090842]

28 **Ahn H**, Kim J, Jeung EB, Lee GS. Dimethyl sulfoxide inhibits NLRP3 inflammasome activation. *Immunobiology* 2014; **219**: 315-322 [PMID: 24380723 DOI: 10.1016/j.imbio.2013.11.003]

29 **Schottelius AJ**, Mayo MW, Sartor RB, Baldwin AS. Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. *J Biol Chem* 1999; **274**: 31868-31874 [PMID: 10542212]

30 **Driessler F**, Venstrom K, Sabat R, Asadullah K, Schottelius AJ. Molecular mechanisms of interleukin-10-mediated inhibition of NF-kappaB activity: a role for p50. *Clin Exp Immunol* 2004; **135**: 64-73 [PMID: 14678266]

31 **Frasnelli ME**, Tarussio D, Chobaz-Péclat V, Busso N, So A. TLR2 modulates inflammation in zymosan-induced arthritis in mice. *Arthritis Res Ther* 2005; **7**: R370-R379 [PMID: 15743485]

32 **Tian B**, Brasier AR. Identification of a nuclear factor kappa B-dependent gene network. *Recent Prog Horm Res* 2003; **58**: 95-130 [PMID: 12795416]

33 **Janero DR**. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med* 1990; **9**: 515-540 [PMID: 2079232]

34 **Stein HJ**, Oosthuizen MM, Hinder RA, Lamprechts H. Oxygen free radicals and glutathione in hepatic ischemia/reperfusion injury. *J Surg Res* 1991; **50**: 398-402 [PMID: 2020191]

35 **Akyürek N**, Kafali EM, Muhtaroglu S. The effects of dimethylsulfoxide on experimental hepatic ischemia. *Swiss Surg* 2000; **6**: 23-27 [PMID: 10709433]

36 **Jo SK**, Hu X, Yuen PS, Aslamkhan AG, Pritchard JB, Dear JW, Star RA. Delayed DMSO administration protects the kidney from mercuric chloride-induced injury. *J Am Soc Nephrol* 2004; **15**: 2648-2654 [PMID: 15466269]

37 **Xie K**, Yu Y, Zhang Z, Liu W, Pei Y, Xiong L, Hou L, Wang G. Hydrogen gas improves survival rate and organ damage in zymosan-induced generalized inflammation model. *Shock* 2010; **34**: 495-501 [PMID: 20351628 DOI: 10.1097/SHK.0b013e3181def9aa]

38 **Iwamoto M**, Koji T, Makiyama K, Kobayashi N, Nakane PK. Apoptosis of crypt epithelial cells in ulcerative colitis. *J Pathol* 1996; **180**: 152-159 [PMID: 8976873]

39 **Martin CA**, Panja A. Cytokine regulation of human intestinal primary epithelial cell susceptibility to Fas-mediated apoptosis. *Am J Physiol Gastrointest Liver Physiol* 2002; **282**: G92-G104 [PMID: 11751162]

40 **Lu J**, Caplan MS, Saraf AP, Li D, Adler L, Liu X, Jilling T. Platelet-activating factor-induced apoptosis is blocked by Bcl-2 in rat intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G340-G350 [PMID: 14512286]

P- Reviewer: Vorobjova T **S- Editor**: Ma YJ **L- Editor**: Filipodia **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10866

World J Gastroenterol 2015 October 14; 21(38): 10866-10873
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Clinical characteristics of hepatoduodenal lymph node metastasis in gastric cancer

Taisuke Imamura, Shuhei Komatsu, Daisuke Ichikawa, Toshiyuki Kosuga, Kazuma Okamoto, Hirotaka Konishi, Atsushi Shiozaki, Hitoshi Fujiwara, Eigo Otsuji

Taisuke Imamura, Shuhei Komatsu, Daisuke Ichikawa, Toshiyuki Kosuga, Kazuma Okamoto, Hirotaka Konishi, Atsushi Shiozaki, Hitoshi Fujiwara, Eigo Otsuji, Division of Digestive Surgery, Department of Surgery, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

Author contributions: Imamura T and Komatsu S contributed equally to this work; Imamura T, Komatsu S, Ichikawa D and Otsuji E designed the research; Imamura T, Kosuga T, Okamoto K and Shiozaki A performed the research; Imamura T, Konishi H and Fujiwara H analyzed the data; Imamura T and Komatsu S wrote the paper.

Institutional review board statement: This study was institutionally reviewed and comprehensively approved by the Kyoto Prefectural University of Medicine.

Informed consent statement: The patients provided informed written consent prior to study enrollment.

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

Data sharing statement: Technical appendix and study data are available from the corresponding author at skomatsu@koto.kpu-m.ac.jp (Shuhei Komatsu) under the permission of Shuhei Komatsu. Participants gave informed consent for data sharing. No additional data are available.

Correspondence to: Shuhei Komatsu, MD, PhD, Division of Digestive Surgery, Department of Surgery, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kawaramachihirokoji, Kamigyo-ku, Kyoto 602-8566, Japan. skomatsu@koto.kpu-m.ac.jp
Telephone: +81-75-2515527
Fax: +81-75-2515522

Received: March 29, 2015
Peer-review started: March 31, 2015
First decision: June 19, 2015
Revised: July 1, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 14, 2015

Abstract

AIM: To assess the clinical features of hepatoduodenal lymph node (HDLN) metastasis and to clarify the optimal indication of HDLN dissection.

METHODS: We investigated a total of 276 patients who underwent gastrectomy with extended lymphadenectomy, including HDLN dissection, for gastric cancer between 1999 and 2012. Of these, 26 patients (9.4%) had HDLN metastasis. First, we investigated the clinicopathological characteristics, their perioperative clinical outcomes, such as postoperative complications, and prognostic outcomes between patients with and without HDLN metastasis. Second, we detected the prognostic factors, particularly in patients with HDLN metastasis. Third, we assessed the therapeutic value of HDLN dissection to determine its optimal indication.

RESULTS: The five-year overall survival rate of the patients with HDLN metastasis was 29%. Univariate and multivariate logistic regression analyses revealed that the tumour location (the middle or lower stomach [P = 0.005, OR = 5.88 (95%CI: 1.61-38.1)] and pT category [T3 or T4, P = 0.017, OR = 4.45 (95%CI: 1.28-21.3)] were independent risk factors for HDLN

metastasis. Cox proportional hazard analysis identified pN3 as an independent poor prognostic factor in the patients with HDLN metastasis [P = 0.021, HR = 5.17 (95%CI: 1.8-292)]. For patients who underwent radical HDLN dissection, HDLN metastasis was a prognostic indicator in pN3 gastric cancer (P < 0.0001), but not pN1-2 (P = 0.602). Furthermore, the index of therapeutic value of HDLN dissection for gastric cancer in the middle or lower stomach and the upper stomach was 3.4 and 0.0, respectively.

CONCLUSION: We suggest that HDLN dissection should be indicated for pN1 or pN2 gastric cancers located at the middle or lower stomach.

Key words: Gastric cancer; Hepatoduodenal lymph node; D2 lymphadenectomy; Prognostic factor; Tumor location

Core tip: Gastric cancer located at the middle or lower stomach is a risk factor of hepatoduodenal lymph node (HDLN) metastasis and indicates relatively high therapeutic value of HDLN dissection. N-category, in especially pN3, is an independent poor prognostic factor in gastric cancer patients with HDLN metastasis. HDLN dissection should be indicated for N1 or N2 gastric cancers located at the middle or lower stomach.

Imamura T, Komatsu S, Ichikawa D, Kosuga T, Okamoto K, Konishi H, Shiozaki A, Fujiwara H, Otsuji E. Clinical characteristics of hepatoduodenal lymph node metastasis in gastric cancer. *World J Gastroenterol* 2015; 21(38): 10866-10873 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10866.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10866

INTRODUCTION

Gastric cancer is one of the most common causes of death from cancer worldwide[1]. Recent advances in diagnostic techniques, less invasive treatment techniques, and perioperative management have increased the early detection of gastric cancer and decreased the mortality and morbidity[1,2]. Patients with advanced stage disease, however, still present a poor prognostic outcome and have a high incidence of lymph node metastasis. Therefore, nodal status is the strongest predictor of the prognosis of gastric cancer patients, and treatment strategy against metastatic lymph nodes is the most important clinical issue[3-10].

Although radical gastrectomy with lymphadenectomy is recognized as the best strategy for macroscopic tumour clearance for advanced gastric cancer, criteria for optimal lymphadenectomy are different between Eastern and Western countries, because of differences in the number of obese patients, for whom a surgical approach is difficult, and differences in the epidemiologic characteristics of gastric cancer[11,12]. For example, D1 lymphadenectomy, which is a dissection of the perigastric lymph nodes, is mainly performed for advanced gastric cancer in Western countries, whereas D2 lymphadenectomy, which is a dissection of the nodes along the celiac artery and its branches in addition to the perigastric lymph nodes, is routinely performed for advanced gastric cancer in Eastern countries[12].

Recently, a 15-year Dutch trial demonstrated fewer locoregional recurrences of gastric cancer and better long-term survival benefit in patients with D2 lymphadenectomy compared with those with D1 lymphadenectomy[13]. Therefore, the therapeutic value of D2 lymphadenectomy has started to be re-evaluated in Western countries[14,15]. In this study, we focused on the hepatoduodenal lymph node (HDLN), especially the lymph node at station No. 12a. This node is defined as a hepatoduodenal ligament lymph node along the proper hepatic artery[16], and the Japanese treatment guidelines[17] recommend it to be routinely removed as a standard procedure for D2 lymphadenectomy. On the other hand, the HDLN is not removed in Western countries, and HDLN metastasis is classified as distant metastasis according to the 7th American Joint Committee on Cancer (AJCC) staging manual[18], although a recent report indicates the inappropriateness of including the HDLN in the distant metastatic lymph node group in all gastric cancers[19].

In this study, we hypothesized that HDLN metastasis could be an indicator of poor prognosis in some subgroups of gastric cancer and that it could also be a governor of local metastatic control in other subgroups. We aimed to verify these hypotheses and to clarify the optimal indication of HDLN dissection retrospectively from patients' hospital records.

MATERIALS AND METHODS

Study population of gastric cancer patients

A total of 276 consecutive patients that underwent gastrectomy with HDLN dissection, with curative intention, for gastric cancer in the Division of Digestive Surgery, Department of Surgery, Kyoto Prefectural University of Medicine, between January 1999 and December 2012 were retrospectively analysed from their hospital records. Surgical procedures comprised a distal gastrectomy in 211 patients, a total gastrectomy in 59 patients, a pancreaticoduodenectomy in 5 patients, and a proximal gastrectomy in 1 patient according to the preoperative stage and tumour location. Resected specimens were examined and evaluated by pathologists based on classifications of the 14th JCGC[16] and the AJCC staging manual[18]. As a result, 90 patients were staged as pT1, 37 as pT2, 64 as pT3, and 85 as pT4. Histological types were classified as differentiated (papillary adenocarcinoma,

Table 1 Comparison of the clinicopathological features between patients with and without hepatoduodenal lymph node metastasis *n* (%)

	n	HDLN metastasis		Univariate	Multivariate	
		Positive	Negative	*P* value	OR (95%CI)	*P* value
Total	276	26	250			
Sex						
Male	186	16 (62)	170 (68)			
Female	90	10 (38)	80 (32)	0.509	-	
Age (yr)						
< 65	134	10 (38)	124 (50)			
⩾ 65	142	16 (62)	126 (50)	0.277	-	
Tumor location						
U	64	2 (8)	62 (25)			
M and L	212	24 (92)	188 (75)	0.029	5.88 (1.61-38.1)	0.005
CA 19-9 (U/mL)						
< 10	132	9 (35)	123 (49)			
⩾ 10	144	17 (65)	127 (51)	0.153	-	
CEA (ng/mL)						
< 3	195	16 (62)	179 (72)			
⩾ 3	81	10 (38)	71 (28)	0.295	-	
Histopathological type						
Differentiated	135	10 (38)	125 (50)			
Undifferentiated	141	16 (62)	125 (50)	0.260	-	
Venous invasion						
(-)	173	12 (46)	161 (64)			
(+)	103	14 (54)	89 (36)	0.072	-	
Lymphatic invasion						
(-)	105	3 (12)	102 (42)			
(+)	171	23 (88)	148 (58)	0.002	-	
Tumor size (mm)						
< 45	119	5 (19)	114 (46)			
⩾ 45	157	21 (81)	136 (54)	0.007	-	
T category						
T1 and T2	127	3 (12)	124 (50)			
T3 and T4	149	23 (88)	126 (50)	< 0.0001	4.45 (1.28-21.3)	0.017

HDLN: Hepatoduodenal lymph node; CEA: Carcinoembryonic antigen; CA 19-9: Carbohydrate antigen 19-9; U: Upper gastric body; M: Middle gastric body; L: Lower gastric body.

or moderately or well-differentiated adenocarcinoma) or undifferentiated (poorly differentiated or undifferentiated adenocarcinoma, signet-ring cell carcinoma, or mucinous adenocarcinoma) based on the 14th JCGC[16].

Of all 276 patients, 182 patients (66%) received adjuvant chemotherapy, but 94 patients (34%) did not. S-1 or 5-fluorouracil was administered as a key drug of adjuvant chemotherapy. None of the patients received adjuvant radiotherapy or chemoradiotherapy. All patients were examined in the outpatient clinic, where abdominal ultrasound, computed tomography (CT), and measurement of levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were performed every 3-6 mo after surgery. All patients gave their informed consent in writing.

Analysis of surgical outcomes and clinicopathological factors

To reconfirm the feasibility and safety of HDLN dissection, we first investigated the clinicopathological characteristics of all patients who underwent HDLN dissection (Table 1) and their perioperative clinical outcomes, such as postoperative complications (Table 2). Second, to evaluate the prognostic differences, survival rates after surgery were compared between patients with and without HDLN metastasis (Figure 1). Risk factors for HDLN metastasis were also investigated (Table 1). Moreover, we detected the prognostic factors, particularly in patients with HDLN metastasis (Table 3 and Figure 2). Third, we assessed the therapeutic value of HDLN dissection to determine its optimal indication (Table 4). The incidence of each lymph node metastasis was calculated by dividing the number of patients with pathological lymph node metastasis by the number of the patients with the lymph node dissection. The index of therapeutic value of lymphadenectomy was calculated by multiplying the incidence of each lymph node metastasis by the five-year survival rate of the patients with metastasis at each nodal station and then dividing by 100[20].

Statistical analysis

All continuous variables were classified into two groups based on the median value of each parameter. The χ^2 test and Fisher's exact test were used to compare clinicopathological characteristics between patients with and without HDLN metastasis. Multivariate stepwise logistic regression analysis was performed to identify the independent risk factors associated with HDLN

Table 2 Complications after gastrectomy with extended lymphadenectomy

Complications	*n* (%)
Anastomotic leakage	12 (4.3)
Pancreatic fistula	8 (2.9)
Intra-abdominal abscess	4 (1.4)
Surgical site infection	26 (9.4)
Pneumonia	4 (1.4)
Cholecystitis	3 (1.1)
Pancreatitis	6 (2.2)
Hepatic dysfunction	2 (0.7)
Stenosis at anastomotic site	10 (3.6)
Ileus	11 (4.0)
Post-operative hemorrhage	2 (0.7)

metastasis. Survival curves were estimated using the Kaplan-Meier method, and statistical differences were examined using the log-rank test. Univariate and multivariate survival analyses were performed using the likelihood ratio test of the stratified Cox proportional hazards model. $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathological characteristics and perioperative clinical outcomes in patients who underwent HDLN dissection

We investigated clinicopathological characteristics in the patients who underwent gastrectomy with D2 lymphadenectomy, including HDLN dissection, based on Japanese guidelines (Table 1). Tumours in the middle or lower stomach ($P = 0.029$), tumour size of 45 mm and more ($P = 0.007$), the presence of lymphatic invasion ($P = 0.002$), and T category of T3 or T4 ($P < 0.0001$) were more frequently observed in the patients with HDLN metastasis. Multivariate stepwise logistic regression analysis identified tumours in the middle or lower stomach ($P = 0.005$, OR = 5.88) and T category of T3 or T4 ($P = 0.017$, OR = 4.45) as independent risk factors of HDLN metastasis. We also analysed complications that were observed after D2 gastrectomy, including HDLN lymphadenectomy, which were defined by the Clavien-Dindo classification as type Ⅱ or more[21] (Table 2). The incidence of anastomotic leakage, pancreatic fistula, and intra-abdominal abscess was 4.3%, 2.9%, and 1.4%, respectively, and these were not as high as those in previous reports[11,22]. One patient (0.3%) died as a result of surgery.

Long-term prognosis of patients with or without HDLN metastasis

Next, we analysed the long-term prognosis of the patients. The five-year overall survival (OS) rate of those with or without HDLN metastasis was 29% or 72%, respectively ($P < 0.0001$) (Figure 1A). Limited to node-positive patients, the five-year OS rate of those with or without HDLN metastasis was 29% or 56%, respectively ($P < 0.0001$) (Figure 1B). Median survival time (MST) of the node-positive patients with or without HDLN metastasis was 14.1 mo or 29.7 mo, respectively (Figure 1B).

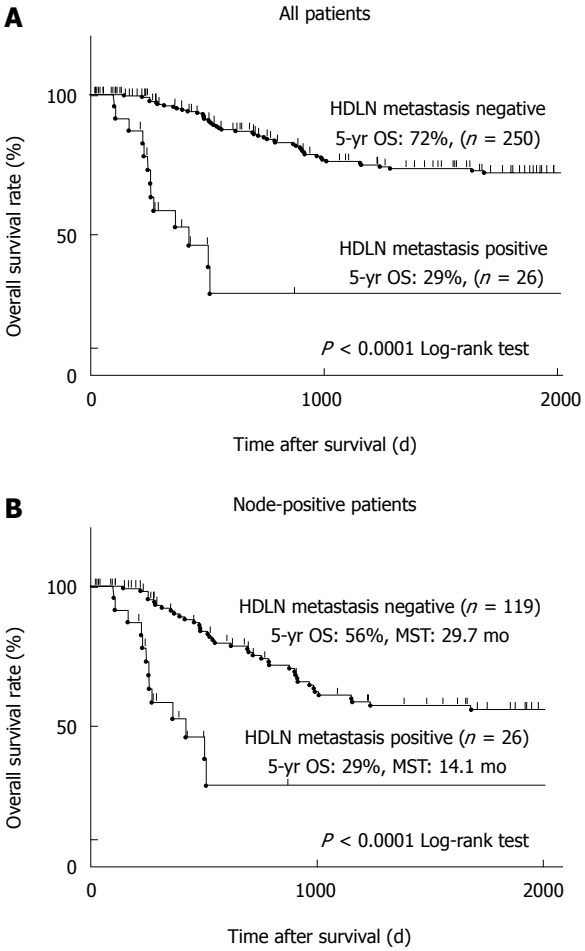


Figure 1 Five-year overall survival of gastric cancer patients based on hepatoduodenal lymph node metastasis. A: All 276 patients analysed in this study were divided into two groups based on hepatoduodenal lymph node (HDLN) metastasis. A group with HDLN metastasis ($n = 26$) and that without HDLN metastasis ($n = 250$) were compared. Kaplan-Meier survival curves were statistically analysed by the log-rank test ($P < 0.0001$); B: The patients who had lymph node metastasis ($n = 145$) were divided into two groups and analysed as described in panel a (26 patients with HDLN metastasis and 119 patients without HDLN metastasis, $P < 0.0001$).

Prognostic factors of patients with HDLN metastasis and correlation between the prognosis and nodal status

We next investigated the prognostic factors of patients with HDLN metastasis. Univariate analysis revealed that pN3 patients, whose total number of metastatic lymph nodes is seven or more, showed significantly poorer prognosis than pN1 or pN2 patients ($P = 0.002$, the five-year survival rate of pN3 or pN1-2 was 0% or 62.5%, respectively) (Table 3 and Figure 2A). Multivariate analysis using the Cox proportional hazard model showed that pN3 was an independent poor prognostic factor in patients with HDLN metastasis

Table 3 Univariate and multivariate analyses of the prognostic factors in patients with hepatoduodenal lymph node metastasis

	n	5-yr OS (%)	MST (mo)	Univariate	Multivariate	
				P value	HR (95%CI)	*P* value
	26	28.7	14.1			
Sex						
Male	16	27.1	12.2			
Female	10	25.7	16.9	0.753	-	
Age (yr)						
< 65	10	38.1	14.1			
⩾ 65	16	0	12.2	0.483	-	
CA 19-9 (U/mL)						
< 10	17	35.7	16.9			
⩾ 10	9	0	12.2	0.327	-	
CEA (ng/mL)						
< 3	16	35.8	14.1			
⩾ 3	10	0	9.1	0.382	-	
Tumor location						
U	2	0	8.7			
M and L	24	30.0	14.1	0.569	-	
Histopathological type						
Differentiated	10	0	16.9			
Undifferentiated	16	40.9	14.1	0.529	-	
Venous invasion						
(-)	12	0	14.1			
(+)	14	16.7	16.9	0.411	-	
Lymphatic invasion						
(-)	3	0	9.1			
(+)	23	26.9	14.1	0.624	-	
Tumor size (mm)						
< 45	5	0	9.1			
⩾ 45	21	31.4	14.1	0.274	-	
T-category						
T1 and T2	3	0	9.1			
T3 and T4	23	31.5	16.9	0.222	-	
N-category						
N1 or N2	10	62.5	16.8			
N3	16	0	8.8	0.002	5.17 (1.8-292.7)	0.021

OS: Overall survival; MST: Median survival time; CEA: Carcinoembryonic antigen; CA 19-9: Carbohydrate antigen 19-9; U: Upper gastric body; M: Middle gastric body; L: Lower gastric body.

(P = 0.021, HR = 5.17) (Table 3). Limited to pN1 and pN2 patients analysed, there was no significant prognostic difference between the patients with and without HDLN metastasis (P = 0.602) (Figure 2B). On the other hand, in pN3 patients, the five-year survival rate of those with or without HDLN metastasis was 0% or 32.2%, and the median survival time (MST) of those with or without HDLN metastasis was 8.8 mo or 30.2 mo (Figure 2C), suggesting that the prognosis of those patients with HDLN metastasis was significantly poorer than that of those without HDLN metastasis (P < 0.001).

Therapeutic value index and therapeutic benefit of HDLN dissection

Lastly, we assessed the index of therapeutic value of lymphadenectomy[20] at each nodal station in all patients who underwent D2 gastrectomy (Table 4). The therapeutic value of lymphadenectomy of HDLN was 2.7, which was relatively low in comparison with those of the perigastric nodes at stations No. 1 to No. 7, which were in the range of 2.7 to 18.2. However, the index of HDLN (No. 12a) for tumours in the middle or lower stomach was 3.4, which was relatively higher than that for tumours in the upper stomach, which was 0.0, suggesting that HDLN dissection could provide some advantages to patients with gastric cancer in the middle or lower stomach.

DISCUSSION

It is still unclear whether HDLN metastasis is a poor prognostic indicator or a determining factor of local metastasis such that its removal by surgery provides survival benefit. Moreover, the indication of HDLN dissection is controversial between Eastern and Western countries. In this study, we hypothesized that there are some far advanced patient subgroups for whom HDLN metastasis could be a poor prognostic indicator, and that there are other subgroups for whom dissection of HDLN with metastasis could provide an advantage. To verify these hypotheses, the clinicopathological factors and survival rates of patients who underwent HDLN dissection were analysed

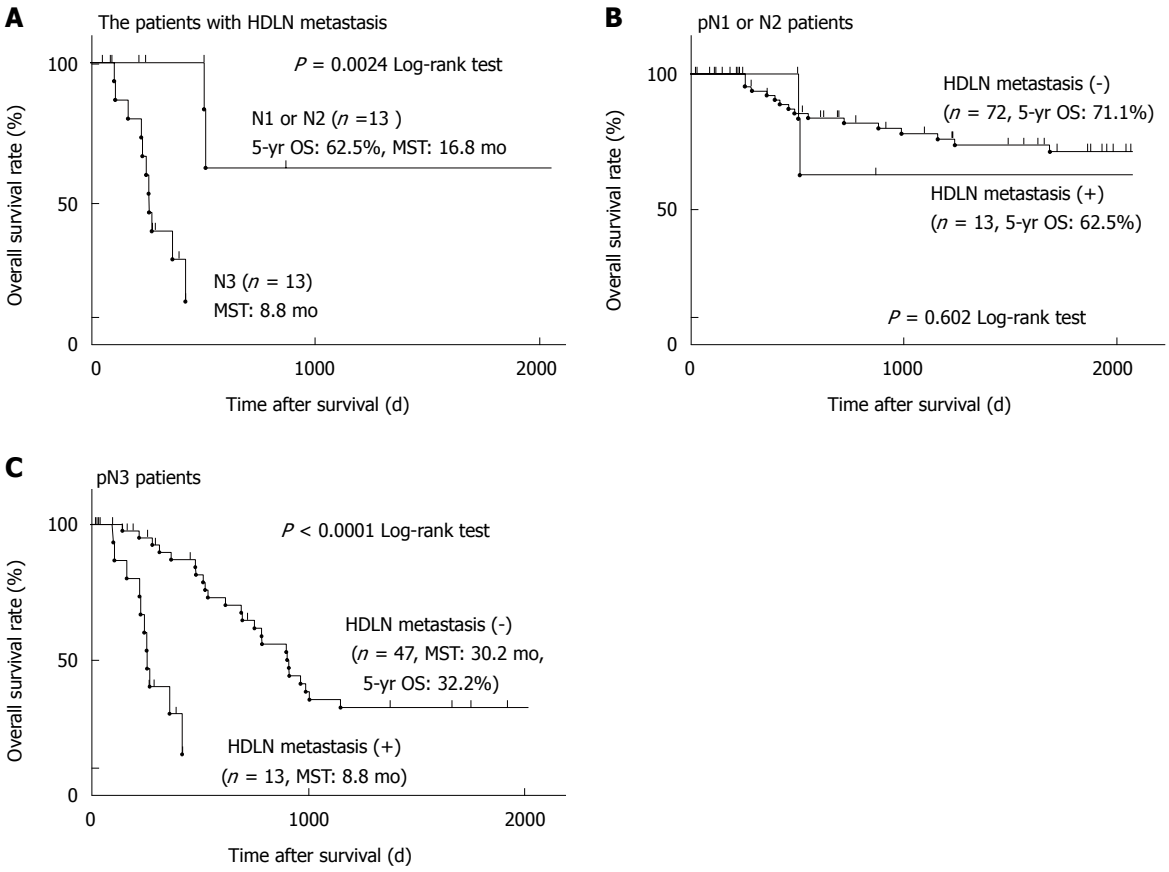


Figure 2 Five-year overall survival curves of gastric cancer patients based on the extent of lymph node metastasis. A: Twenty-six patients who had hepatoduodenal lymph node (HDLN) metastasis were divided into two groups based on the extent of lymph node metastasis. A group of pathological (p)N1 or N2 (n = 10) and that of pN3 (n = 16) were compared as described in Figure 1 (P = 0.0024); B: All 82 pN1 or N2 patients were divided into two groups based on HDLN metastasis and analysed as described in Figure 1 (10 patients with HDLN metastasis and 72 patients without HDLN metastasis, P = 0.602); C: All 63 pN3 patients were analysed as described in Figure 1 (16 patients with HDLN metastasis and 47 patients without HDLN metastasis, P < 0.0001).

retrospectively. Consequently, HDLN metastasis was clearly identified as a poor prognostic indicator in patients with a tumour in the upper stomach or with pN3 gastric cancer, as well as a key factor controlling local recurrence in patients with pN1and pN2 gastric cancer.

According to the JCGC and the treatment guidelines[16,17], the classification of regional lymph nodes and the indication of lymphadenectomy were defined by the extent of gastrectomy, regardless of tumour location. Currently, HDLN is included in the lymph nodes which are routinely removed in advanced gastric cancer patients, even when a tumour is located in the upper stomach. However, our study revealed that patients with a tumour in the upper stomach as well as HDLN metastasis had extremely poor prognosis, and that the index of therapeutic value of HDLN dissection for those patients was 0.0, suggesting it provided no advantages, which is consistent with the previous report[20]. Thus, HDLN dissection for tumours in the upper stomach should not be recommended as a standard procedure of D2 lymphadenectomy. As described above, because the 7th AJCC staging manual[18] defines HDLN metastasis as distant metastasis, HDLN is not removed in Western countries. Our study, however, demonstrated that HDLN dissection provided a relatively high survival rate to the patients who had pN1 or pN2 gastric cancer in the middle or lower stomach, regardless of the presence of HDLN metastasis (Figure 2 and Table 3). Therefore, the tumour location is an important factor in making a preoperative decision about whether HDLN dissection should be performed.

There is no doubt that differences in treatment indications for gastric cancer between Eastern and Western countries are greatly influenced by the features of cancer biology, epidemiology, and surgical difficulties in those countries[11,22-24]. Concerning tumour location, the incidence of upper gastric cancer in the United States (51%) was higher than that in Japan (17%)[22]. This might greatly influence the treatment indication of lymphadenectomy in each country. Recently, however, the incidence of upper gastric cancer is increasing in Eastern countries, as shown in this study [23% (64/276)] (Table 1). Using the multivariate logistic regression analysis (Table 1) and the index of therapeutic values (Table 4), we revealed that the tumour location is an important factor in making a decision about HDLN dissection. Based on this, therefore, we suggest that the guidelines

Table 4 Index of estimated benefit from hepatoduodenal lymph node dissection

Nodal station		Incidence of LNM (%)	5-yr OS of patients with LNM (%)	Index
No. 1		41.0	43.9	18.0
No. 2		16.2	45.8	7.4
No. 3		32.2	56.4	18.2
No. 4sa		8.1	75.0	6.1
No. 4sb		6.6	40.6	2.7
No. 4d		22.9	59.8	13.7
No. 5		12.5	53.2	6.7
No. 6		19.2	60.6	11.6
No. 7		12.8	47.5	6.1
No. 8a		13.3	55.9	7.4
No. 9		19.7	32.1	6.3
No. 10		1.2	100.0	1.2
No. 11p		23.7	56.5	13.4
No. 11d		0.0	NA	NA
No. 12a (HDLN)		9.4	28.7	2.7
	Tumor location			
No. 12a (HDLN)	U	3.1	0.0	0.0
No. 12a (HDLN)	M and L	11.3	30.0	3.4

The index of therapeutic value of lymph node dissection was calculated by multiplying the incidence of metastasis by the 5-year survival rate of patients with metastasis at each nodal station and then dividing by 100[12]. OS: Overall survival; LNM: Lymph node metastasis; HDLN: Hepatoduodenal lymph node; U: Upper gastric body; M: Middle gastric body; L: Lower gastric body; NA: Not applicable.

should include this factor and should be standardized worldwide.

Our results indicate that HDLN dissection could salvage HDLN metastasis in patients with pN2 or lesser lymph node metastasis, because there was no significant prognostic difference between the patients with and without HDLN metastasis (P = 0.602) after radical HDLN dissection. Other studies, however, reported the potential survival benefit of lymphadenectomy, such as prophylactic para-aortic lymph node (PAN) dissection, for curable gastric cancer in a limited number of patients[25,26], whereas it was not demonstrated in a randomized clinical trial (RCT) (JCOG9501)[27]. Moreover, prospective RCTs, such as JCOG0001 and JCOG0405[28,29], demonstrated the survival benefit of neoadjuvant chemotherapy followed by extended surgery with PAN dissection for gastric cancer patients with only PAN or bulky N2 metastasis (limited putative pN3 metastasis). Similarly to these studies, we speculate that the survival benefit may also be obtained in pN3 patients by neoadjuvant chemotherapy followed by extended surgery with HDLN dissection. For this issue, further prospective studies are warranted.

Our results indicate the significance in making a decision about HDLN dissection depending on the tumour location and pathological N-category. However, there is a major problem in diagnosing metastatic lymph nodes accurately before surgery using current imaging methods. We previously generated a simple math formula to estimate preoperative metastatic nodal counts using multidetector row computed tomography (MDCT); Pathologic counts = 1.63 × (counts by MDCT) + 2.5[30]. Based on this formula, 3 or more nodal counts by MDCT might be considered pN3. Therefore, for patients with less than 3 putative metastatic nodal counts by MDCT, who are considered pN1 or pN2, D2 lymphadenectomy with HDLN dissection should be performed. On the other hand, for patients with 3 or more nodal counts by MDCT, neoadjuvant chemotherapy may be considered as a treatment choice before surgical resection with extended lymphadenectomy, including HDLN dissection.

Our study, however, have some limitations. The population of the cohort was relatively small. A prospective study may be needed to validate the indication of HDLN dissection. Therefore, although the therapeutic value of HDLN dissection for gastric cancer is currently limited, we would suggest that HDLN dissection can provide a prognostic benefit to pN1 and pN2 gastric cancer patients whose tumour is located in the middle or lower stomach.

COMMENTS

Background

It is still unclear whether hepatoduodenal lymph node (HDLN) metastasis is a poor prognostic indicator or a determining factor of local metastasis such that its removal by surgery provides survival benefit. Moreover, the indication of HDLN dissection is controversial between Eastern and Western countries.

Research frontiers

This study was designed to evaluate the clinical characteristics and impact of HDLN metastasis on the clinical course and to clarify the optimal indication of HDLN dissection.

Innovations and breakthroughs

HDLN metastasis was clearly identified as a poor prognostic indicator in patients with a tumour in the upper stomach or with pN3 gastric cancer, as well as a key factor controlling local recurrence in patients with pN1and pN2 gastric cancer.

Applications

The results indicate the significance in making a decision about HDLN dissection depending on the tumour location and pathological N-category. For patients with lesions considered as pN1 or pN2, D2 lymphadenectomy with HDLN dissection should be performed.

Terminology

Hepatoduodenal lymph node is defined as a hepatoduodenal ligament lymph node along the proper hepatic artery, and the Japanese treatment guidelines recommend it to be routinely removed as a standard procedure for D2 lymphadenectomy. On the other hand, the HDLN is not removed in Western countries, and HDLN metastasis is classified as distant metastasis according to the 7th American Joint Committee on Cancer staging manual.

Peer-review

The study is of high level, the results shown in this work could be a contribution for that the medical teams take a good decision that can help a better survival of the patient. The results could be taken in account in different hospitals in the world.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global

cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
2 **Wadhwa R**, Song S, Lee JS, Yao Y, Wei Q, Ajani JA. Gastric cancer-molecular and clinical dimensions. *Nat Rev Clin Oncol* 2013; **10**: 643-655 [PMID: 24061039 DOI: 10.1038/nrclinonc.2013.170]
3 **Hartgrink HH**, Jansen EP, van Grieken NC, van de Velde CJ. Gastric cancer. *Lancet* 2009; **374**: 477-490 [PMID: 19625077 DOI: 10.1016/S0140-6736(09)60617-6]
4 **Zhang J**, Zhou Y, Jiang K, Shen Z, Ye Y, Wang S. Evaluation of the seventh AJCC TNM staging system for gastric cancer: a meta-analysis of cohort studies. *Tumour Biol* 2014; **35**: 8525-8532 [PMID: 24696259 DOI: 10.1007/s13277-014-1848-6]
5 **Dikken JL**, Verheij M, Cats A, Jansen EP, Hartgrink HH, van de Velde CJ. Extended lymph node dissection for gastric cancer from a European perspective. *Gastric Cancer* 2011; **14**: 396-398 [PMID: 21837457 DOI: 10.1007/s10120-011-0081-x]
6 **Brar S**, Law C, McLeod R, Helyer L, Swallow C, Paszat L, Seevaratnam R, Cardoso R, Dixon M, Mahar A, Lourenco LG, Yohanathan L, Bocicariu A, Bekaii-Saab T, Chau I, Church N, Coit D, Crane CH, Earle C, Mansfield P, Marcon N, Miner T, Noh SH, Porter G, Posner MC, Prachand V, Sano T, van de Velde C, Wong S, Coburn N. Defining surgical quality in gastric cancer: a RAND/UCLA appropriateness study. *J Am Coll Surg* 2013; **217**: 347-357.e1 [PMID: 23664139 DOI: 10.1016/j.jamcollsurg.2013.01.067]
7 **Allum WH**. Optimal surgery for gastric cancer: is more always better? *Recent Results Cancer Res* 2012; **196**: 215-227 [PMID: 23129377 DOI: 10.1007/978-3-642-31629-6_15]
8 **Russell MC**, Mansfield PF. Surgical approaches to gastric cancer. *J Surg Oncol* 2013; **107**: 250-258 [PMID: 22674546 DOI: 10.1002/jso.23180]
9 **Schmidt B**, Yoon SS. D1 versus D2 lymphadenectomy for gastric cancer. *J Surg Oncol* 2013; **107**: 259-264 [PMID: 22513454 DOI: 10.1002/jso.23127]
10 **Wong J**, Jackson P. Gastric cancer surgery: an American perspective on the current options and standards. *Curr Treat Options Oncol* 2011; **12**: 72-84 [PMID: 21274666 DOI: 10.1007/s11864-010-0136-y]
11 **Bonenkamp JJ**, Hermans J, Sasako M, van de Velde CJ, Welvaart K, Songun I, Meyer S, Plukker JT, Van Elk P, Obertop H, Gouma DJ, van Lanschot JJ, Taat CW, de Graaf PW, von Meyenfeldt MF, Tilanus H. Extended lymph-node dissection for gastric cancer. *N Engl J Med* 1999; **340**: 908-914 [PMID: 10089184]
12 **Bickenbach K**, Strong VE. Comparisons of Gastric Cancer Treatments: East vs. West. *J Gastric Cancer* 2012; **12**: 55-62 [PMID: 22792517 DOI: 10.5230/jgc.2012.12.2.55]
13 **Songun I**, Putter H, Kranenbarg EM, Sasako M, van de Velde CJ. Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial. *Lancet Oncol* 2010; **11**: 439-449 [PMID: 20409751 DOI: 10.1016/S1470-2045(10)70070-X]
14 **Strong VE**, Yoon SS. Extended lymphadenectomy in gastric cancer is debatable. *World J Surg* 2013; **37**: 1773-1777 [PMID: 23649527 DOI: 10.1007/s00268-013-2070-1]
15 **Degiuli M**, Sasako M, Ponti A. Morbidity and mortality in the Italian Gastric Cancer Study Group randomized clinical trial of D1 versus D2 resection for gastric cancer. *Br J Surg* 2010; **97**: 643-649 [PMID: 20186890 DOI: 10.1002/bjs.6936]
16 **Japanese Gastric Cancer Association**. Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer* 2011; **14**: 101-112 [PMID: 21573743 DOI: 10.1007/s10120-011-0041-5]
17 **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]
18 **Edge SB**, Byrd DR, Compton CC. American Joint Committee on Cancer (AJCC) Cancer Staging Manual (7th edn). Springer: New York, 2010
19 **Lee SL**, Lee HH, Ko YH, Song KY, Park CH, Jeon HM, Kim SS. Relevance of hepatoduodenal ligament lymph nodes in resectional surgery for gastric cancer. *Br J Surg* 2014; **101**: 518-522 [PMID: 24615472 DOI: 10.1002/bjs.9438]
20 **Sasako M**, McCulloch P, Kinoshita T, Maruyama K. New method to evaluate the therapeutic value of lymph node dissection for gastric cancer. *Br J Surg* 1995; **82**: 346-351 [PMID: 7796005]
21 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542]
22 **Noguchi Y**, Yoshikawa T, Tsuburaya A, Motohashi H, Karpeh MS, Brennan MF. Is gastric carcinoma different between Japan and the United States? *Cancer* 2000; **89**: 2237-2246 [PMID: 11147594]
23 **Shim JH**, Song KY, Jeon HM, Park CH, Jacks LM, Gonen M, Shah MA, Brennan MF, Coit DG, Strong VE. Is gastric cancer different in Korea and the United States? Impact of tumor location on prognosis. *Ann Surg Oncol* 2014; **21**: 2332-2339 [PMID: 24599411 DOI: 10.1245/s10434-014-3608-7]
24 **Strong VE**, Song KY, Park CH, Jacks LM, Gonen M, Shah M, Coit DG, Brennan MF. Comparison of gastric cancer survival following R0 resection in the United States and Korea using an internationally validated nomogram. *Ann Surg* 2010; **251**: 640-646 [PMID: 20224369 DOI: 10.1097/SLA.0b013e3181d3d29b]
25 **Maeta M**, Yamashiro H, Saito H, Katano K, Kondo A, Tsujitani S, Ikeguchi M, Kaibara N. A prospective pilot study of extended (D3) and superextended para-aortic lymphadenectomy (D4) in patients with T3 or T4 gastric cancer managed by total gastrectomy. *Surgery* 1999; **125**: 325-331 [PMID: 10076618]
26 **Kunisaki C**, Akiyama H, Nomura M, Matsuda G, Otsuka Y, Ono H, Nagahori Y, Hosoi H, Takahashi M, Kito F, Shimada H. Comparison of surgical results of D2 versus D3 gastrectomy (para-aortic lymph node dissection) for advanced gastric carcinoma: a multi-institutional study. *Ann Surg Oncol* 2006; **13**: 659-667 [PMID: 16538414]
27 **Sasako M**, Sano T, Yamamoto S, Kurokawa Y, Nashimoto A, Kurita A, Hiratsuka M, Tsujinaka T, Kinoshita T, Arai K, Yamamura Y, Okajima K. D2 lymphadenectomy alone or with para-aortic nodal dissection for gastric cancer. *N Engl J Med* 2008; **359**: 453-462 [PMID: 18669424 DOI: 10.1056/NEJMoa0707035]
28 **Yoshikawa T**, Sasako M, Yamamoto S, Sano T, Imamura H, Fujitani K, Oshita H, Ito S, Kawashima Y, Fukushima N. Phase II study of neoadjuvant chemotherapy and extended surgery for locally advanced gastric cancer. *Br J Surg* 2009; **96**: 1015-1022 [PMID: 19644974 DOI: 10.1002/bjs.6665]
29 **Tsuburaya A**, Mizusawa J, Tanaka Y, Fukushima N, Nashimoto A, Sasako M. Neoadjuvant chemotherapy with S-1 and cisplatin followed by D2 gastrectomy with para-aortic lymph node dissection for gastric cancer with extensive lymph node metastasis. *Br J Surg* 2014; **101**: 653-660 [PMID: 24668391 DOI: 10.1002/bjs.9484]
30 **Kawaguchi T**, Komatsu S, Ichikawa D, Okamoto K, Shiozaki A, Fujiwara H, Murayama Y, Kuriu Y, Ikoma H, Nakanishi M, Ochiai T, Kokuba Y, Nishimura T, Otsuji E. Nodal counts on MDCT as a surrogate marker for surgical curability in gastric cancer. *Ann Surg Oncol* 2012; **19**: 2465-2470 [PMID: 22395992 DOI: 10.1245/s10434-012-2283-9]

P- Reviewer: Beltran MA, Bernal G, Caboclo JLF, Hsieh CC
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10874

World J Gastroenterol 2015 October 14; 21(38): 10874-10882
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Management of entecavir-resistant chronic hepatitis B with adefovir-based combination therapies

Hyoung Su Kim, Hyung Joon Yim, Myoung Kuk Jang, Ji Won Park, Sang Jun Suh, Yeon Seok Seo, Ji Hoon Kim, Bo Hyun Kim, Sang Jong Park, Sae Hwan Lee, Sang Gyune Kim, Young Seok Kim, Jung Il Lee, Jin-Woo Lee, In Hee Kim, Tae Yeob Kim, Jin-Wook Kim, Sook-Hyang Jeong, Young Kul Jung, Hana Park, Seong Gyu Hwang; on behalf of Antiviral Resistance Study Group

Hyoung Su Kim, Myoung Kuk Jang, Ji Won Park, Department of Internal Medicine, Hallym University College of Medicine, Seoul 134-701, South Korea

Hyung Joon Yim, Sang Jun Suh, Young Kul Jung, Department of Internal Medicine, Korea University Ansan Hospital, Ansan 425-707, South Korea

Yeon Seok Seo, Ji Hoon Kim, Department of Internal Medicine, Korea University Medical College, Seoul 136-705, South Korea

Bo Hyun Kim, Sang Jong Park, Department of Internal Medicine, Bundang Jesaeng Hospital, Seongnam 463-774, South Korea

Sae Hwan Lee, Department of Internal Medicine, Soon Chun Hyang University College of Medicine, Cheonan 330-721, South Korea

Sang Gyune Kim, Young Seok Kim, Department of Internal Medicine, Soon Chun Hyang University College of Medicine, Bucheon 330-721, South Korea

Jung Il Lee, Jin-Woo Lee, Department of Internal Medicine, Inha University School of Medicine, Incheon 400-711, South Korea

In Hee Kim, Department of Internal Medicine, Chonbuk National University Medical School, Jeonju 561-712, South Korea

Tae Yeob Kim, Department of Internal Medicine, Hanyang University College of Medicine, Guri 471-701, South Korea

Jin-Wook Kim, Sook-Hyang Jeong, Department of Internal Medicine, Seoul National University College of Medicine, Seongnam 463-707, South Korea

Young Kul Jung, Department of Internal Medicine, Gachon University School of Medicine, Incheon 405-760, South Korea

Hana Park, Seong Gyu Hwang, Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam 463-712, South Korea

Author contributions: Kim HS, Yim HJ and Hwang SG designed the research; Kim HS, Yim HJ, Jang MK, Park JW, Suh SJ, Seo YS, Kim JH, Kim BH, Park SJ, Lee SH, Kim SG, Kim YS, Lee JI, Lee JW, Kim IH, Kim TY, Kim JW, Jeong SH, Jung YK, Park H and Hwang SG performed research; Yim HJ collected data; Kim HS analyzed the data and wrote the paper; and Yim HJ and Hwang SG share corresponding authorship.

Supported by Research Funds from the Korean Association for the Study of the Liver (in part).

Institutional review board statement: The study was reviewed and approved by the local ethics committee (Korea University Ansan Hospital, approval No. AS11102-001).

Informed consent statement: All participants provided written informed consent prior to study enrollment.

Conflict-of-interest statement: The authors have no conflicts of interest to declare with respect to this manuscript.

Data sharing statement: Participants gave informed consent for data sharing. Technical appendix, statistical code, and dataset are available from one of the corresponding author at gudwns21@medimail.co.kr. No additional data are available.

Correspondence to: Hyung Joon Yim, MD, PhD, Department of Internal Medicine, Korea University Ansan Hospital, 123

Jeokgeum-ro, Danwon-Gu, Ansan-Si, Gyeonggi-Do 425-707, South Korea. gudwns21@medimail.co.kr
Telephone: +82-31-4126565
Fax: +82-31-4125582

Received: February 2, 2015
Peer-review started: February 5, 2015
First decision: March 26, 2015
Revised: May 13, 2015
Accepted: July 18, 2015
Article in press: July 18, 2015
Published online: October 14, 2015

Abstract

AIM: To evaluate the long-term efficacy adefovir (ADV)-based combination therapies in entecavir (ETV)-resistant chronic hepatitis B (CHB) patients.

METHODS: Fifty CHB patients with genotypic resistance to ETV at 13 medical centers in South Korea were included for the analysis. All the patients received rescue therapy with the combination of ADV plus ETV (ADV/ETV, *n* = 23) or ADV plus lamivudine (LMV) (ADV/LMV, *n* = 27) for more than 12 mo. Patients were monitored at least every 3-4 mo during ADV-based combination therapy by clinical examination as well as biochemical and virological assessments. Hepatitis B virus (HBV) DNA levels were measured by real-time PCR and logarithmically transformed for analysis. Cumulative rates of virologic response (VR; HBV DNA < 20 IU/mL) were calculated using the Kaplan-Meier method, and the difference was determined by a log-rank test. Multivariate logistic regression and Cox proportional hazards models were used to identify independent risk factors significantly associated with short-term and long-term VR, respectively.

RESULTS: Baseline median HBV DNA levels were 5.53 (2.81-7.63) $\log_{10}$ IU/mL. The most commonly observed ETV genotypic mutation sites were rt184 and rt202. Patients were treated for a median of 27 (12-45) mo. Overall, cumulative VR rates at 6, 12, 24, and 36 mo were 26%, 36%, 45%, and 68%, respectively. Patients treated with the ADV/ETV combination showed higher cumulative VR rates (35%, 43%, 65%, and 76%, respectively) than those with the ADV/LAM combination (18%, 30%, 30%, and 62%, respectively; $P = 0.048$). In the multivariate analysis, low baseline HBV DNA levels (< 5.2 $\log_{10}$ IU/mL) and initial virologic response at 3 mo (IVR-3; HBV DNA < 3.3 $\log_{10}$ IU/mL after 3 mo) were independent predictive factors for VR. Patients with favorable predictors achieved cumulative VR rates up to 90% at 36 mo. During the same period, the cumulative incidence of virologic breakthrough was as low as 6% in patients with the both favorable predictors.

CONCLUSION: If tenofovir is not available, ADV/ETV combination could be considered in ETV-resistant patients with low HBV DNA titers, and may be continued if IVR-3 is achieved.

Key words: Adefovir; Chronic hepatitis B; Entecavir; Lamivudine; Resistance

Core tip: Studies regarding optimal treatment strategies for entecavir-resistant chronic hepatitis B are sparse. Tenofovir may be the best option, but it is still not available in many countries. Where tenofovir is not available, adefovir plus entecavir can be considered an alternative treatment option in patients with favorable predictive factors. These factors included lower baseline hepatitis B virus (HBV) DNA levels (< 5.2 $\log_{10}$ IU/mL) and reduction of HBV DNA < 3.3 $\log_{10}$ IU/mL after 3 mo of treatment in our study. The present study will guide the treatment of entecavir-resistant chronic hepatitis B.

Kim HS, Yim HJ, Jang MK, Park JW, Suh SJ, Seo YS, Kim JH, Kim BH, Park SJ, Lee SH, Kim SG, Kim YS, Lee JI, Lee JW, Kim IH, Kim TY, Kim JW, Jeong SH, Jung YK, Park H, Hwang SG; on behalf of Antiviral Resistance Study Group. Management of entecavir-resistant chronic hepatitis B with adefovir-based combination therapies. *World J Gastroenterol* 2015; 21(38): 10874-10882 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10874.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10874

INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains an important global health problem, and 15%-40% of infected patients may develop cirrhosis-related complications and/or hepatocellular carcinoma (HCC)[1]. Over the past decades, there have been great advances in the management of chronic hepatitis B (CHB) owing to the development of oral nucleos(t)ide analogues (NAs)[2]. The sustained suppression of serum HBV DNA by these agents has been associated with the prevention of liver disease progression and inhibition of HCC development[3,4]. However, a major shortcoming of these NAs is the high rate of virological relapse when treatment is discontinued[5,6]. Therefore, long-term or indefinite treatment with NAs is needed. Unfortunately, the risk of drug resistance increases in proportion to the duration of NAs therapy[7]. For example, cumulative lamivudine (LMV) resistance rates were reported to be 23% and 71% after 1 and 4 years of LMV therapy, respectively[8,9]. Moreover, NAs discontinuation sometimes results in hepatitis flares that may lead to fulminant hepatic failure and death[10]. Thus, the benefits of therapy are attenuated and subsequent therapeutic options may be limited.

Of the NAs, entecavir (ETV) is one of the most potent and safest antiviral agents for HBV infection, with a superior potency to LMV and adefovir (ADV)[11-13].

A previous study showed that the cumulative probability of ETV resistance in treatment naïve patients remained at only 1.2% after up to 5 years of treatment[14]. However, the rate is higher in LMV-resistant patients[15,16], and it may increase to 51% after 5 years of ETV therapy[14]. Resistance to ETV appears to occur through a two-hit mechanism with an initial selection of the M204V/I mutation followed by amino acid substitutions at rtT184, rtS202, or rtM250[17]. Consequently, for CHB patients with LMV resistance, current international guidelines recommend switching to tenofovir disoproxil fumarate (TDF), adding on TDF, or adding on ADV, but not switching to ETV monotherapy[18,19]. However, earlier international guidelines had recommended switching to 1 mg of ETV per day as a treatment option for CHB patients infected with HBV resistant to LMV due to insufficient clinical data[2,20]. As a result of sequential ETV monotherapy in LMV-resistant patients, resistance to ETV developed in a substantial number of patients currently.

For patients with an ETV-resistant CHB, switching to or adding on TDF or TDF-emtricitabine combination therapy are considered as therapeutic options, and combination therapy with ADV plus NAs may still be used in countries where TDF is not available[19,21,22]. It has been shown that both ADV and TDF are active *in vitro* against ETV-resistant HBV infection, but clinical data on the efficacy of ADV or TDF in patients infected with ETV-resistant HBV strains are limited[21,23-26].

Although there have been few reports on the short-term effects of ADV combination therapy for ETV-resistant HBV infection, especially for that developed after LMV-ETV sequential monotherapy[23,24,27], there is little available clinical information regarding the long-term effects of ADV combination therapy in such patients. Therefore, this study aimed to evaluate the long-term efficacy of combined ADV regimens over 48 wk in CHB patients with ETV resistance.

MATERIALS AND METHODS

Patients and study design

A total of 50 CHB patients with genotypic ETV resistance, who subsequently received rescue ADV-based combination therapy for more than 12 mo at 13 medical centers in South Korea between January 2008 and October 2012, were enrolled in this retrospective cohort study. ETV resistance was documented in all patients by genotypic analyses at the time of switching to ADV-based combination therapy. We excluded patients infected with other viruses such as hepatitis C virus, human immunodeficiency virus, or hepatitis D virus and those with other concomitant liver diseases such as alcoholic liver disease, autoimmune liver disease, or HCC. All patients were monitored at least every 3-4 mo during ADV-based combination therapy by clinical examination as well as biochemical and virological assessments.

The study was approved by the Institutional Review Boards of each institution, and informed written consent was obtained from all study participants, or their legal guardian. The protocol conforms to the ethical guidelines of the Declaration of Helsinki.

Laboratory assay

Routine biochemical tests were performed using standard laboratory procedures. Hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), and antibody to HBeAg (anti-HBe) levels were measured using a microparticle enzyme immunoassay (Abbott Laboratories, North Chicago, IL, United States). Serum HBV DNA levels were measured by the COBAS TaqMan PCR assay (Roche, Branchburg, NJ, United States; lower limit of detection: 20 IU/mL). Genotypic resistance to LMV, ADV, and ETV was determined by direct sequencing (TRUGENE HBV, Siemens Health Care Diagnostic Solutions, Tarrytown, NY, United States) or restriction fragment mass polymorphism analysis, as previously described[28].

Definitions

Primary non-response was defined as a failure to reduce serum HBV DNA levels by > 1 $\log_{10}$ IU/mL after 3 mo of treatment[29]. Initial virologic response at 3 mo (IVR-3) and virologic response (VR) were defined as an HBV DNA level < 3.3 $\log_{10}$ IU/mL after 3 mo of treatment[28,30] and an undetectable HBV DNA level (< 20 IU/mL) during treatment, respectively. A biochemical response was defined as normalization of serum alanine aminotransferase (ALT) levels. Virological breakthrough (VBT) was defined as an increase in serum HBV DNA level > 1 $\log_{10}$ IU/mL from the nadir during therapy.

Statistical analysis

HBV DNA levels were logarithmically transformed for analysis. Continuous variables were analyzed using the Mann-Whitney *U*-test, whereas categorical variables were analyzed using the χ^2 test. A repeated measure analysis was used to compare HBV DNA level reductions according to ADV combination regimens. Cumulative rates of VR and VBT were calculated using the Kaplan-Meier method, and the difference was determined by a log-rank test. Multivariate logistic regression and Cox proportional hazards models were used to identify independent risk factors significantly associated with short-term and long-term VR, respectively. Candidate variables with a *P*-value < 0.1 on univariate analysis were entered into the regression analysis. A *P*-value < 0.05 was considered significant. Statistical analyses were performed using SPSS, version 16 (SPSS Inc., Chicago, IL, United States) and the statistical review of the study was performed by a biomedical statistician.

Table 1 Baseline characteristics of the patients *n* (%)

Variables	Total (*n* = 50)
Age (yr)[1]	46.5 (22-74)
Male	37 (74)
HBeAg-positive	47 (94)
Cirrhosis	12 (24)
Antiviral history before ETV (naïve/clevudine/LMV)	2/2/46 (4/4/92)
Duration of ETV (mo)[1]	24 (13-58)
Serum ALT (IU/L)[1]	31 (5-1704)
Serum total bilirubin level (mg/dL)[1]	0.84 (0.28-4.30)
Serum albumin level (g/dL)[1]	4.2 (3.6-5.1)
INR[1]	1.01 (0.87-1.30)
Serum HBV DNA level (log_{10} IU/mL)[1]	5.53 (2.81-7.63)
Duration of ADV combination therapy (mo)[1]	27 (12-45)
Site of ETV-resistant mutations added on rtM204V/I	
rt184	19 (38)
rt202	22 (44)
rt173	1 (2)
rt169 + rt184	1 (2)
rt184 + rt202	6 (12)
rt184 + rt250	1 (2)
Patients with elevated ALT level above ULN	18 (36)
Rescue therapy regimens [(ADV + LMV)/(ADV + ETV)]	27/23 (54/46)

[1]Data are expressed as median (range). ADV: Adefovir; ALT: Alanine aminotransferase; ETV: Entecavir; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; INR: International normalized ratio; LMV: Lamivudine; ULN: Upper limit of normal.

RESULTS

Baseline characteristics of the patients

A total of 50 patients who met the inclusion criteria were analyzed. The patients' baseline characteristics are summarized in Table 1. Thirty-seven (74%) patients were men and the median age was 46.5 (22-74) years. Twelve patients (24%) had liver cirrhosis and 47 patients (94%) were positive for HBeAg. The median HBV DNA level was 5.53 (2.81-7.63) log_{10} IU/mL and 18 patients had elevated serum ALT levels above the upper limit of normal (40 IU/L). The most commonly observed ETV genotypic mutation sites were rt184 and rt202. The median duration of ETV therapy was 24 (13-58) mo. Out of the total 50 patients, 27 received ADV/LMV combination therapy and 23 received ADV/ETV combination therapy. The median duration of ADV combination therapy was 27 (12-45) mo.

Treatment response

Figure 1 shows the changes in mean HBV DNA levels during the first 12 mo of treatment. After the start of ADV combination therapy, serum HBV DNA levels declined continuously with overall mean changes of -2.14 log_{10} IU/mL, -2.37 log_{10} IU/mL, and -2.67 log_{10} IU/mL at months 3, 6, and 12, respectively. The mean reduction in serum HBV DNA levels from baseline to month 12 was significantly greater in the ADV/ETV combination group than in the ADV/LMV combination group (-2.77 *vs* -2.57 log_{10} IU/mL, P = 0.028) by repeated measure analysis (Figure 1). During the first year of treatment, VR (HBV DNA levels < 20 IU/mL)

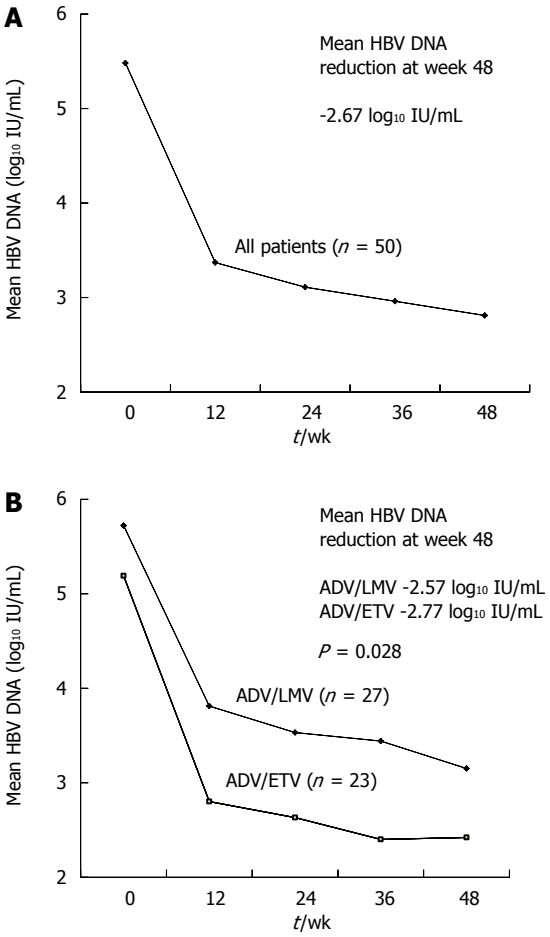


Figure 1 Changes of hepatitis B virus DNA levels during 48 wk. A: The overall mean changes of hepatitis B virus (HBV) DNA levels from baseline; B: The mean reduction of serum HBV DNA levels in adefovir plus entecavir (ADV/ETV) combination group and in the adefovir plus lamivudine (ADV/LMV) combination group.

and primary non-response were observed in 18 (36%) and 9 (18%) patients, respectively. Eight of the 18 patients who showed elevated serum ALT levels at baseline experienced normalization of serum ALT levels (44.4%). During the first year of ADV combination therapy, HBeAg loss occurred in 6 (12.8%) of the 47 HBeAg positive patients. Of these, one patient experienced HBeAg seroconversion.

During the long-term treatment period that lasted a median of 27 mo, VR, HBeAg loss, and biochemical response were achieved in an additional 9, 3, and 6 patients, respectively.

Cumulative VR rates at 6, 12, 24, and 36 mo were 26%, 36%, 45%, and 68%, respectively (Figure 2A). Cumulative VR rates at 6, 12, 24, and 36 mo were, respectively, 35%, 43%, 65%, and 76% in the ADV/ETV combination group and 18%, 30%, 30%, and 62% in the ADV/LMV combination group. There was a significant difference between the two groups (P = 0.048; Figure 2B).

Predictive factors of virologic response

Of the clinical features, a longer duration of ETV

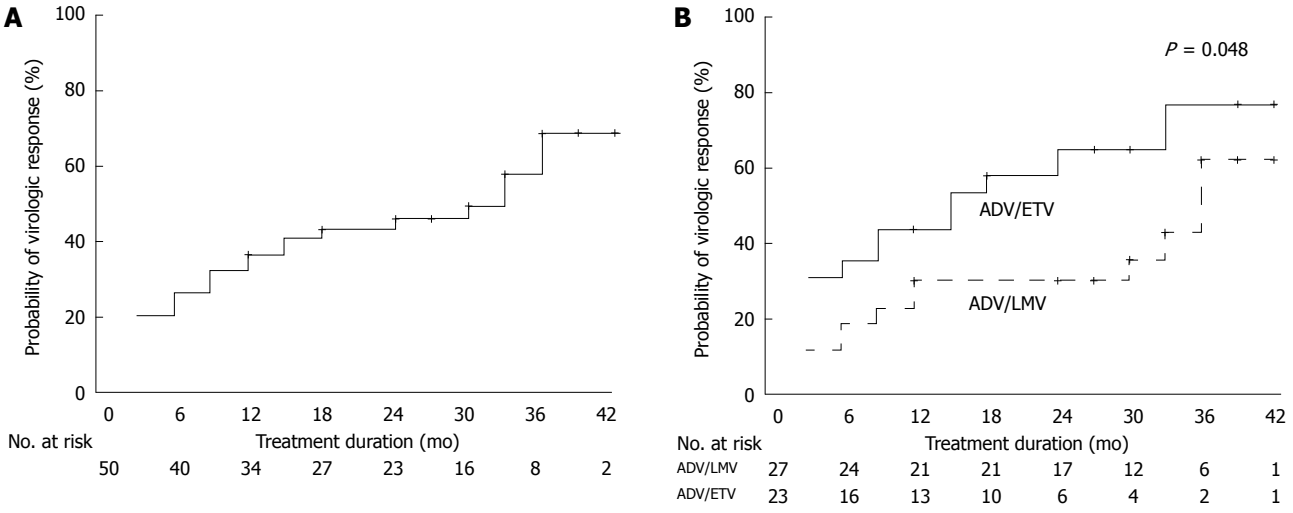


Figure 2 Virologic responses according to type of treatments up to 36 mo. A: Overall cumulative virologic response rates at 6, 12, 24, and 36 mo; B: Cumulative virologic response rates in the adefovir plus entecavir (ADV/ETV) combination group and in the adefovir plus lamivudine (ADV/LMV) combination group (P = 0.048).

Table 2 Comparison of clinical features between groups according to 1-year virologic response *n* (%)

	Patients without VR (n = 32)	Patients with VR (n = 18)	P value
Age (yr)[1]	47 (22-70)	42.5 (33-74)	0.413
Male	22 (68.8)	15 (83.3)	0.328
HBeAg-positive	31 (96.9)	16 (88.9)	0.291
Cirrhosis	8 (25)	4 (22.2)	1.000
Duration of ETV therapy (mo)[1]	24 (13-48)	36 (17-58)	0.003
Serum ALT level (IU/L)[1]	34.5 (12-918)	29 (5-1704)	0.210
Serum total bilirubin level (mg/dL)[1]	0.84 (0.31-1.99)	0.79 (0.28-4.30)	0.869
Serum albumin level (g/dL)[1]	4.2 (3.6-5.1)	4.3 (3.6-4.9)	0.691
INR[1]	1.01 (0.93-1.23)	1.02 (0.87-1.30)	0.848
Serum HBV DNA level (log_{10} IU/mL)[1]	6.16 (3.85-7.63)	4.24 (2.81-7.08)	< 0.001
Site of ETV-resistant mutations			0.441
rt184	12 (37.5)	7 (38.9)	
rt202	14 (43.8)	8 (44.4)	
rt173	0 (0)	1 (5.6)	
rt169 + rt184	0 (0)	1 (5.6)	
rt184 + rt202	5 (15.6)	1 (5.6)	
rt184 + rt250,	1 (3.1)	0 (0)	
Presence of IVR-3	7 (21.9)	17 (94.4)	< 0.001
Rescue therapy regimens (ADV/LMV *vs* ADV/ETV)	19 *vs* 13 (59.4 *vs* 40.6)	8 *vs* 10 (44.4 *vs* 55.6)	0.382

[1]Data are expressed as median (range). ADV: Adefovir; ALT: Alanine aminotransferase; ETV: Entecavir; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; INR: International normalized ratio; IVR-3: Initial virologic response at 3 mo; LMV: Lamivudine; VR: Virologic response.

Table 3 Multivariate analyses of clinical factors affecting one-year virologic response

	RR	95%CI	P value
Duration of ETV therapy (mo)	1.039	0.936-1.153	0.473
Serum HBV DNA level (< 5.2 log_{10} IU/mL)	7.614	1.160-49.986	0.034
Presence of IVR-3	24.862	2.398-257.781	0.007

ETV: Entecavir; HBV: Hepatitis B virus; IVR-3: Initial virologic response at 3 mo.

treatment prior to ADV combination therapy, low serum HBV DNA levels, and the achievement of IVR-3 were considered favorable factors for VR after 1-year of treatment. Other factors such as age, sex, cirrhosis, HBeAg status, serum ALT levels, international normalized ratio (INR), serum bilirubin levels, serum albumin levels, type of ETV resistance mutation, and type of ADV combination regimen were not significantly associated with VR (Table 2).

A multivariate logistic regression model was used to identify independent risk factors significantly associated with VR during the first year. In the univariate analysis, duration of ETV treatment prior to ADV combination therapy, serum HBV DNA levels, and IVR-3 were candidate variables for multivariate analysis (P < 0.1). In the multivariate analysis, IVR-3 and serum HBV DNA levels remained independent predictors of VR (Table 3).

A Cox proportional hazards model was used to identify independent risk factors significantly

Table 4 Univariate and multivariate analyses of factors affecting long-term virologic response

	Univariate analysis			Multivariate analysis		
	RR	95%CI	*P* value	RR	95%CI	*P* value
Age (yr)	1.011	0.973-1.050	0.586			
Sex (male)	1.156	0.488-2.740	0.741			
HBeAg positivity (-)	1.905	0.568-6.383	0.296			
Disease status (LC)	0.775	0.293-2.054	0.609			
Duration of ETV (mo)	1.077	1.036-1.119	< 0.001	1.022	0.970-1.076	0.419
Serum ALT (IU/L)	1.000	0.998-1.002	0.976			
Serum total bilirubin level (mg/dL)	1.405	0.774-2.550	0.264			
Serum albumin level (g/dL)	1.214	0.384-3.836	0.741			
INR	0.137	0.001-22.543	0.445			
Serum HBV DNA level (< 5.2 $\log_{10}$ IU/mL)	5.084	2.231-11.581	< 0.001	2.870	1.049-7.854	0.040
Type of ETV-resistant mutation (rtT184)	0.780	0.359-1.693	0.529			
Presence of IVR-3	8.822	3.228-24.114	< 0.001	4.417	1.402-13.918	0.011
Rescue therapy regimens (ADV/ETV)	2.007	0.928-4.338	0.077	1.678	0.683-4.119	0.259

ADV: Adefovir; ALT: Alanine aminotransferase; CHB: Chronic hepatitis B; ETV: Entecavir; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; INR: International normalized ratio; IVR-3: Initial virologic response at 3 mo; LC: Liver cirrhosis; LMV: Lamivudine.

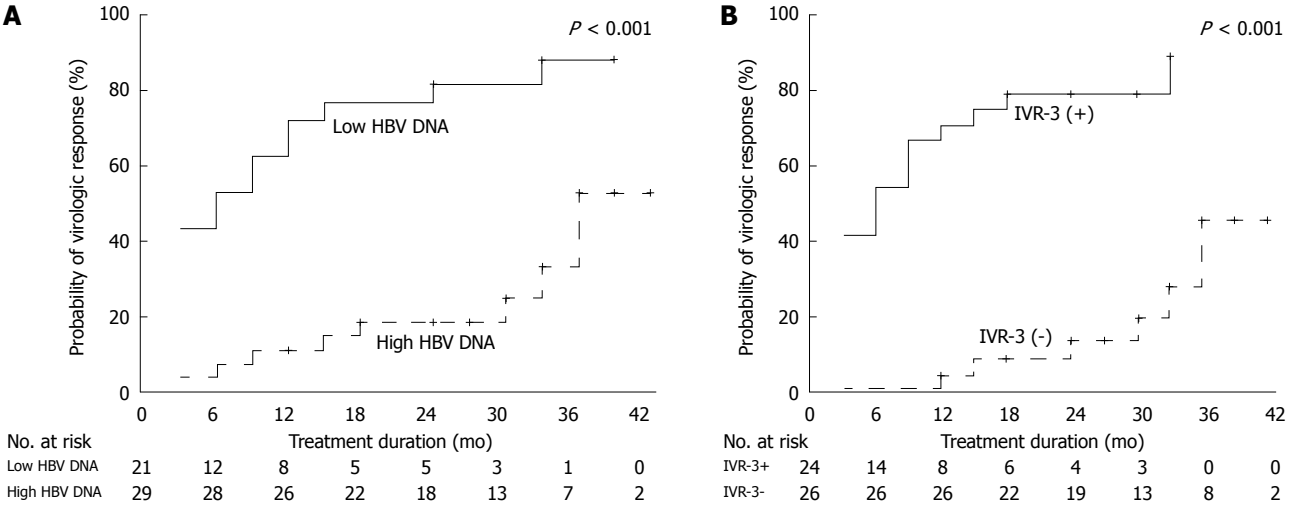


Figure 3 Virologic responses according to the presence of favorable factors. A: Cumulative virologic response rates in patients with low baseline serum hepatitis B virus (HBV) DNA levels and in patients with high baseline serum HBV DNA levels ($P < 0.001$); B: Cumulative virologic response rates in patients with and without initial virologic response-3 (IVR-3) ($P < 0.001$).

associated with long-term VR. The results were similar to the 1-year results detailed above (Table 4).

Impact of predictive factors on the long-term efficacy of ETV

Twenty-one patients (42%) had low baseline serum HBV DNA levels (< 5.2 $\log_{10}$ IU/mL) and IVR-3 was achieved in 24 of 50 (48%) patients. Patients with a low serum HBV DNA level or IVR-3 had a significantly higher probability of achieving VR. Cumulative VR rates at 6, 12, 24, and 36 mo were 52%, 71%, 81%, and 87% in patients with low baseline serum HBV DNA levels and 7%, 10%, 18%, and 52% in patients with high baseline serum HBV DNA levels, respectively($P < 0.001$; Figure 3A). Cumulative VR rates at 6, 12, 24, and 36 mo were 0%, 4%, 13%, and 46% in patients without IVR-3 and 54%, 71%, 80%, and 90% in patients with IVR-3, respectively ($P < 0.001$; Figure 3B). VR was achieved in only 18% (4/22) of patients without favorable predictors (no IVR-3 and a high HBV DNA level) and in 73% (8/11) of patients with one predictor. However, patients with two favorable predictors achieved VR in 88% of cases (15/17). During the treatment period, the respective cumulative incidence of VR at 36 mo according to the increasing number of favorable predictors was 38%, 85%, and 88%. There was a significant difference among the groups ($P < 0.001$; Figure 4).

Virological breakthrough

VBT was observed in 10 patients during the follow-up period. Cumulative VBT rates at 6, 12, 24, and 36 mo were 2%, 6%, 18%, and 26%, respectively (Figure 5A). Only one patient with VR (3.7%, 1/27) and one patient with two favorable predictors (4.5%, 1/22) experienced VBT. During the treatment period, the respective cumulative incidence of VBT at 36 mo according to the increasing number of favorable

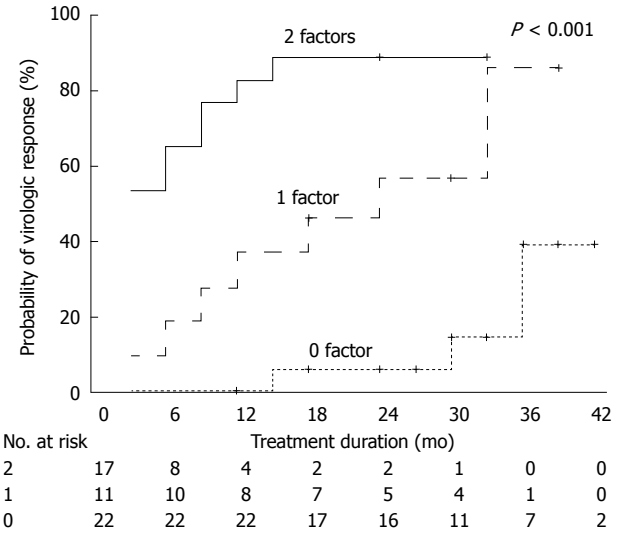


Figure 4 Virologic responses according to the number of predictive factors. Cumulative virologic response rates in patients with 2, 1, and 0 favorable factors are presented ($P < 0.001$).

predictors was 40%, 21%, and 6% (Figure 5B).

DISCUSSION

Although highly potent NAs with optimal genetic resistance profiles (ETV and TDF) have been introduced, prior NAs with lower genetic barriers continue to cause drug resistance, which is an important clinical problem. In particular, sequential monotherapy leads to the emergence of multi-drug resistant mutants, a matter of great concern in the management of CHB patients. So far, few studies have evaluated the efficacy of ADV combination therapy for ETV-resistant HBV infection. However, previous studies included small numbers of patients and/or patients with concurrent ADV resistance[21,23,27]. To our knowledge, this is one of the largest studies and the first long-term follow-up study (up to 4 years) of the efficacy of ADV-based combination therapy in ETV-resistant CHB patients.

Previous studies showed VR rates of about 50% to ADV/ETV combination therapy in patients with LMV- and ETV-resistant HBV infection[21,23,24]. In the present study, however, 27 of 50 (54%) patients showed a VR with respective cumulative VR rates of 36% and 68% at 12 and 36 mo. The reason for the relatively high VR in our study may be due to the difference in the study population and follow-up duration compared to previous studies. Our study excluded patients with prior ADV exposure in order to accurately evaluate the antiviral efficacy of ADV-based regimens in those with resistance to ETV, and the patients were followed up for a median of 27 mo (up to 4 years).

This study demonstrated that the antiviral efficacy of ADV/ETV combination therapy is superior to that of ADV/LMV combination therapy in patients with ETV resistance. During the first year of therapy, the mean

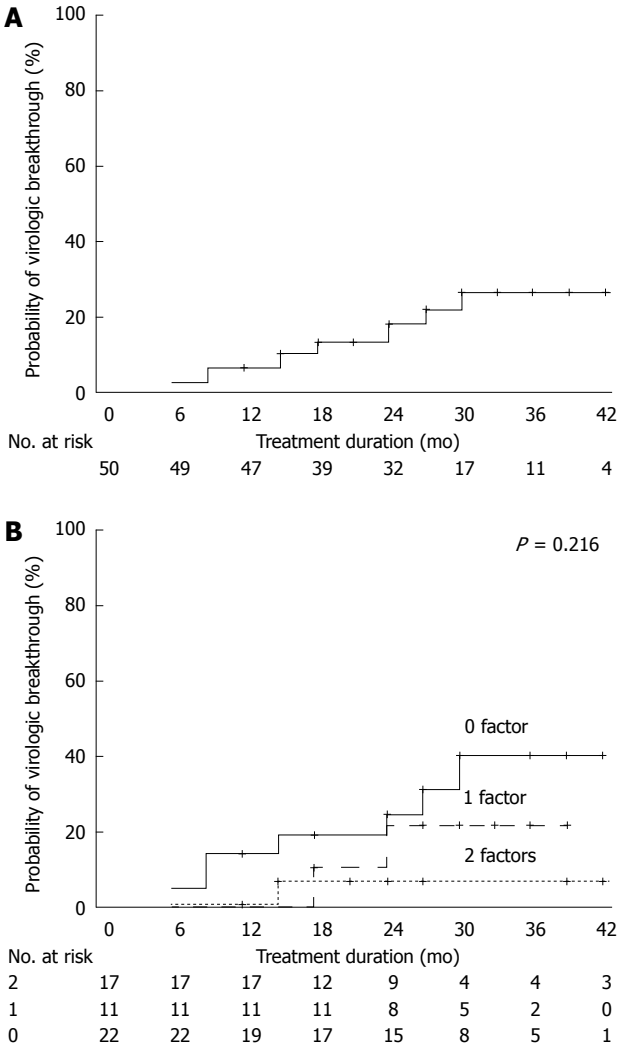


Figure 5 Development of virological breakthrough. A: Overall cumulative virological breakthrough (VBT) rates; B: Cumulative incidence of VBT at 36 mo according to the number of favorable predictors.

reduction in serum HBV DNA levels was significantly greater in the ADV/ETV combination group than in the ADV/LMV combination group (-2.77 *vs* -2.57 $\log_{10}$ IU/mL, $P = 0.028$) by repeated measure analysis. In addition, during the long-term follow-up period, the respective cumulative VR rates at 12 and 36 mo were 43% and 76% in the ADV/ETV combination group and 30% and 62% in the ADV/LMV combination group. There was a significant difference between the two groups ($P = 0.048$). This is the first such finding in ETV-resistant CHB patients; previous studies did not demonstrate the superiority of ADV/ETV combination therapy over ADV/LMV combination therapy in LMV- and ETV-resistant patients[24,27]. However, in a previous study, ADV/ETV combination therapy was used as rescue therapy in only 18 patients[24], which is a relatively small number for a comparison of the efficacy of the ADV/ETV and ADV/LMV regimens.

Another interesting finding of this study is the prognostic role of lower baseline HBV DNA levels and IVR-3, which are predictive factors for short-term and

long-term VR. ADV-based combination therapy has proven to be highly effective in patients with lower baseline HBV DNA levels or IVR-3. In fact, cumulative VR rates in patients with lower baseline HBV DNA levels or IVR-3 were very high, reaching 90% at 36 mo. In clinical practice, the ADV/ETV combination can be considered for ETV-resistant CHB patients with lower HBV DNA levels, and IVR-3 may help determine whether ADV/ETV combination therapy could be maintained or should be switched to TDF-based regimens.

A VBT was observed in 10 out of 50 patients during the follow-up period, with a cumulative VBT rate of 26% at 36 mo. Interestingly, only one patient with favorable predictors experienced VBT during the follow-up period, with a 6% cumulative VBT rate at 36 mo. No ADV mutations were found in this patient, and serum HBV DNA levels declined again despite maintaining therapy. This indicates a clinically useful long-term efficacy of ADV-based combination therapy in ETV-resistant patients in the presence of favorable predictors of VR such as a lower HBV DNA level and IVR-3.

TDF is a potent HBV inhibitor with a high genetic barrier to resistance and doesn't exhibit cross resistance with LMV or ETV[22,31]. In recent studies, TDF/ETV combination therapy showed excellent efficacy in patients with multi-drug resistance (MDR) and resulted in a relatively high rate of complete VR at an early time point, even in patients with triple resistance to LAM, ETV, and ADV[25,26]. When considering the potencies of TDF and ADV, a TDF/ETV combination should be superior to an ADV/ETV combination in CHB patients with MDR although comparative data of this is lacking. As there are countries where TDF is still not available, ADV/ETV combination could be considered an alternative option.

Our study has some limitations. First, the sample size was relatively small. However, considering the difficulty of including ETV-resistant CHB patients, the present study would be accepted as a valuable multicenter study and the largest one evaluating ADV-based combination therapy in ETV-resistant CHB patients. Second, the study was performed retrospectively. In future, a prospective study based on TDF mono- or combination therapy should be considered in ETV resistant CHB patients depending on TDF availability.

In conclusion, an ADV/ETV combination was superior to an ADV/LMV combination, and ADV-based combination therapy was effective in patients with favorable predictors.

In countries where tenofovir is not available, the ADV/ETV combination could be considered an alternative treatment option in ETV-resistant patients with a low HBV DNA titer, and may be continued if IVR-3 is achieved.

COMMENTS

Background

Antiviral resistance to hepatitis B virus (HBV) leads to attenuation of the therapeutic benefits and limits subsequent treatment options. Entecavir (ETV) is one of the most potent and the safest antiviral agents with high genetic barrier. Studies regarding optimal treatment strategies ETV-resistant chronic hepatitis B (CHB) are sparse.

Research frontiers

Both adefovir (ADV) and tenofovir (TDF) are active against ETV-resistant HBV infection *in vitro*, but clinical data on the efficacy of ADV or TDF in those patients are lacking. Therefore, additional study is needed to determine optimal treatment strategies in ETV-resistant CHB patients.

Innovations and breakthroughs

Previous few studies regarding the efficacy of ADV combination therapy for ETV-resistant CHB were conducted in small numbers of patients and evaluated short- term efficacy. This study is one of the largest studies and the first long-term follow-up study (up to 4 years). Furthermore, it shows predictive factors for virologic response (VR), which will be useful for guidance of the treatment strategy.

Applications

This study results suggest the ADV/ETV combination therapy could be considered an alternative treatment option in ETV-resistant CHB patients, especially in those with favorable predictive factors.

Terminology

Initial virologic response at 3 mo (IVR-3) is defined as an HBV DNA level < 3.3 $\log_{10}$ IU/mL after 3 mo of treatment and demonstrated as a predictive factor for VR.

Peer-review

Here the authors report original data on long term efficacy of ADV-based combination therapies, *i.e.,* ADV/ETV and ADV/LMV, on 50 CHB patients with genotypic resistance to ETV. They find higher rates of virological response in patients treated with ADV/ETV *vs* ADV/LMV and they identify low baseline HBV DNA levels and IVR-3 as independent predictive factor for VR. Although its interest is limited to countries where TDF is not available or not reimbursed, this study will be the largest one on this topic, hence worthy of attention and consideration.

REFERENCES

1 **Bosch FX**, Ribes J, Cléries R, Díaz M. Epidemiology of hepatocellular carcinoma. *Clin Liver Dis* 2005; **9**: 191-211, v [PMID: 15831268]

2 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539 [PMID: 17256718 DOI: 10.1002/hep.21513]

3 **Liaw YF**. Hepatitis B virus replication and liver disease progression: the impact of antiviral therapy. *Antivir Ther* 2006; **11**: 669-679 [PMID: 17310811]

4 **Chang TT**, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, Safadi R, Lee SS, Halota W, Goodman Z, Chi YC, Zhang H, Hindes R, Iloeje U, Beebe S, Kreter B. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology* 2010; **52**: 886-893 [PMID: 20683932 DOI: 10.1002/hep.23785]

5 **Kim JH**, Lee SJ, Joo MK, Kim CH, Choi JH, Jung YK, Yim HJ, Yeon JE, Park JJ, Kim JS, Bak YT, Byun KS. Durability of antiviral response in HBeAg-positive chronic hepatitis B patients who maintained virologic response for one year after lamivudine discontinuation. *Dig Dis Sci* 2009; **54**: 1572-1577 [PMID: 18975080 DOI: 10.1007/s10620-008-0508-3]

6 **Kim YJ**, Kim K, Hwang SH, Kim SS, Lee D, Cheong JY, Cho SW. Durability after discontinuation of nucleos(t)ide therapy in chronic

HBeAg negative hepatitis patients. *Clin Mol Hepatol* 2013; **19**: 300-304 [PMID: 24133668 DOI: 10.3350/cmh.2013.19.3.300]
7 **Bartholomeusz A**, Locarnini SA. Antiviral drug resistance: clinical consequences and molecular aspects. *Semin Liver Dis* 2006; **26**: 162-170 [PMID: 16673294 DOI: 10.1055/s-2006-939758]
8 **Lai CL**, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, Brown N, Woessner M, Boehme R, Condreay L. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin Infect Dis* 2003; **36**: 687-696 [PMID: 12627352]
9 **Lok AS**, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD, Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; **125**: 1714-1722 [PMID: 14724824]
10 **Kim JK**, Hwang SG, Park H, Choi HY, Cho HJ, Ko KH, Hong SP, Park PW, Kim NK, Rim KS. [Clinical outcomes after discontinuation of Lamivudine in chronic hepatitis B patients with Lamivudine resistant HBV mutant]. *Korean J Hepatol* 2005; **11**: 227-242 [PMID: 16177549]
11 **Chang TT**, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001-1010 [PMID: 16525137]
12 **Lai CL**, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, Colonno R, Fernandes L. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; **354**: 1011-1020 [PMID: 16525138]
13 **Leung N**, Peng CY, Hann HW, Sollano J, Lao-Tan J, Hsu CW, Lesmana L, Yuen MF, Jeffers L, Sherman M, Min A, Mencarini K, Diva U, Cross A, Wilber R, Lopez-Talavera J. Early hepatitis B virus DNA reduction in hepatitis B e antigen-positive patients with chronic hepatitis B: A randomized international study of entecavir versus adefovir. *Hepatology* 2009; **49**: 72-79 [PMID: 19065670 DOI: 10.1002/hep.22658]
14 **Tenney DJ**, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, Wichroski MJ, Xu D, Yang J, Wilber RB, Colonno RJ. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009; **49**: 1503-1514 [PMID: 19280622 DOI: 10.1002/hep.22841]
15 **Preda CM**, Baicus C, Negreanu L, Tugui L, Olariu SV, Andrei A, Zambatu I, Diculescu MM. Effectiveness of entecavir treatment and predictive factors for virologic response. *Rev Esp Enferm Dig* 2014; **106**: 305-311 [PMID: 25287232]
16 **Yim HJ**, Seo YS, Yoon EL, Kim CW, Lee CD, Park SH, Lee MS, Park CK, Chae HB, Kim MY, Baik SK, Kim YS, Kim JH, Lee JI, Lee JW, Hong SP, Um SH. Adding adefovir vs. switching to entecavir for lamivudine-resistant chronic hepatitis B (ACE study): a 2-year follow-up randomized controlled trial. *Liver Int* 2013; **33**: 244-254 [PMID: 23295056 DOI: 10.1111/liv.12036]
17 **Warner N**, Locarnini S. Mechanisms of hepatitis B virus resistance development. *Intervirology* 2014; **57**: 218-224 [PMID: 25034491 DOI: 10.1159/000360940000360940]
18 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010S0168-8278(12)00167-5]
19 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]
20 **Liaw YF**, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263-283 [PMID: 19669255 DOI: 10.1007/s12072-008-9080-3]
21 **Yang HJ**, Lee JH, Kim YJ, Yoon JH, Lee HS. Antiviral efficacy of combination therapy with entecavir and adefovir for entecavir/lamivudine-resistant hepatitis B virus with or without adefovir resistance. *J Med Virol* 2012; **84**: 424-430 [PMID: 22246827 DOI: 10.1002/jmv.23229]
22 **Yim HJ**, Hwang SG. Options for the management of antiviral resistance during hepatitis B therapy: reflections on battles over a decade. *Clin Mol Hepatol* 2013; **19**: 195-209 [PMID: 24133659 DOI: 10.3350/cmh.2013.19.3.195]
23 **Jeon JW**, Shin HP, Lee JI, Joo KR, Cha JM, Park JJ, Lim JU, Lim K, Kim S. Efficacy of entecavir and adefovir combination therapy for patients with lamivudine- and entecavir-resistant chronic hepatitis B. *Dig Dis Sci* 2012; **57**: 1358-1365 [PMID: 22134785 DOI: 10.1007/s10620-011-1988-0]
24 **Lee YB**, Lee JH, Choi WM, Cho YY, Yoo JJ, Lee M, Lee DH, Cho Y, Yu SJ, Kim YJ, Yoon JH, Kim CY, Lee HS. Efficacy of adefovir-based combination therapy for patients with Lamivudine- and entecavir-resistant chronic hepatitis B virus infection. *Antimicrob Agents Chemother* 2013; **57**: 6325-6332 [PMID: 24100506 DOI: 10.1128/AAC.01742-13AAC.01742-13]
25 **Lee YB**, Lee JH, Lee DH, Cho H, Ahn H, Choi WM, Cho YY, Lee M, Yoo JJ, Cho Y, Cho EJ, Yu SJ, Kim YJ, Yoon JH, Kim CY, Lee HS. Efficacy of entecavir-tenofovir combination therapy for chronic hepatitis B patients with multidrug-resistant strains. *Antimicrob Agents Chemother* 2014; **58**: 6710-6716 [PMID: 25155601 DOI: 10.1128/AAC.03845-14AAC.03845-14]
26 **Petersen J**, Ratziu V, Buti M, Janssen HL, Brown A, Lampertico P, Schollmeyer J, Zoulim F, Wedemeyer H, Sterneck M, Berg T, Sarrazin C, Lutgehetmann M, Buggisch P. Entecavir plus tenofovir combination as rescue therapy in pre-treated chronic hepatitis B patients: an international multicenter cohort study. *J Hepatol* 2012; **56**: 520-526 [PMID: 22037226 DOI: 10.1016/j.jhep.2011.09.018S0168-8278(11)00788-4]
27 **Yim HJ**, Lee HJ, Suh SJ, Seo YS, Kim CW, Lee CD, Park SH, Lee MS, Park CK, Chae HB, Kim MY, Baik SK, Kim YS, Kim JH, Lee JI, Lee JW, Hong SP, Um SH. Adefovir and lamivudine combination therapy in patients with entecavir-resistant chronic hepatitis B: antiviral responses and evolution of mutations. *Intervirology* 2014; **57**: 239-247 [PMID: 24993731 DOI: 10.1159/000360399000360399]
28 **Park JW**, Kim HS, Seo DD, Jang JS, Shin WG, Kim KH, Jang MK, Lee JH, Kim HY, Kim DJ, Lee MS, Park CK. Long-term efficacy of entecavir in adefovir-refractory chronic hepatitis B patients with prior lamivudine resistance. *J Viral Hepat* 2011; **18**: e475-e481 [PMID: 21914066 DOI: 10.1111/j.1365-2893.2011.01479.x]
29 **Zoulim F**, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 2009; **137**: 1593-1608.e1-2 [PMID: 19737565 DOI: 10.1053/j.gastro.2009.08.063]
30 **Lee JM**, Kim HJ, Park JY, Lee CK, Kim do Y, Kim JK, Lee HW, Paik YH, Lee KS, Han KH, Chon CY, Hong SP, Nguyen T, Ahn SH. Rescue monotherapy in lamivudine-resistant hepatitis B e antigen-positive chronic hepatitis B: adefovir versus entecavir. *Antivir Ther* 2009; **14**: 705-712 [PMID: 19704174]
31 **Jung SK**, Kim KA, Ha SY, Lee HK, Kim YD, Lee BH, Paik WH, Kim JW, Bae WK, Kim NH, Lee JS, Jwa YJ. Tenofovir disoproxil fumarate monotherapy for nucleos(t)ide analogue-naïve and nucleos(t)ide analogue-experienced chronic hepatitis B patients. *Clin Mol Hepatol* 2015; **21**: 41-48 [PMID: 25834801 DOI: 10.3350/cmh.2015.21.1.41]

P- Reviewer: De Vincentis A, Preda CM **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10883

World J Gastroenterol 2015 October 14; 21(38): 10883-10889
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Geographical distribution of the incidence of gastric cancer in Bhutan

Tashi Dendup, James M Richter, Yoshio Yamaoka, Kinley Wangchuk, Hoda M Malaty

Tashi Dendup, Department of Surgery, Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan

James M Richter, Massachusetts General Hospital, Harvard Medical School, MGH Gastroenterology Associates, Harvard University, Boston, MA 02114, United States

Yoshio Yamaoka, Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Yufu 879-5593, Japan

Kinley Wangchuk, Microbiology Unit, Department of Laboratory Medicine, Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan

Hoda M Malaty, Department of Medicine, Baylor College of Medicine, Houston, TX 77030, United States

Author contributions: Dendup T and Malaty HM contributed equally to this work; Malaty HM and Dendup T designed research; Malaty HM, Richter JM, Yamaoka Y and Wangchuk K performed research and contributed new reagents/analytic tools; and Malaty HM analyzed data and wrote the paper.

Supported by The Center for Eye Research and Education, Boston Massachusetts (partly).

Institutional review board statement: The project was approved by National Referral Hospital, Thimphu.

Informed consent statement: This is a retrospective Study used data for the analysis.

Conflict-of-interest statement: There is no any conflict of interest to any of the contributed authors.

Data sharing statement: Excel file of data is attached.

Correspondence to: Hoda M Malaty, MD, PhD, Department of Medicine, Baylor College of Medicine, Houston, Veterans Affairs Medical Center (111D), 2002 Holcombe Blvd., Houston, TX 77030, United States. hmalaty@bcm.edu
Telephone: +1-713-7950232

Received: March 22, 2015
Peer-review started: March 23, 2015
First decision: April 23, 2015
Revised: May 9, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 14, 2015

Abstract

AIM: To estimate the prevalence of gastric cancer (GC) in a cohort of patients diagnosed with GC and to compare it with patients diagnosed with all other types of gastro-intestinal (GI) cancer during the same period.

METHODS: Between 2008 and 2013, five-year period, the medical records of all GI cancer patients who underwent medical care and confirm diagnosis of cancer were reviewed at the National Referral Hospital, Thimphu which is the only hospital in the country where surgical and cancer diagnosis can be made. Demographic information, type of cancer, and the year of diagnosis were collected.

RESULTS: There were a total of 767 GI related cancer records reviewed during the study period of which 354 (46%) patients were diagnosed with GC. There were 413 patients with other GI cancer including; esophagus, colon, liver, rectum, pancreas, gall bladder, cholangiocarcinoma and other GI tract cancers. The GC incidence rate is approximately 0.9/10000 per year (367 cases/5

years per 800000 people). The geographic distribution of GC was the lowest in the south region of Bhutan 0.3/10000 per year compared to the central region 1.4/10000 per year, Eastern region 1.2/10000 per year, and the Western region 1.1/10000 per year. Moreover, GC in the South part was significantly lower than the other GI cancer in the same region (8% *vs* 15%; OR = 1.8, 95%CI: 1.3-3.1, *P* = 0.05). Among GC patients, 38% were under the age of 60 years, mean age at diagnosis was 62.3 (± 12.1) years with male-to-female ratio 1:0.5. The mean age among patients with all other type GI cancer was 60 years (± 13.2) and male-to-female ratio of 1:0.7. At time of diagnosis of GC, 342 (93%) were at stage 3 and 4 of and by the year 2013; 80 (23%) GC patients died compared to 31% death among patients with the all other GI cancer (*P* = 0.08).

CONCLUSION: The incidence rate of GC in Bhutan is twice as high in the United States but is likely an underestimate rate because of unreported and undiagnosed cases in the villages. The high incidence of GC in Bhutan could be attributed to the high prevalence of *Helicobacter pylori* infection that we previously reported. The lowest incidence of GC in Southern part of the country could be due to the difference in the ethnicity as most of its population is of Indian and Nepal origin. Our current study emphasizes on the importance for developing surveillance and prevention strategies for GC in Bhutan.

Key words: Gastric cancer; Mortality; Epidemiology; Bhutan

Core tip: The incidence rate of gastric cancer (GC) in Bhutan is twice as high in the United States but is likely an underestimate rate because of unreported and undiagnosed cases in the villages. The high incidence of GC in Bhutan could be attributed to the high prevalence of *Helicobacter pylori* infection that we previously reported. The lowest incidence of GC in Southern part of the country could be due to the difference in the ethnicity as most of its population is of Indian and Nepal origin. Our current study emphasizes on the importance for developing surveillance and prevention strategies for GC in Bhutan.

Dendup T, Richter JM, Yamaoka Y, Wangchuk K, Malaty HM. Geographical distribution of the incidence of gastric cancer in Bhutan. *World J Gastroenterol* 2015; 21(38): 10883-10889 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10883.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10883

INTRODUCTION

Gastric cancer (GC) is the second leading cause of cancer-related mortality and the fourth most common cancer globally[1-3]. However, its incidence rates in different geographical regions are distinctly varied[2]. It been reported that in East Asia, high-risk countries include South Korea, Japan and China, where the age-standardized incidence rate is higher than 20 cases of GC per 100000 inhabitants per year[2]. The World Health Organization reports the incidence of GC in Bhutan to be very high[4]; however there is no published data yet. Bhutan is a small mountainous country bordering India and China and consists of four geographical regions, west, east, central, and south with a population consists of 800000 citizens.

It has been established that a *Helicobacter pylori* (*H. pylori*) infection is an etiologic agent of chronic gastritis and peptic ulcer disease, GC and mucosal associated lymphoid tissue lymphoma (MALT) and it is listed as a number one carcinogen[5-8]. We previously reported a very high prevalence of *H. pylori* in Bhutan[9,10]. Childhood hygiene practices and family education determine the prevalence of *H. pylori* infection[11,12]. The current study aimed to estimate the incidence of GC in Bhutan and to compare the geographic distribution of GC to our previously reported geographic distribution of *H. pylori* infection.

MATERIALS AND METHODS

Data source and case identification

Between 2008 and 2013, five-year period, the medical records of all Gastro-Intestinal Cancer patients who underwent medical care and confirm diagnosis of cancer were reviewed at the National Referral Hospital, Thimphu which is the only hospital in the country where surgical and cancer diagnosis can be made. Diagnosis and ascertainment of the GC cases was based on endoscopic and pathological examination. There is only one surgeon in the country (TD) who operates on most/all the oncology cases in the country and there not yet established GC registry in Bhutan. Demographic information, *e.g.*, age, gender, place of residence, and date of diagnosis were collected. For each patient, type of gastro-intestinal cancer was retrieved.

Geography, population, and climate of Bhutan

Bhutan is a remote Himalayan country between India and Tibet (China) with a population consists of only 800000 citizens residing in 18147 sq mi (47000 sq km) (Figure 1). Seventy percent of country is rural and agriculture based and the literacy rate is 47% (2011

Figure 1 Geographic of the map of Bhutan.

Census). More than 30% of Bhutan populations live below poverty level. The climate in Bhutan varies with elevation, from subtropical in the south to temperate in the highlands and polar-type climate, with year-round snow in the north.

Bhutan is demographically divided into four main regions, Southern, Western, Eastern, and central regions. The Southern region shares border with India and ethnically they are of Indian and Nepal origin. The Western region is mostly on higher altitudes and socioeconomic standard is higher than Southern region. The normal water supply is through rural water scheme that is supported by the government and most people use local streams, rivers and piped water supply. The Central region shares similarity with the Western region both socioeconomically and geographically though it is little warmer. The Eastern region is lower in altitude than the Western region and have similar rural water supply scheme as the Western region.

Statistical analysis

Calculation of the crude incidence rate was based on the number of recorded cases divided by the overall population by the number of years. The estimated Bhutan population in 2005 was 800000.

RESULTS

There were a total of 767 GI related cancer records reviewed during the years of 2008 and 2013. There were 354 (46%) patients diagnosed with GC and 413 patients with diagnosed with esophagus, colon, liver, rectum, pancreas, gall bladder, cholangio-carcinoma and other types of GI cancers. The GC incidence rate was approximately 0.9/10000 per year (354 cases/5 years/800000 people). Among GC patients, 38% were under the age of 60 years, mean age at diagnosis was 62.3 (SD ± 12.1) years and the male-to-female ratio 1:0.5. The mean age among patients with all other type GI cancer was 60 years (± 13.2) and male-to-female ratio of 1:0.7. GC in the South part was significantly lower than the other GI cancer in the same region (8% *vs* 15%, OR = 1.8, 95%CI: 1.3-3.1, $P = 0.05$).

The geographic distribution of the overall number of GC cases by each city is presented in Figure 1. We calculated the incidence rate of GC by each region; we found that the geographic distribution of GC was the lowest in the Southern region of Bhutan 0.3/10000 per year compared to the central region 1.4/100000 per year, Eastern region 1.2/10000 per year, and the Western region 1.1/10000 per year (Figure 2). As we previously reported, the geographic distribution of *H. pylori* infection was the lowest in the south region of the country (Figure 3).

At time of diagnosis of GC, 10 (3%) of the patients were at stage 1, 15 (4%) at stage 2, 211 (60%) at stage 3 and 118 (33%) stage 4. By the year 2013; 79 GC patients (22%) died.

DISCUSSION

The latest estimate of the global incidence rates of GC was updated by the International Agency for Research on Cancer in 2008 report estimated that there were 989000 new cases of GC or (7.8% of all reported cancer cases)[1-3]. We found that the incidence rate

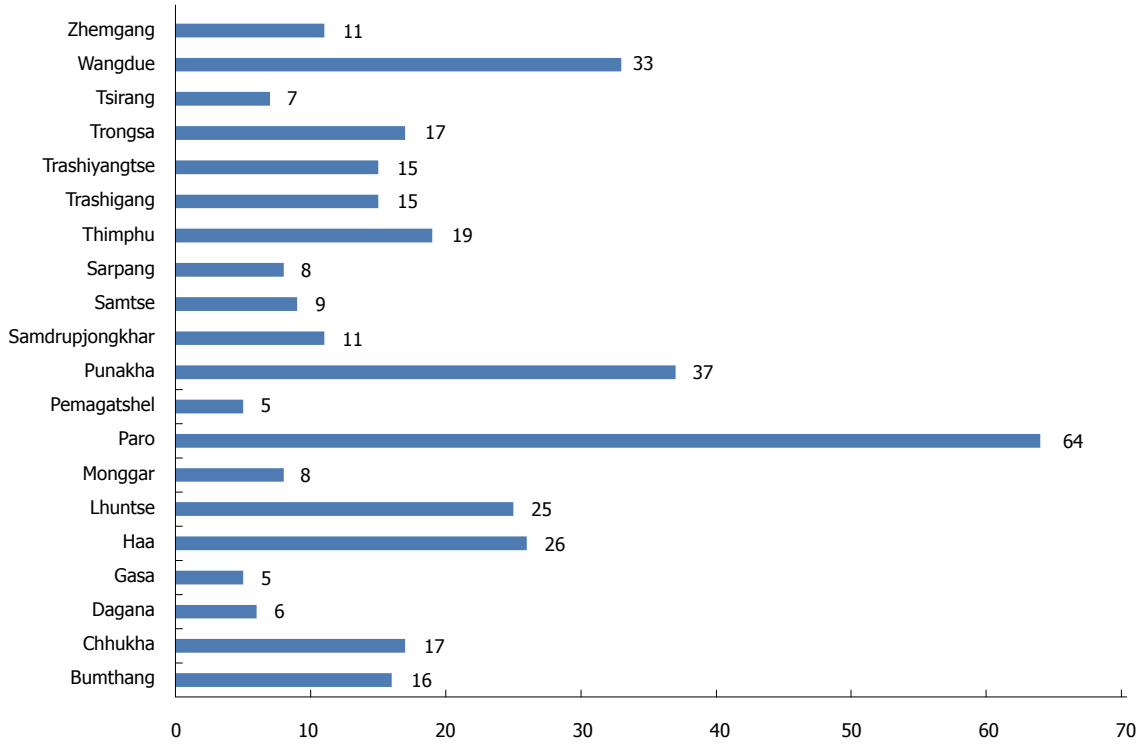


Figure 2 Gastric cancer cases distribution by area in Bhutan.

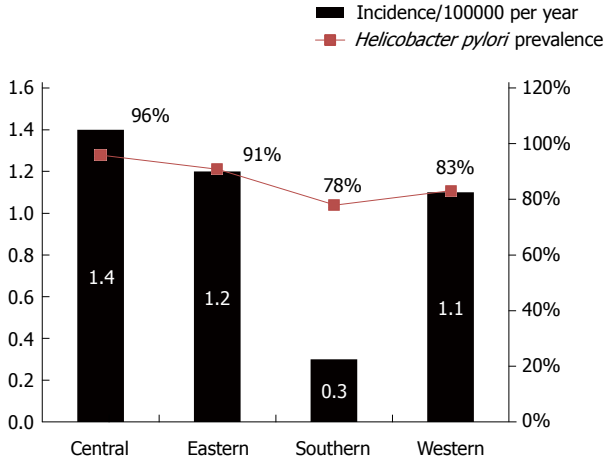


Figure 3 Geographic distribution of the incidence of gastric cancer and *Helicobacter pylori* infection.

of GC in Bhutan is high as it is second higher rate of cancer in the country after the incidence of cervical cancer (Unpublished data). The results of the current study add more emphasis to literature of the high GC incidence rate in South Asia, as the current study is the first report of the incidence of GC in Bhutan. In our previous study in Bhutan when we assessed subjects for gastric mucosal atrophy, we found that PG Ⅰ/Ⅱ ratio was significantly inversely correlated with the atrophy score in the antrum and the corpus. Furthermore, we found that the PG status was significantly associated with the presence of atrophy in the corpus and the prevalence of the PG-positive status was significantly higher among *H. pylori*-positive subjects than among *H. pylori*-negative subjects. We concluded that the high incidence of GC in Bhutan can be attributed to the high prevalence of *H. pylori* infection and gastric mucosal atrophy[13].

Geographic variations in GC incidence have been reported in both the West and the East[14-16]. Of interest our results revealed the lowest GC rate was found in south Bhutan than the rest of the country. This variation is consistent with our previous published results of the finding of the lowest *H. pylori* prevalence in the south part Bhutan[9]. The marked lower prevalence in the Southern region could be due is the different of the ethnicity in the region as they are of Indian and Nepal origin and they have different food habits than the original Bhutanese. It is known that Bhutanese are broadly from three ethnic backgrounds. The first ethnic group is from Tibetan descent that mainly from the Western parts of the country while the second is the Indo-Burmese ethnic group where mostly from the population in the Eastern parts of the country, the Southern Bhutanese, the third group, is of the Nepali origin and mainly Aryan descent. Migration studies have shown that first-generation migrants from countries with a high incidence of *H. pylori* infection relocating to countries of low incidence rates had similar risks as that of the country of origin, but the incidence rate tended toward that of the host country in subsequent generations, suggesting the important role of environmental risk factors[17-20]. Moreover, various biological strains could be present in different

parts of the country and within individuals. A study published that Indian *H. pylori* isolates have been shown to have European origins and are widely held to be only mildly pathogenic[21].

The high incidence of GC in Bhutan could be attributed to the high prevalence of *H. pylori* infection that we previously reported. Moreover, it was also reported that host genetic have an impact on host responses to gastric inflammation and acid secretion, thereby interacting with *H. pylori* infection in gastric carcinogenesis. Therefore, host genetic factors may determine why some individuals infected with *H. pylori* develop GC, while others do not[22].

It has been established that a *H. pylori* infection is an etiologic agent of chronic gastritis and peptic ulcer disease, GC and MALT and it is listed as a number one carcinogen[1-4]. Several studies and randomized controlled trials showed that *H. pylori* eradication reduce GC incidence by at least 35%[23-28]. Current consensus is that *H. pylori* screening and treatment is effective only in high-risk populations[29-32]. However, up till today such screening/surveillance had not taken place yet in Bhutan in spite the high prevalence of *H. pylori* infection and high GC rate among all age groups.

The current study revealed that 95% of GC patients were diagnosed at stages 3 and 4 and accordingly this could result in a higher mortality than early diagnosis. Early cancer detection is important because countries that perform GC surveillance, such as Japan and Korea, have lower mortality rates[3]. The Asian-Pacific Consensus Group recommended the screening and treatment of *H. pylori* as an evidenced-based and reasonable strategy for primary prevention of GC in selected communities where the burden of GC is high[32].

It has been well documented that treatment of *H. pylori* infection has an impact on the precursors of GC. A study from Colombia, a region with high GC risk, assessed the effect of *H. pylori* eradication therapy on intestinal metaplasia, multifocal atrophy and dysplasia in reported significant regression in histopathology score after treatment[33-36]. A recent study from Taiwan reported that mass eradication of *H. pylori* infection resulted in significant reduction in incidence of gastric atrophy resulting from chemoprevention[37]. Adopting the 2008 Asia-Pacific guidelines for a low threshold for treatment of symptomatic patients, as well as low cost follow-up testing could significantly lower the prevalence of peptic ulcer disease, GC. Therefore, there it is a great need for developing surveillance and prevention strategies for GC in Bhutan.

The utilization of the current data for constructing our study has some shortcomings that should be addressed. The main limitation is that we relied on the hospital records and not GC registry to identify the cancer cases. However, up till today, there is no cancer registry in Bhutan and the Timphu National hospital is the only hospital that diagnoses all cancer cases in the country, so our data is valid as representative GC cases in Bhutan. The second limitation of the study that we did not have enough data to calculate the overall mortality rate due GC and further studies are highly needed to address that topic.

In conclusion, this study demonstrates clear evidence of the high GC incidence in Bhutan that could be attributed to the high prevalence of *H. pylori* infection that we previously reported. The lowest incidence of GC in Southern part of the country could be due to the difference in the ethnicity as most of its population is of Indian and Nepal origin. Our current study emphasizes on the importance for developing surveillance and prevention strategies for GC in Bhutan.

COMMENTS

Background

The World Health Organization reports the incidence of gastric cancer (GC) in Bhutan to be very high; however there is no published data yet. GC is the second leading cause of cancer-related mortality and the fourth most common cancer globally. However, its incidence rates in different geographical regions are distinctly varied. It has been established that a *Helicobacter pylori* (*H. pylori*) infection is an etiologic agent of chronic gastritis and peptic ulcer disease, GC and mucosal associated lymphoid tissue lymphoma and it is listed as as a number one carcinogen.

Research frontiers

The authors performed a retrospective study on a cohort of patients estimate the incidence of GC in Bhutan and to compare the geographic distribution of GC to our previously reported geographic distribution of *H. pylori* infection.

Innovations and breakthroughs

The incidence rate of GC in Bhutan is twice as high in the United States but is likely an underestimate rate because of unreported and undiagnosed cases in the villages.

Applications

The high incidence of GC in Bhutan could be attributed to the high prevalence of *H. pylori* infection that the authors previously reported. The lowest incidence of GC in Southern part of the country could be due to the difference in the ethnicity as most of its population is of Indian and Nepal origin. It is of importance for developing surveillance and prevention strategies for GC in Bhutan.

Peer-review

This is an interesting and well written paper.

REFERENCES

1 **Ang TL**, Fock KM. Clinical epidemiology of gastric cancer. *Singapore Med J* 2014; **55**: 621-628 [PMID: 25630323 DOI: 10.11622/smedj.2014174]

2 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

3 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Online]. Available from: URL: http://globocan.iarc.fr

4 Stomach cancer statistics. Stomach cancer is the fifth most common cancer in the world, with 952000 new cases diagnosed in

2012. Available from: URL: http://www.wcrf.org/cancer_statistics/data_specific_cancers/stomach_cancer_statistics.php
5 **Correa P**, Fox J, Fontham E, Ruiz B, Lin YP, Zavala D, Taylor N, Mackinley D, de Lima E, Portilla H. Helicobacter pylori and gastric carcinoma. Serum antibody prevalence in populations with contrasting cancer risks. *Cancer* 1990; **66**: 2569-2574 [PMID: 2249197]
6 **Forman D**. The etiology of gastric cancer. *IARC Sci Publ* 1991; (**105**): 22-32 [PMID: 1855854]
7 **Cao XY**, Jia ZF, Jin MS, Cao DH, Kong F, Suo J, Jiang J. Serum pepsinogen II is a better diagnostic marker in gastric cancer. *World J Gastroenterol* 2012; **18**: 7357-7361 [PMID: 23326145 DOI: 10.3748/wjg.v18.i48.7357]
8 **Graham DY**, Malaty HM, Go MF. Are there susceptible hosts to Helicobacter pylori infection? *Scand J Gastroenterol Suppl* 1994; **205**: 6-10 [PMID: 7863244]
9 **Dorji D**, Dendup T, Malaty HM, Wangchuk K, Yangzom D, Richter JM. Epidemiology of Helicobacter pylori in Bhutan: the role of environment and Geographic location. *Helicobacter* 2014; **19**: 69-73 [PMID: 24102940 DOI: 10.1111/hel.12088]
10 **Vilaichone RK**, Mahachai V, Shiota S, Uchida T, Ratanachu-ek T, Tshering L, Tung NL, Fujioka T, Moriyama M, Yamaoka Y. Extremely high prevalence of Helicobacter pylori infection in Bhutan. *World J Gastroenterol* 2013; **19**: 2806-2810 [PMID: 23687418]
11 **Malaty HM**, Kim JG, Kim SD, Graham DY. Prevalence of Helicobacter pylori infection in Korean children: inverse relation to socioeconomic status despite a uniformly high prevalence in adults. *Am J Epidemiol* 1996; **143**: 257-262 [PMID: 8561159 DOI: 10.1093/oxfordjournals.aje.a008736]
12 **Mitchell HM**, Li YY, Hu PJ, Liu Q, Chen M, Du GG, Wang ZJ, Lee A, Hazell SL. Epidemiology of Helicobacter pylori in southern China: identification of early childhood as the critical period for acquisition. *J Infect Dis* 1992; **166**: 149-153 [PMID: 1607687 DOI: 10.1093/infdis/166.1.149]
13 **Shiota S**, Mahachai V, Vilaichone RK, Ratanachu-ek T, Tshering L, Uchida T, Matsunari O, Yamaoka Y. Seroprevalence of Helicobacter pylori infection and gastric mucosal atrophy in Bhutan, a country with a high prevalence of gastric cancer. *J Med Microbiol* 2013; **62**: 1571-1578 [PMID: 23831768 DOI: 10.1099/jmm.0.060905-0]
14 **Aragonés N**, Pérez-Gómez B, Pollán M, Ramis R, Vidal E, Lope V, García-Pérez J, Boldo E, López-Abente G. The striking geographical pattern of gastric cancer mortality in Spain: environmental hypotheses revisited. *BMC Cancer* 2009; **9**: 316 [PMID: 19737377 DOI: 10.1186/1471-2407-9-316]
15 **Mezzanotte G**, Cislaghi C, Decarli A, La Vecchia C. Cancer mortality in broad Italian geographical areas, 1975-1977. *Tumori* 1986; **72**: 145-152 [PMID: 3705187]
16 **Wong BC**, Ching CK, Lam SK, Li ZL, Chen BW, Li YN, Liu HJ, Liu JB, Wang BE, Yuan SZ, Xu CP, Hou XH, Zhang AT, Zheng ZT. Differential north to south gastric cancer-duodenal ulcer gradient in China. China Ulcer Study Group. *J Gastroenterol Hepatol* 1998; **13**: 1050-1057 [PMID: 9835323 DOI: 10.1111/j.1440-1746.1998.tb00569.x]
17 **Arnold M**, Razum O, Coebergh JW. Cancer risk diversity in non-western migrants to Europe: An overview of the literature. *Eur J Cancer* 2010; **46**: 2647-2659 [PMID: 20843493 DOI: 10.1016/j.ejca.2010.07.050]
18 **Ronellenfitsch U**, Kyobutungi C, Ott JJ, Paltiel A, Razum O, Schwarzbach M, Winkler V, Becher H. Stomach cancer mortality in two large cohorts of migrants from the Former Soviet Union to Israel and Germany: are there implications for prevention? *Eur J Gastroenterol Hepatol* 2009; **21**: 409-416 [PMID: 19242359 DOI: 10.1097/MEG.0b013e3283155220]
19 **Nguyen EV**. Cancer in Asian American males: epidemiology, causes, prevention, and early detection. *Asian Am Pac Isl J Health* 2003; **10**: 86-99 [PMID: 15509149]
20 **Maskarinec G**, Noh JJ. The effect of migration on cancer incidence among Japanese in Hawaii. *Ethn Dis* 2004; **14**: 431-439 [PMID: 15328946]
21 **Misra V**, Misra SP, Dwivedi M, Singh PA. Point prevalence of peptic ulcer and gastric histology in healthy Indians with Helicobacter pylori infection. *Am J Gastroenterol* 1997; **92**: 1487-1491 [PMID: 9317069]
22 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402 [PMID: 10746728 DOI: 10.1038/35006081]
23 **Peterson WL**, Graham DY, Marshall B, Blaser MJ, Genta RM, Klein PD, Stratton CW, Drnec J, Prokocimer P, Siepman N. Clarithromycin as monotherapy for eradication of Helicobacter pylori: a randomized, double-blind trial. *Am J Gastroenterol* 1993; **88**: 1860-1864 [PMID: 8237933]
24 **Graham DY**, Lew GM, Malaty HM, Evans DG, Evans DJ, Klein PD, Alpert LC, Genta RM. Factors influencing the eradication of Helicobacter pylori with triple therapy. *Gastroenterology* 1992; **102**: 493-496 [PMID: 1732120]
25 **Pounder RE**, Williams MP. The treatment of Helicobacter pylori infection. *Aliment Pharmacol Ther* 1997; **11** Suppl 1: 35-41 [PMID: 9146789 DOI: 10.1046/j.1365-2036.11.s1.9.x]
26 **Carlson SJ**, Yokoo H, Vanagunas A. Progression of gastritis to monoclonal B-cell lymphoma with resolution and recurrence following eradication of Helicobacter pylori. *JAMA* 1996; **275**: 937-939 [PMID: 8598622 DOI: 10.1001/jama.1996.03530360047037]
27 **Moayyedi P**, Dixon MF. Significance of Helicobacter pylori infection and gastric cancer: implications for screening. *Gastrointest Endosc Clin N Am* 1997; **7**: 47-64 [PMID: 8995112]
28 **Tanahashi T**, Tatsumi Y, Sawai N, Yamaoka Y, Nakajima M, Kodama T, Kashima K. Regression of atypical lymphoid hyperplasia after eradication of Helicobacter pylori. *J Gastroenterol* 1997; **32**: 543-547 [PMID: 9250905 DOI: 10.1007/BF02934097]
29 **Peterson WL**, Fendrick AM, Cave DR, Peura DA, Garabedian-Ruffalo SM, Laine L. Helicobacter pylori-related disease: guidelines for testing and treatment. *Arch Intern Med* 2000; **160**: 1285-1291 [PMID: 10809031 DOI: 10.1001/archinte.160.9.1285]
30 **Lam SK**, Talley NJ. Report of the 1997 Asia Pacific Consensus Conference on the management of Helicobacter pylori infection. *J Gastroenterol Hepatol* 1998; **13**: 1-12 [PMID: 9737564 DOI: 10.1111/j.1440-1746.1998.tb00537.x]
31 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781 [PMID: 17170018 DOI: 10.1136/gut.2006.101634]
32 **Fock KM**, Talley N, Moayyedi P, Hunt R, Azuma T, Sugano K, Xiao SD, Lam SK, Goh KL, Chiba T, Uemura N, Kim JG, Kim N, Ang TL, Mahachai V, Mitchell H, Rani AA, Liou JM, Vilaichone RK, Sollano J. Asia-Pacific consensus guidelines on gastric cancer prevention. *J Gastroenterol Hepatol* 2008; **23**: 351-365 [PMID: 18318820 DOI: 10.1111/j.1440-1746.2008.05314.x]
33 **Leung WK**, Lin SR, Ching JY, To KF, Ng EK, Chan FK, Lau JY, Sung JJ. Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on Helicobacter pylori eradication. *Gut* 2004; **53**: 1244-1249 [PMID: 15306578 DOI: 10.1136/gut.2003.034629]
34 **Rokkas T**, Pistiolas D, Sechopoulos P, Robotis I, Margantinis G. The long-term impact of Helicobacter pylori eradication on gastric histology: a systematic review and meta-analysis. *Helicobacter* 2007; **12** Suppl 2: 32-38 [PMID: 17991174]
35 **Wang J**, Xu L, Shi R, Huang X, Li SW, Huang Z, Zhang G. Gastric atrophy and intestinal metaplasia before and after Helicobacter pylori eradication: a meta-analysis. *Digestion* 2011; **83**: 253-260 [PMID: 21282951 DOI: 10.1159/000280318]
36 **Uemura N**, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, Sasaki N, Haruma K, Sumii K, Kajiyama G. Effect of Helicobacter pylori eradication on subsequent development of

cancer after endoscopic resection of early gastric cancer. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 639-642 [PMID: 9264278]
37 **Lee YC**, Chen TH, Chiu HM, Shun CT, Chiang H, Liu TY, Wu MS, Lin JT. The benefit of mass eradication of Helicobacter pylori infection: a community-based study of gastric cancer prevention. *Gut* 2013; **62**: 676-682 [PMID: 22698649 DOI: 10.1136/gutjnl-2012-302240]

P- Reviewer: Razis E **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10890

World J Gastroenterol 2015 October 14; 21(38): 10890-10897
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Upper gastrointestinal bleeding in Scotland 2000-2010: Improved outcomes but a significant weekend effect

Asma Ahmed, Matthew Armstrong, Ishbel Robertson, Allan John Morris, Oliver Blatchford, Adrian J Stanley

Asma Ahmed, Allan John Morris, Adrian J Stanley, GI Unit, Glasgow Royal Infirmary, G4 0SF Glasgow, United Kingdom

Matthew Armstrong, Ishbel Robertson, ISD Scotland, G2 6QE Glasgow, United Kingdom

Oliver Blatchford, Health Protection Scotland, G2 6QE Glasgow, United Kingdom

Author contributions: All authors contributed equally to this work.

Institutional review board statement: All data records were extracted from the permanently linked dataset held by Information Services Division (ISD), Scotland and were managed subject to ISD information governance rules and processes.

Conflict-of-interest statement: The authors declare that we have no conflicting interest.

Data sharing statement: No additional data are available.

Correspondence to: Dr. Adrian J Stanley, Consultant Gastroenterologist, GI Unit, Glasgow Royal Infirmary, Castle Street, G4 OSF Glasgow,
United Kingdom. adrian.stanley@ggc.scot.nhs.uk
Telephone: +44-141-2114073
Fax: +44-141-2115131

Received: January 18, 2015
Peer-review started: January 30, 2015
First decision: March 10, 2015
Revised: April 3, 2015
Accepted: June 10, 2015

Article in press: June 10, 2015
Published online: October 14, 2015

Abstract

AIM: To assess numbers and case fatality of patients with upper gastrointestinal bleeding (UGIB), effects of deprivation and whether weekend presentation affected outcomes.

METHODS: Data was obtained from Information Services Division (ISD) Scotland and National Records of Scotland (NRS) death records for a ten year period between 2000-2001 and 2009-2010. We obtained data from the ISD Scottish Morbidity Records (SMR01) database which holds data on inpatient and day-case hospital discharges from non-obstetric and non-psychiatric hospitals in Scotland. The mortality data was obtained from NRS and linked with the ISD SMR01 database to obtain 30-d case fatality. We used 23 ICD-10 (International Classification of diseases) codes which identify UGIB to interrogate database. We analysed these data for trends in number of hospital admissions with UGIB, 30-d mortality over time and assessed effects of social deprivation. We compared weekend and weekday admissions for differences in 30-d mortality and length of hospital stay. We determined comorbidities for each admission to establish if comorbidities contributed to patient outcome.

RESULTS: A total of 60643 Scottish residents were admitted with UGIH during January, 2000 and October, 2009. There was no significant change in annual number of admissions over time, but there was a statistically significant reduction in 30-d case fatality from 10.3% to 8.8% ($P < 0.001$) over these 10 years. Number of admissions with UGIB was higher for the patients from most deprived category ($P < 0.05$),

although case fatality was higher for the patients from the least deprived category ($P < 0.05$). There was no statistically significant change in this trend between 2000/01-2009/10. Patients admitted with UGIB at weekends had higher 30-d case fatality compared with those admitted on weekdays ($P < 0.001$). Thirty day mortality remained significantly higher for patients admitted with UGIB at weekends after adjusting for comorbidities. Length of hospital stay was also higher overall for patients admitted at the weekend when compared to weekdays, although only reached statistical significance for the last year of study 2009/10 ($P < 0.0005$).

CONCLUSION: Despite reduction in mortality for UGIB in Scotland during 2000-2010, weekend admissions show a consistently higher mortality and greater lengths of stay compared with weekdays.

Key words: Gastrointestinal Haemorrhage; Mortality; Endoscopy; Length of stay; Emergency service

Core tip: In this study we have used a large administrative database to demonstrate a significant reduction in mortality from upper gastrointestinal bleeding in Scotland from 2000 to 2010, with stable number of admissions over this time. It is interesting to see this trend during a period of increased incidence of variceal bleeding with a rising burden of chronic liver disease. This is the first report from Scotland demonstrating a "weekend effect" for upper gastrointestinal bleeding. Patients admitted at weekends have significantly higher mortality and a greater length of hospital stay compared with those admitted on weekdays, despite adjustments for comorbidities. These data can help inform resource planning for hospitals at weekends.

Ahmed A, Armstrong M, Robertson I, Morris AJ, Blatchford O, Stanley AJ. Upper gastrointestinal bleeding in Scotland 2000-2010: Improved outcomes but a significant weekend effect. *World J Gastroenterol* 2015; 21(38): 10890-10897 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10890.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10890

INTRODUCTION

Upper gastrointestinal bleeding (UGIB) is a common medical emergency with an incidence of 103-172 per 100000 in the United Kingdom[1-3]. This condition accounts for approximately 25000 hospital admissions annually in the United Kingdom[4]. Some studies have suggested an improved outcome over recent years, with others describing a reduced incidence and an association with social deprivation[1,3,5,6].

An increased case fatality among patients presenting to hospitals at weekends has been reported for a number of medical emergencies, including pulmonary embolism[7], myocardial infarction[8] and stroke[9,10]. This has been described as a "weekend effect". Although some recent studies have suggested a worse outcome for patients presenting with UGIB at weekends, reports on this issue are inconsistent[3,11-13]. A study based on the 2007 United Kingdom national audit did not find a weekend effect for UGIB[14]. There are several processes involved in early management of acute UGIB including risk stratification, early resuscitation, specialist involvement and early endoscopy. Many of these can be affected by variations in hospital staffing levels and resource availability, particularly at weekends. These may impact on patient outcomes including durations of hospital admission and risk of death.

Our aims were to assess trends over time in numbers and case fatality of patients admitted with UGIB in Scotland and examine whether there is an association with social deprivation. We also assessed whether outcomes including case fatality and duration of hospital stay are different for patients who presented at the weekend, compared with those presenting on weekdays. Finally, we examined whether patient comorbidities accounted for any weekend variation.

MATERIALS AND METHODS

We sourced data from Information Services Division (ISD) Scotland and National Records of Scotland (NRS) death records for a ten year period between 2000/01 and 2009/10. ISD Scotland is a division of National Services Scotland and part of National Health Services Scotland. It works in partnership with a wide range of organisations to build and maintain high quality national health related datasets and statistical services. We obtained data from the Scottish Morbidity Records (ISD) SMR01 database which holds data on inpatient and day-case hospital discharges from non-obstetric and non-psychiatric hospitals in Scotland. SMR01 episode records are used to identify individual hospital stays. The data is based on Scottish residents only. The mortality data was obtained from NRS and linked with the ISD SMR01 database to obtain 30-d case fatality. This was expressed as percentage of patients who died within 30 d from a hospital admission with a main diagnosis of UGIB. Case fatality figures have been reported in this manuscript as "mortality", to ensure consistency with other reports. All data records were extracted from the ISD-held permanently linked dataset and were managed subject to ISD information governance rules and processes.

Upper GI bleeding was defined using ICD-10 (International Classification of diseases) codes. It is a standard tool used to classify diseases and maintain medical records allowing later retrieval of information

Table 1 ICD-10 codes used for upper gastrointestinal bleeding

ICD10 code	Description
I850	Oesophageal varices with bleeding
K226	Gastro-oesophageal laceration - haemorrhage syndrome
K228	Other specified diseases of oesophagus
K250	Gastric ulcer, acute with haemorrhage
K252	Gastric ulcer, acute with both haemorrhage and perforation
K254	Gastric ulcer, chronic or unspecified with haemorrhage
K256	Chronic or unspecified Gastric ulcer with both haemorrhage and perforation
K260	Duodenal ulcer, acute with haemorrhage
K262	Duodenal ulcer, acute with both haemorrhage and perforation
K264	Duodenal ulcer, chronic or unspecified with haemorrhage
K266	Chronic or unspecified Duodenal ulcer with both haemorrhage and perforation
K270	Peptic ulcer, acute with haemorrhage
K272	Peptic ulcer, acute with both haemorrhage and perforation
K274	Peptic ulcer, chronic or unspecified with haemorrhage
K276	Chronic or unspecified Peptic ulcer with both haemorrhage and perforation
K280	Gastrojejunal ulcer, acute with haemorrhage
K282	Gastrojejunal ulcer, acute with both haemorrhage and perforation
K284	Gastrojejunal ulcer, chronic or unspecified with haemorrhage
K286	Chronic or unspecified Gastrojejunal ulcer with both haemorrhage and perforation
K290	Acute haemorrhagic gastritis
K920	Haematemesis
K921	Melaena
K922	Gastrointestinal haemorrhage, unspecified

for epidemiological purposes. ICD-10 codes used to define UGIB are summarised in Table 1.

Data on length of hospital admission was calculated using the number of days between date of admission and discharge. The date of discharge was used to allocate an admission to a financial year.

The measure of deprivation used was the Scottish Index of Multiple Deprivation (SIMD) 2009. The SIMD is a composite index of multiple deprivations using data from seven domains including income, employment, education, housing, health, crime and geographical access. The SIMD 2009 scores are calculated for residential areas and divides areas of Scottish population into quintiles, giving five equal sized groups with 20% of the population falling into each quintile. Quintile 1 is the most deprived and quintile 5 is the least deprived. Patients' residential postal code at the time of hospital admission was used to allocate their SIMD 2009 quintile.

We analysed these data for trends in both number of hospital admissions with UGIB and 30-d mortality over time. We compared weekend and weekday admissions for differences in 30-d mortality and length of hospital stay. Weekdays were defined as Monday to Friday with weekends being Saturday and Sunday (days defined as midnight to midnight). Deaths were recorded within 30 d of patients' admissions; where patients had more than one admission in the 30 d prior to death, the death was only linked to the admission closest to their death, to avoid double-counting.

ISD SMR01 episodic data is not suitable for calculating co-morbidities prevalent at the time of admission, due to coding guidance which requires that only other conditions related to the current diagnosis should be recorded in the secondary diagnosis fields. Therefore to correct for the effect of comorbidities on mortality for weekday and weekend admissions, a five year look back for each admission with UGIB was carried out to determine comorbidities. Comorbidity was measured using the revised Charlson's comorbidity score as described in Department of Health, information centre's Summary Hospital-level Mortality Indicator (SHMI)[15]. Scores assigned over the five year look back period were combined to give a final score at the point of admission. Data were analysed using SPSS version 21[16].

Statistical analysis

We used linear regression analysis to assess the trends in number of admissions with UGIB, and 30 d mortality, and to compare trends in relationship between 30 d mortality and deprivation over the 10-year period. *Z* test of proportions was used to compare proportion of deaths for patients who were admitted on weekdays with proportion of deaths for patients who were admitted on weekends. Two sample *t*-test was used to compare average length of stay between weekends and weekday admissions with UGIB.

RESULTS

A total of 60643 Scottish residents were admitted to Scottish hospitals with a diagnosis of UGIB during the 10 year period between 2000/01-2009/10. Altogether, there were 73834 admissions as some patients had more than one admission for UGIB during this period. There was no significant variation in the numbers of annual hospital admissions with UGIB over this study period.

Patients admitted at weekends were younger than those admitted on weekdays (median age 60 years *vs* 62 years, $P < 0.0005$). Trends in number of hospital admissions, 30-d mortality and length of hospital stay are shown in Table 2.

There was a significant trend in 30-d mortality which reduced from 10.3% of patients in 2000/01 to 8.8% in 2009/10 (χ^2 for trend $P < 0.0005$). The durations of patients' hospital admissions fell significantly between 2000/01 and 2009/10 (median from 3.0 to 2.0 d; mean from 9.2 to 7.9 d, both $P < 0.0005$).

Effect of deprivation

There was a statistically significant association

Table 2 Trends in number of hospital admissions and outcome for patients with upper gastrointestinal bleeding in Scotland

	2000/1	2001/2	2002/3	2003/4	2004/5	2005/6	2006/7	2007/8	2008/9	2009/10
Number of admissions[1]	7674	7717	7365	7106	7145	7236	7316	7363	7397	7717
Median length of stay (d)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	2.0
Mean length of stay (d)	9.2	9.8	10.0	9.8	10.1	9.6	9.2	8.2	8.6	7.9
Total number of patients	6973	7002	6659	6480	6508	6582	6618	6634	6690	6813
Number of deaths	718	744	703	705	663	646	650	625	623	599
30-d mortality (%)[2]	10.3	10.6	10.6	10.9	10.2	9.8	9.8	9.4	9.3	8.8

[1]Same patient may be counted more than once if they had more than one upper gastrointestinal bleeding admission during the year; [2]Patient are counted only once during each year but may be counted more than once across all 10 years.

Table 3 Charlson's co-morbidity score at time of admission

	Charlson's co-morbidity score				
Point of Admission	Number	Median	mean	SD	95%CI (mean)
Weekday	59061	3	7.055	9.452	6.98-7.13
Weekend	15442	4	7.603	9.613	7.45-7.75

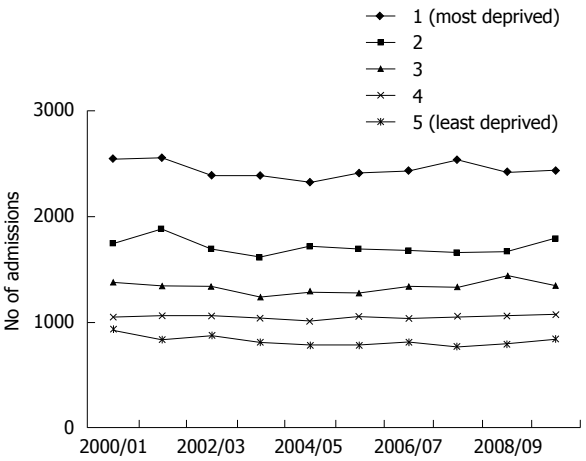


Figure 1 Number of admissions with a diagnosis of upper gastrointestinal bleeding by deprivation quintile (Scottish Index of Multiple Deprivation deprivation quintiles 1 and 5).

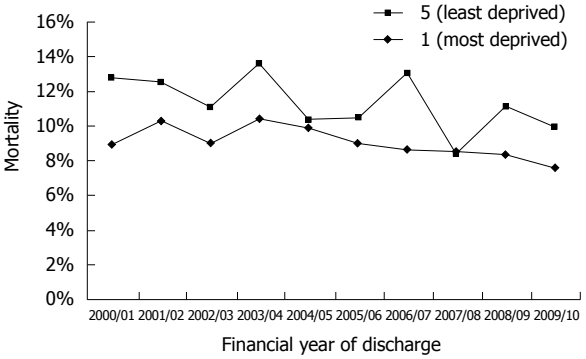


Figure 2 30-d mortality following upper gastrointestinal bleeding for patients in Scottish Index of Multiple Deprivation deprivation quintiles 1 and 5.

between UGIB and deprivation with a higher number of hospital admissions for patients who were more deprived during this 10 years period ($P < 0.05$; Figure 1). However patients in the least deprived SIMD category had a higher 30-d mortality compared with the most deprived SIMD category ($P < 0.05$; Figure 2). Over the ten year study period there was a significant decrease in 30-d mortality for patients in SIMD deprivation quintiles 1, 4 and 5; (P values of χ^2 for trend in quintiles 1 to 5 = 0.002, 0.13, 0.08, 0.02, 0.02 respectively).

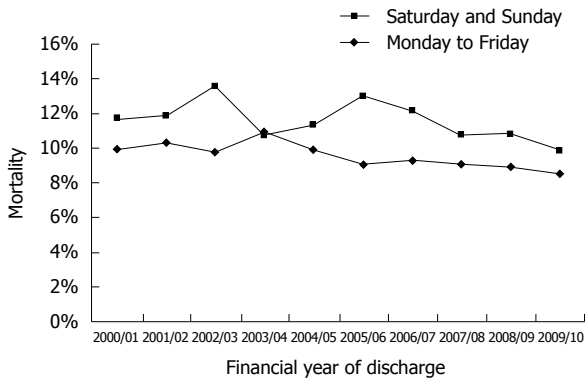


Figure 3 30-d mortality, comparing weekend and weekday admissions.

Weekend effect - mortality

Compared to patients admitted on a weekday, weekend admissions had a significantly higher mortality overall and for seven of the ten years (all but 2003/04, 2004/05 and 2009/10; $P < 0.001$; Figure 3)

Logistic regression analysis was performed including effects of age, gender, day of the week and comorbidity measured by Charlson's comorbidity score. People admitted at the weekend with a diagnosis of UGIB had a higher comorbidity score than those admitted during the week ($P < 0.001$; see Table 3). However, after adjusting for comorbidity, 30 d mortality remained significantly higher for patients admitted with UGIB at weekends.

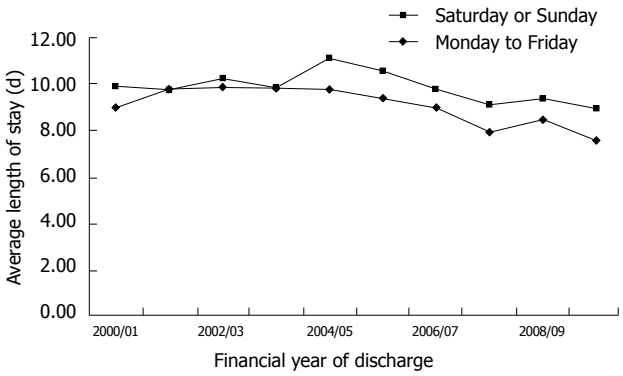


Figure 4 Mean length of hospital stay, comparing weekend and weekday admissions.

Weekend effect- length of admission

Length of hospital stay was higher overall for patients admitted at the weekend when compared to weekdays ($P < 0.0005$), although only reached significance for an individual year in the last year of study (2009/10) as shown in Figure 4.

DISCUSSION

In this study we have used a large administrative database to demonstrate a significant reduction in mortality from UGIB in Scotland from 2000-2010, with a stable number of admissions with UGIB over this time. Admissions with UGIB were closely related to deprivation with a greater number of admissions in the most deprived categories, but higher mortality among the least deprived. Patients admitted at weekends with UGIB had higher mortality than those admitted on weekdays and a longer duration of hospital stay. Although patients admitted at weekend had a higher comorbidity score than those admitted on weekdays, this did not account for the mortality difference. This would suggest that factors other than comorbidity contribute to a worse outcome at weekends.

Our finding of a significant reduction in 30-d mortality from 10.3% to 8.8% over the ten year study period is consistent with some other studies reported from the United Kingdom. Button *et al*[3] found mortality from UGIB in Wales fell from 11.4% to 8.6% over a seven year period. Crooks *et al*[5] reported a reduction in 28 d mortality in England for both variceal and non variceal haemorrhage, which fell by 2% and 3% respectively. Similar findings have been reported recently from other European countries. Cavallaro *et al*[17] found a significant improvement in GI bleeding outcomes in Veneto Italy during the decade 2001-2010; including reduced in-hospital mortality and length of hospital stay. This reduction in mortality may be explained by several factors including advances in endoscopic haemostatic therapies, the use of proton pump inhibitors for ulcer bleeding, vasopressors and antibiotics for variceal haemorrhage and use of risk scoring systems for patients with UGIB. It is interesting that the reduction in overall mortality has occurred during a period of increased incidence of variceal bleeding due to the rising burden of chronic liver disease[6].

Cavallaro *et al*[17] also reported a reduction in overall admissions with UGIB over the 10 year period between 2001-2010. A recent study from Finland reported a decline in incidence of bleeding gastric and duodenal ulcers between the years 2000-2008[18]. This is in contrast to our finding of a stable incidence in Scotland over a similar time period. The reasons for this are unclear but may be explained by different population characteristics such as social deprivation rates and changing incidence of chronic liver disease.

We found a very strong association between the incidence of UGIB and social deprivation with the highest number of admissions among the most deprived groups. A previous West of Scotland study found a 2.2 fold increased incidence for the most deprived quarter of patients when compared with the least deprived[1]. Recent English and Welsh studies found a similar increased admission rates in the most deprived quintile[3,19]. Similar to our study, the English and Welsh studies did not find an increased mortality gradient with deprivation. One plausible explanation for these findings could be the possibility that patients in most deprived quintile presented with UGIB of lesser severity (such as gastritis or Mallory Weiss bleeding after acute alcohol intoxication) thereby resulting in consistently higher number of admissions but lower mortality. On the other hand, patients in least deprived category had fewer episodes of minor UGIB secondary to gastritis and Mallory Weiss bleeding after alcohol intoxication resulting in fewer admissions. It is possible that the majority of presentations in this least deprived category were due to more severe causes of UGIB, thereby increasing overall mortality.

A higher mortality has been reported for patients admitted at weekends with a variety of medical emergencies, including acute myocardial infarction, stroke, UGIB, abdominal aortic aneurysm, pulmonary embolus and acute epiglottitis[3,7,11,12,20-22]. The UGIB study from Wales found that mortality was 13% higher for patients admitted on the weekends compared with weekdays[3]. They found mortality to be even higher for patients admitted on public holidays. Due to methods of coding, we were unable to separately assess outcome for patients presenting on public holidays.

Two large cohort studies from the United States reported a 10%-20% increased mortality for patients admitted with UGIB at weekends compared with weekdays[12,13]. On the contrary, a recent study based on data collected from the 2007 United Kingdom national UGIB audit did not show a difference in risk adjusted mortality for patients presenting at weekends compared with weekdays, despite a delay in endoscopy for those admitted at weekends[14,23]. This

may be due to non-consecutive recording of data in the United Kingdom national audit, with some hospitals contributing a small number of cases which may have created a selection bias[23]. Our data provides a complete national picture by including all hospital admissions for UGIB in Scotland for each year, thereby minimising case selection bias.

There are several possible explanations for our findings of a higher mortality for weekend admissions. Firstly, it may relate to staffing and resource issues. On weekends, hospitals are typically staffed by fewer, less experienced health care providers with poor continuity of care. Many hospitals have relatively limited specialist cover at weekends, including endoscopy staff and interventional radiologists. Some of these issues have been associated with lower quality of care and worse outcome[24,25]. The availability of urgent or next day endoscopy is variable in many hospitals and regions, with the 2007 United Kingdom audit revealing that 52% hospitals had no formal on-call endoscopy rota for emergency procedures, with only 50% patients having endoscopy within 24 h of presentation with acute UGIB[23]. Interestingly a recent study from South Korea suggested that early endoscopy for peptic ulcer bleeding could prevent the deleterious "weekend effect" on outcome[26].

Secondly, it has been suggested that patients admitted over the weekend with a variety of medical conditions have increased co-morbidities or more severe illness[27,28]. It is possible that patients with minor bleeding delay seeking medical attention over the weekend and see their General Practitioner on Monday, while those with more severe bleeding seek emergency care. Due to the observational nature of our study we were unable to determine bleeding severity for individual cases. We found that patients admitted at the weekend with UGIB had a higher Charlson's co-morbidity score than those admitted during the week. However, even after correction for co-morbidity, patients admitted at the weekend had higher 30-d mortality than those admitted on weekdays. Therefore differences in comorbidity do not fully account for the higher weekend mortality.

Median length of hospital stay for patients admitted at the weekend was also significantly longer over the whole study period, with a numerically higher in-patient stay for patients admitted at weekends compared with weekdays for each year from April, 2003. Dorn *et al*[11] examined for weekend effect using a large population based data from North America and reported length of hospital stay to be 1.7% longer for weekend admissions with UGIB. Similar findings were reported by Shaheen *et al*[12] from Canada. In contrast, Button *et al*[3] reported shorter duration for weekend admissions and a younger patient age group suggesting possibly less severe bleeding, but higher case fatality. The reasons for this remain unclear.

There are several potential limitations of our study. Firstly, the weekend was defined as midnight on Friday to midnight on Sunday. We know that for practical purposes this is not an exact reflection of variations in staffing levels and resources. However for coding reasons, this was the only way to define the weekend for the purposes of this study.

It is possible that coding misclassified some patients with UGIB. In order to minimise this we used a broad combination of ICD 10 codes including some very specific, and others more sensitive but less specific (see Table 1). Another potential weakness could be the accuracy of the coding itself. However, the accuracy of ICD coding has improved in Scotland over time, with the most recent audit from 2011 showing an accuracy of 88%[29]. Therefore error resulting from this is likely to be small.

Thirdly, we were unable to assess the timing of endoscopy and use of drug therapy which may have affected case fatality and duration of hospital admission. Although most international guidelines recommend endoscopy within 24 h of admission with UGIB[30,31], as stated above, during the 2007 audit many United Kingdom hospitals had no formal out-of-hours endoscopy rota and many patients did not undergo endoscopy within 24 h, particularly at weekends[23].

In conclusion, this is the first study from Scotland demonstrating "weekend effect" for UGIB. Although there has been a gradual reduction in mortality for patients admitted with UGIB in Scotland over the decade 2000-2010, those admitted at the weekend have consistently higher mortality and a greater length of stay compared with those admitted on weekdays.

ACKNOWLEDGMENTS

Richard Hunter and John Quinn (Information Statistics Division), for their help with data analysis for the Charlson's comorbidity scores.

COMMENTS

Background

Upper gastrointestinal bleeding (UGIB) is a common medical emergency accounting for 25000 hospital admissions annually in the United Kingdom. UGIB has been associated with a high mortality which remains significant but has improved over the years. Recent studies have shown increased mortality for patients presenting to hospitals at weekends for a number of medical emergencies. However, there is inconsistent data on whether UGIB demonstrates a "weekend effect" with worse outcome for patients admitted at weekend with UGIB. In this study the authors aimed to assess the effect of weekend admission on outcome of patients attending hospital with UGIB.

Research frontiers

There is a growing interest in hospital resource availability and staffing level at weekends and its impact on patient outcome. This has been examined for several medical emergencies which can inform resource planning for hospitals at weekends.

Innovations and breakthroughs

This is the first report from Scotland confirming a reduction in 30 d mortality

from UGIB over the ten year period. These findings are consistent with other reports from the United Kingdom and Europe. However, the present study also found higher mortality and longer length of stay for admissions over the weekend in comparison with weekday admissions.

Applications

The authors suggest further studies to identify and understand deficiencies in available staffing and resources at the weekend followed by introduction of measures to improve provision of care at the weekends including availability of formal out of hours endoscopy.

Terminology

"Weekend effect" describes worse outcome of patients admitted over the weekend when compared to those admitted over the weekend. This effect reflects staffing and resource issues at the weekend, which requires better understanding of these issues, thereby allowing implementation of changes.

Peer-review

This is an interesting study evaluating the upper gastrointestinal bleeding within ten years in Scotland. Interesting data and concerning about the weekend effect. Given the advent of 7 d working in the National Health Services, hopefully this is re-examined for 2005-2015 for example, this effect might be lessened.

REFERENCES

1 **Blatchford O**, Davidson LA, Murray WR, Blatchford M, Pell J. Acute upper gastrointestinal haemorrhage in west of Scotland: case ascertainment study. *BMJ* 1997; **315**: 510-514 [PMID: 9329304]

2 **Rockall TA**, Logan RF, Devlin HB, Northfield TC. Incidence of and mortality from acute upper gastrointestinal haemorrhage in the United Kingdom. Steering Committee and members of the National Audit of Acute Upper Gastrointestinal Haemorrhage. *BMJ* 1995; **311**: 222-226 [PMID: 7627034]

3 **Button LA**, Roberts SE, Evans PA, Goldacre MJ, Akbari A, Dsilva R, Macey S, Williams JG. Hospitalized incidence and case fatality for upper gastrointestinal bleeding from 1999 to 2007: a record linkage study. *Aliment Pharmacol Ther* 2011; **33**: 64-76 [PMID: 21128984 DOI: 10.1111/j.1365-2036.2010.04495.x]

4 **Palmer K**. Management of haematemesis and melaena. *Postgrad Med J* 2004; **80**: 399-404 [PMID: 15254304 DOI: 10.1383/medc.31.1.19.2801]

5 **Crooks C**, Card T, West J. Reductions in 28-day mortality following hospital admission for upper gastrointestinal hemorrhage. *Gastroenterology* 2011; **141**: 62-70 [PMID: 21447331 DOI: 10.1053/j.gastro.2011.03.048]

6 **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]

7 **Bell CM**, Redelmeier DA. Mortality among patients admitted to hospitals on weekends as compared with weekdays. *N Engl J Med* 2001; **345**: 663-668 [PMID: 11547721 DOI: 10.1056/NEJMsa003376]

8 **Kostis WJ**, Demissie K, Marcella SW, Shao YH, Wilson AC, Moreyra AE. Weekend versus weekday admission and mortality from myocardial infarction. *N Engl J Med* 2007; **356**: 1099-1109 [PMID: 17360988 DOI: 10.1056/NEJMoa063355]

9 **Hasegawa Y**, Yoneda Y, Okuda S, Hamada R, Toyota A, Gotoh J, Watanabe M, Okada Y, Ikeda K, Ibayashi S. The effect of weekends and holidays on stroke outcome in acute stroke units. *Cerebrovasc Dis* 2005; **20**: 325-331 [PMID: 16131801 DOI: 10.1159/000087932]

10 **Saposnik G**, Baibergenova A, Bayer N, Hachinski V. Weekends: a dangerous time for having a stroke? *Stroke* 2007; **38**: 1211-1215 [PMID: 17347472 DOI: 10.1161/01.STR.0000259622.78616.ea]

11 **Dorn SD**, Shah ND, Berg BP, Naessens JM. Effect of weekend hospital admission on gastrointestinal hemorrhage outcomes. *Dig Dis Sci* 2010; **55**: 1658-1666 [PMID: 19672711 DOI: 10.1007/s10620-009-0914-1]

12 **Shaheen AA**, Kaplan GG, Myers RP. Weekend versus weekday admission and mortality from gastrointestinal hemorrhage caused by peptic ulcer disease. *Clin Gastroenterol Hepatol* 2009; **7**: 303-310 [PMID: 18849015 DOI: 10.1016/j.cgh.2008.08.033]

13 **Ananthakrishnan AN**, McGinley EL, Saeian K. Outcomes of weekend admissions for upper gastrointestinal hemorrhage: a nationwide analysis. *Clin Gastroenterol Hepatol* 2009; **7**: 296-302e1 [PMID: 19084483 DOI: 10.1016/j.cgh.2008.08.013]

14 **Jairath V**, Kahan BC, Logan RF, Hearnshaw SA, Travis SP, Murphy MF, Palmer KR. Mortality from acute upper gastrointestinal bleeding in the United kingdom: does it display a "weekend effect"? *Am J Gastroenterol* 2011; **106**: 1621-1628 [PMID: 21606977 DOI: 10.1038/ajg.2011.172]

15 Indicator Specification Summary Hospital level Mortality Indicator methodology. Accessed Jan 2014. Available from: URL: http://www.hscic.gov.uk/media/11151/Indicator-SpecificationSummary-Hospital-level-Mortality-Indicator-methodology/pdf/SHMI_Specification.pdf

16 **IBM Corp**. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp, 2012

17 **Cavallaro LG**, Monica F, Germanà B, Marin R, Sturniolo GC, Saia M. Time trends and outcome of gastrointestinal bleeding in the Veneto region: a retrospective population based study from 2001 to 2010. *Dig Liver Dis* 2014; **46**: 313-317 [PMID: 24365335 DOI: 10.1016/j.dld.2013.11.005]

18 **Malmi H**, Kautiainen H, Virta LJ, Färkkilä N, Koskenpato J, Färkkilä MA. Incidence and complications of peptic ulcer disease requiring hospitalisation have markedly decreased in Finland. *Aliment Pharmacol Ther* 2014; **39**: 496-506 [PMID: 24461085 DOI: 10.1111/apt.12620]

19 **Crooks CJ**, West J, Card TR. Upper gastrointestinal haemorrhage and deprivation: a nationwide cohort study of health inequality in hospital admissions. *Gut* 2012; **61**: 514-520 [PMID: 21757448 DOI: 10.1136/gutjnl.2011.300186]

20 **Cram P**, Hillis SL, Barnett M, Rosenthal GE. Effects of weekend admission and hospital teaching status on in-hospital mortality. *Am J Med* 2004; **117**: 151-157 [PMID: 15276592]

21 **Schmulewitz L**, Proudfoot A, Bell D. The impact of weekends on outcome for emergency patients. *Clin Med* 2005; **5**: 621-625 [PMID: 16411359]

22 **Smith S**, Allan A, Greenlaw N, Finlay S, Isles C. Emergency medical admissions, deaths at weekends and the public holiday effect. Cohort study. *Emerg Med J* 2014; **31**: 30-34 [PMID: 23345314 DOI: 10.1136/ememed.2012.201881]

23 **Hearnshaw SA**, Logan RF, Lowe D, Travis SP, Murphy MF, Palmer KR. Use of endoscopy for management of acute upper gastrointestinal bleeding in the UK: results of a nationwide audit. *Gut* 2010; **59**: 1022-1029 [PMID: 20357318 DOI: 10.1136/gut.2008.174599]

24 **Tarnow-Mordi WO**, Hau C, Warden A, Shearer AJ. Hospital mortality in relation to staff workload: a 4-year study in an adult intensive-care unit. *Lancet* 2000; **356**: 185-189 [PMID: 10963195 DOI: 10.1016/S0140-6736(00)02478-8]

25 **Meltzer D**, Manning WG, Morrison J, Shah MN, Jin L, Guth T, Levinson W. Effects of physician experience on costs and outcomes on an academic general medicine service: results of a trial of hospitalists. *Ann Intern Med* 2002; **137**: 866-874 [PMID: 12458986 DOI: 10.7326/0003-4819-137-11-200212030-00007]

26 **Youn YH**, Park YJ, Kim JH, Jeon TJ, Cho JH, Park H. Weekend and nighttime effect on the prognosis of peptic ulcer bleeding. *World J Gastroenterol* 2012; **18**: 3578-3584 [PMID: 22826623 DOI: 10.3748/wjg.v.18.i27.3578]

27 **Mikulich O**, Callaly E, Bennett K, O'Riordan D, Silke B. The increased mortality associated with a weekend emergency admission is due to increased illness severity and altered case-mix. *Acute Med* 2011; **10**: 182-187 [PMID: 22111090]

28 **de Groot NL**, Bosman JH, Siersema PD, van Oijen MG, Bredenoord AJ. Admission time is associated with outcome of upper gastrointestinal bleeding: results of a multicentre prospective cohort study. *Aliment Pharmacol Ther* 2012; **36**: 477-484 [PMID: 22747509 DOI: 10.1111/j.1365-2036.2012.05205.x]

29 Information Services Division. Accessed Mar 2015. Available from: URL: http://www.isdscotland.org/Health-Topics/Hospital-Care/Publications/2012-05-08/Assessment-of-SMR01Data-2010-2011-ScotlandReport.pdf
30 **Barkun AN**, Bardou M, Kuipers EJ, Sung J, Hunt RH, Martel M, Sinclair P. International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. *Ann Intern Med* 2010; **152**: 101-113 [PMID: 20083829 DOI: 10.7326/0003-4819-152-2-201001,90-00009]
31 **National Institute for health and Clinical Excellence guideline**. Acute upper gastrointestinal bleeding: management. 2012 Jun 23. London (UK): NICE, 2012

P- Reviewer: Antoniazzi S, Corbett C **S- Editor**: Yu J **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10898

World J Gastroenterol 2015 October 14; 21(38): 10898-10906
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Lymphocyte-to-monocyte ratio predicts survival of patients with hepatocellular carcinoma after curative resection

Ze-Xiao Lin, Dan-Yun Ruan, Yang Li, Dong-Hao Wu, Xiao-Kun Ma, Jie Chen, Zhan-Hong Chen, Xing Li, Tian-Tian Wang, Qu Lin, Jing-Yun Wen, Xiang-Yuan Wu

Ze-Xiao Lin, Dan-Yun Ruan, Dong-Hao Wu, Xiao-Kun Ma, Jie Chen, Zhan-Hong Chen, Xing Li, Tian-Tian Wang, Qu Lin, Jing-Yun Wen, Xiang-Yuan Wu, Department of Medical Oncology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, Guangdong Province, China

Yang Li, Department of Liver Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, Guangdong Province, China

Author contributions: Lin ZX, Ruan DY and Li Y contributed to this work equally and should be regarded as co-first authors; Lin ZX, Ruan DY and Li Y conceived and designed the study; Wu DH, Ma XK, Chen J, Chen ZH, Li X, Wang TT, Lin Q and Wen JY performed the study; Lin ZX, Ruan DY and Li Y analyzed the data; Lin ZX drafted and revised the manuscript; all authors have read and approved the final manuscript.

Supported by National Natural Science Foundation of China, No. 81372374; the Combination Project of Production, Education and Research from Guangdong Province and Ministry of Education, No. 2012B091100460; and the Natural Science Foundation of Guangdong, No. 2014A030313146.

Institutional review board statement: This study protocol was approved by the Clinical Ethics Review Board of The Third Affiliated Hospital of Sun Yat-sen University.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: None of the authors declared a potential conflict of interest related to this manuscript. None of the authors have received a fee for serving as a speaker for any organization, and none have received research funding from any organization. The authors are not employees of any organization and do not own stocks and/or shares in any organization. The authors do not own patents.

Data sharing statement: Technical appendix, statistical code, and dataset are available from the corresponding author at wuxiangy@mail.sysu.edu.cn. Participants provided informed consent for data sharing. No additional data are available.

Correspondence to: Xiang-Yuan Wu, MD, Professor, Chief, Department of Medical Oncology, The Third Affiliated Hospital of Sun Yat-sen University, No. 600 Tianhe Road, Guangzhou 510630, Guangdong Province, China. wuxiangy@mail.sysu.edu.cn
Telephone: +86-20-85252217
Fax: +86-20-85252092

Received: March 5, 2015
Peer-review started: March 6, 2015
First decision: April 24, 2015
Revised: May 11, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 14, 2015

Abstract

AIM: To investigate the prognostic value of pre-operative lymphocyte-to-monocyte ratio (LMR) in patients with hepatocellular carcinoma (HCC) undergoing curative hepatectomy.

METHODS: Clinicopathological data of 210 hepatitis B virus (HBV)-associated HCC patients who were treated by radical hepatic resection between 2003 and 2010 were retrospectively analyzed. None of the patients received any preoperative anticancer therapy

or intraoperative radiofrequency ablation. The diagnosis was confirmed by pathological examination after surgery. Absolute peripheral blood lymphocyte and monocyte counts were derived from serum complete blood cell count before surgery, and LMR was calculated by dividing lymphocyte count by monocyte count. The best cutoff was determined by receiver operating characteristics (ROC) curve analysis. Correlations between LMR levels and clinicopathological features were assessed using the χ^2 test. Survival outcomes were estimated using the Kaplan-Meier method and compared by the log-rank test. Univariate and multivariate analyses were performed to evaluate the prognostic impact of LMR and other clinicopathological factors on overall survival (OS) and recurrence-free survival (RFS), using the Cox proportional hazards model.

RESULTS: The optimal cutoff value of LMR for survival analysis was 3.23, which resulted in the most appropriate sensitivity of 55.3% and specificity of 74.7%, with the area under the curve (AUC) of 0.66 (95%CI: 0.593-0.725). All patients were dichotomized into either a low ($\leqslant$ 3.23) LMR group (n = 66) or a high (> 3.23) LMR group (n = 144). A low preoperative LMR level was significantly correlated with the presence of cirrhosis, elevated levels of total bilirubin and larger tumor size. Patients with a low LMR level had significantly reduced 5-year OS (61.9% *vs* 83.2%, P < 0.001) and RFS (27.8% *vs* 47.6%, P = 0.009) compared to those with a high LMR level. Multivariate analyses indicated that a lower LMR level was a significantly independent predictor of inferior OS (P = 0.003) and RFS (P = 0.006). Subgroup analysis indicated that survival outcome was significantly more favorable in cirrhotic patients with LMR > 3.23. However, there were no differences between low and high LMR groups for OS and RFS in non-cirrhotic patients.

CONCLUSION: Preoperative LMR was demonstrated for the first time to serve as an independent prognostic factor in HBV-associated HCC patients after curative resection. Prospective studies with larger cohorts for validation are warranted.

Key words: Hepatocellular carcinoma; Liver resection; Lymphocyte-to-monocyte ratio; Prognosis; Prognostic factor

Core tip: Inflammatory microenvironment plays an important role in the progression of hepatocellular carcinoma (HCC). Peripheral blood lymphocyte-to-monocyte ratio (LMR), a novel inflammatory biomarker that combines estimates of host immune homeostasis and tumor microenvironment, has been found to serve as a predictor of clinical outcomes in various malignancies. Prior to this study, there have been no reports regarding the prognostic value of LMR in HCC patients. For the first time in literature, our study identified the optimal cutoff value of LMR for survival analysis and concluded that preoperative LMR could serve as an independent prognostic factor in hepatitis B virus-associated HCC patients after curative resection.

Lin ZX, Ruan DY, Li Y, Wu DH, Ma XK, Chen J, Chen ZH, Li X, Wang TT, Lin Q, Wen JY, Wu XY. Lymphocyte-to-monocyte ratio predicts survival of patients with hepatocellular carcinoma after curative resection. *World J Gastroenterol* 2015; 21(38): 10898-10906 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10898.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10898

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading types of malignant tumors worldwide, which primarily develops in the setting of chronic liver inflammation[1]. In China, nearly 90% of HCC patients have underlying hepatitis B virus (HBV) infection. Hepatic resection has been established as a curative treatment for patients who have localized lesions arising in non-cirrhotic livers, or in cirrhotic livers with well-preserved hepatic function[2]. However, the long-term survival after resection remains dismal due to a high frequency of tumor recurrence[3-5]. Clinicopathological factors, such as microvascular invasion, multifocal disease, tumor size and degree of histologic differentiation, have been used to predict survival in patients with HCC after curative resection[3-6]. However, these clinical tumor parameters can only partially explain the prognostic heterogeneity of HCC.

Cumulative evidence has demonstrated that crosstalk between tumor cells and their surrounding inflammatory microenvironment plays a critical role in the initiation and progression of HCC. Inflammatory infiltrates in the tumor microenvironment can largely influence the biological behavior of HCC[7-12]. Tumor-associated macrophages (TAMs), which comprise a major proportion of leukocytes that infiltrate into the stroma, have been found to promote HCC proliferation, angiogenesis and metastasis[7,11-14]. Immunohistochemical studies have validated the association between high TAM density and unfavorable prognosis in HCC patients after curative resection[15,16]. Peripheral blood monocytes, which are precursors of TAMs[7], have also been reported to be a prognostic factor for HCC[17,18]. Tumor-infiltrating lymphocytes (TILs) are another representative component of the immune microenvironment. Specific TIL subtypes are involved in the clinical course of HCC, and TIL phenotypes are informative regarding prognosis[8-10,13].

Recently, the peripheral blood lymphocyte-to-monocyte ratio (LMR), as a simple surrogate biomarker of TILs and TAMs, has been reported to be a predictor of clinical outcomes in various malignancies[19-25]. LMR

also acts as a representative biomarker by combining estimates of host immune homeostasis (*i.e.*, absolute lymphocyte count) and tumor microenvironment (*i.e.*, absolute monocyte count)[19,20]. To date, there have been no reports regarding the prognostic value of LMR in HCC patients. We therefore conducted this study to investigate the impact of preoperative peripheral blood LMR on long-term outcomes after curative hepatic resection for HCC.

MATERIALS AND METHODS

Patient enrollment and clinicopathological variables

From January 2003 to December 2010, 210 patients with HBV-associated HCC who underwent curative hepatectomy at the Third Affiliated Hospital of Sun Yat-sen University were eligible for this retrospective study. All the patients had chronic HBV infection and were negative for hepatitis C virus antibody. Preoperative diagnosis of HCC was based on typical dynamic images evaluated by contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) according to the Asian Pacific Association for the Study of the Liver (APASL) guideline[26]. Pathological examination confirmed the diagnosis after surgical resection. Curative resection was defined as the complete resection of all tumor nodules with clear microscopic margins and no residual tumors as indicated by CT scan at one month after surgery. Neither preoperative anticancer therapy nor intraoperative radiofrequency ablation was performed on the patients. Antiviral therapy with oral nucleos(t)ide analogues was recommended for all the patients after liver resection.

For each patient in the group, demographic information, complete blood cell count, liver function parameters, serum alpha-fetoprotein (AFP) level, Barcelona Clinic Liver Cancer (BCLC) stage, and other tumor-related parameters were recorded. Tumor-related variables, such as maximal tumor diameter, number of tumor nodules, portal vein thrombus and histological differentiation, were obtained from pathology reports. The absolute peripheral blood lymphocyte and monocyte counts were derived from the complete blood cell count before surgery, with LMR calculated by dividing lymphocyte count by monocyte count. None of the patients exhibited clinical manifestations of acute inflammation before treatment or of coexistent hematologic disorders. The study protocol was approved by the Clinical Ethics Review Board of the Third Affiliated Hospital of Sun Yat-sen University. Informed consent was obtained according to the Declaration of Helsinki.

Follow-up

All patients were regularly followed for recurrence at outpatient clinics. None of the patients died within 30 d after surgery. Serum AFP test and abdominal CT scan were performed every 3 mo during the first two postoperative years and every 6 mo thereafter. If clinical recurrence was suspected, CT was performed immediately. Additional diagnostic investigation such as MRI or hepatic arterial angiography was performed in patients with suspicious lesions demonstrated by CT image. Patients with confirmed recurrence received further treatment, such as second hepatectomy, chemoembolization, radiofrequency ablation or percutaneous ethanol injection. Treatment modality after relapse varied among individuals.

Statistical analysis

Receiver operating curve (ROC) analysis was performed to determine the optimal cutoff values for preoperative absolute lymphocyte count (ALC), absolute monocyte count (AMC) and LMR as prognostic factors. The score closest to the point with both maximum sensitivity and specificity was chosen as the best cutoff value. Correlations between LMR levels and clinicopathological features were assessed using the χ^2 test. Survival outcomes were estimated using the Kaplan-Meier method and compared by the log-rank test. The primary endpoint of the present study was overall survival (OS), which was calculated from the time of surgery to the date of death from any cause, or to the date of the last follow-up. The secondary endpoint was recurrence-free survival (RFS), which was defined as the duration from the date of surgery to the date of HCC recurrence, or to the date of the last follow-up. The prognostic values of ALC, AMC, LMR and other clinicopathological factors were analyzed using the Cox proportional hazards model. Significant variables identified in univariate analysis were included in the multivariate model. A P-value < 0.05 was regarded as statistically significant. All statistical analyses were performed using SPSS software (version 17.0, SPSS Inc, Chicago, IL, United States) and MedCalc statistical software (version 11.4.2.0, Broekstraat 52 Mariakerke, Belgium).

RESULTS

Patients' characteristics and outcomes

All the patients had chronic HBV infection and 161 (76.7%) patients had a histological diagnosis of cirrhosis. The median duration of follow-up was 34.8 mo (range: 1.7-106.6 mo). By the last follow-up, 110 (52.4%) patients developed tumor recurrence, 47 (22.9%) died from causes secondary to HCC progression, and one died from cerebrovascular disease. The 1-, 3-, and 5-year OS rates for all the patients in this study were 95.7%, 80.9% and 75.6%, respectively, and the 1-, 3-, and 5-year RFS rates were 69.9%, 51.7% and 42.3%, respectively.

The optimal cutoff values of LMR, ALC and AMC for survival analyses

The best cutoff points of LMR, ALC and AMC for

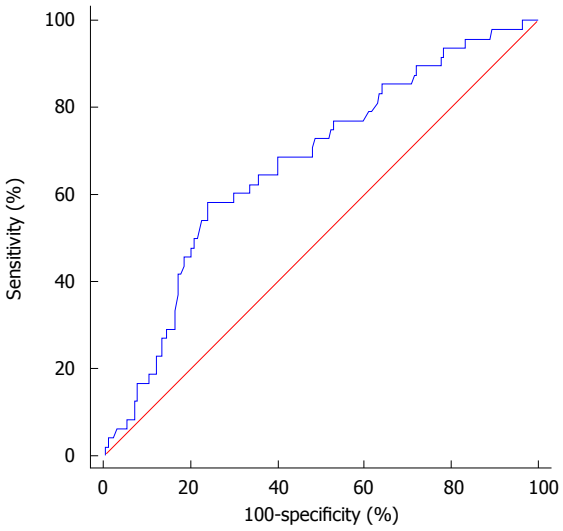


Figure 1 Receiver operating characteristics curve assessing the cutoff value of lymphocyte-to-monocyte ratio for survival analyses in patients with hepatitis B virus-associated hepatocellular carcinoma treated by curative hepatectomy.

survival outcomes were determined by ROC curve analyses, which indicated that the optimal LMR cutoff value for both OS and RFS was 3.23. The LMR cutoff point of 3.23 for OS was selected as the uniform point in survival analyses (Figure 1). The area under the curve (AUC) was recorded as 0.66 (95%CI: 0.593-0.725). Using the LMR value of 3.23 resulted in the most appropriate measures of sensitivity and specificity, which were 55.3% and 74.7%, respectively. Similarly, the most discriminative cutoff values of ALC and AMC were determined to be 1.66 × 10^9/L (AUC: 0.58, 95%CI: 0.511-0.648) and 0.29 × 10^9/L (AUC: 0.61, 95%CI: 0.542-0.678), respectively.

Correlations between preoperative LMR and clinicopathological factors

Based on the cutoff value, all patients were dichotomized into either a low value group or a high value group. The relationship between preoperative peripheral LMR levels and clinicopathological characteristics was summarized in Table 1. Sixty-six patients had an LMR ⩽ 3.23 and one hundred and forty-four patients had an LMR > 3.23. A low LMR level was significantly correlated with ALC ⩽ 1.66 (P < 0.001) and AMC > 0.29 (P < 0.001). Patients with LMR ⩽ 3.23 were also prone to have liver cirrhosis (P = 0.003), elevated levels of total bilirubin (P = 0.002) and larger tumor size (P = 0.030).

Univariate and multivariate analyses

To identify the optimal peripheral blood immunological biomarker for patient prognosis, the impact of ALC, AMC and LMR on survival outcomes was investigated. In univariate analysis for primary endpoint of OS, ALC and AMC were shown to be significant prognostic factors, with a P-value of 0.035 for ALC (HR = 0.511, 95%CI: 0.274-0.953) and a P-value of 0.026 for AMC (HR = 2.644, 95%CI: 1.123-6.223). The association between LMR and OS was also proven to be statistically significant, with a P-value < 0.001 (HR = 0.352, 95%CI: 0.199-0.623), indicating that LMR might provide the strongest prognostic information among these three biomarkers (Table 2). With respect to RFS, significant differences were also observed between low and high LMR groups (P = 0.009, HR = 0.601, 95%CI: 0.410-0.883) (Table 3). Other significant predictors of poorer OS and RFS included a low level

Table 1 Relationship between lymphocyte-to-monocyte ratio and clinicopathological characteristics

Variable	No. of patients	LMR		*P* value
		⩽ 3.23 (*n* = 66)	> 3.23 (*n* = 144)	
Age (yr)				
< 60	165	52	113	0.959
⩾ 60	45	14	31	
Gender				
Female	25	6	19	0.394
Male	185	60	125	
Liver cirrhosis				
Absent	49	7	42	0.003
Present	161	59	102	
ALT (U/L)				
⩽ 75	172	51	121	0.238
> 75	38	15	23	
Total bilirubin (μmol/L)				
⩽ 34	197	57	140	0.002
> 34	13	9	4	
Albumin (g/L)				
< 35	15	7	8	0.303
⩾ 35	195	59	136	
ALP (U/L)				
⩽ 100	171	51	120	0.294
> 100	39	15	24	
AFP (ng/dL)				
⩽ 400	124	38	86	0.769
> 400	86	28	58	
Tumor size (cm)				
⩽ 5	157	43	114	0.030
> 5	53	23	30	
Tumor number				
Single	184	59	125	0.597
Multiple	26	7	19	
Portal vein thrombus				
Absent	196	61	135	0.952
Present	14	5	9	
Microvascular invasion				
Absent	170	55	115	0.552
Present	40	11	29	
Histological differentiation				
Poor	22	8	14	0.598
Well and Moderate	188	58	130	
ALC (× 10^9/L)				
⩽ 1.66	117	50	67	< 0.001
> 1.66	93	16	77	
AMC (× 10^9/L)				
⩽ 0.29	57	3	54	< 0.001
> 0.29	153	63	90	

AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; ALC: Absolute lymphocyte count; ALP: Alkaline phosphatase; AMC: Absolute monocyte count; LMR: Lymphocyte-to-monocyte ratio.

Table 2 Cox proportional hazards model of prognostic factors for overall survival in 210 patients with hepatocellular carcinoma after curative hepatectomy

Variable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Age (yr), ⩾ 60 *vs* < 60	0.766 (0.410-1.433)	0.404		
Gender, male *vs* female	0.829 (0.296-2.321)	0.721		
Liver cirrhosis, yes *vs* no	7.641 (1.853-31.509)	0.005	7.084 (1.694-29.614)	0.007
ALT (U/L), > 75 *vs* ⩽ 75	1.513 (0.771-2.970)	0.229		
Total bilirubin (μmol/L), > 34 *vs* ⩽ 34	2.085 (0.822-5.288)	0.122		
Albumin (g/L), ⩾ 35 *vs* < 35	0.242 (0.112-0.522)	< 0.001		
ALP (U/L), > 100 *vs* ⩽ 100	2.116 (1.148-3.899)	0.016	2.137 (1.153-3.964)	0.016
AFP (ng/dL), > 400 *vs* ⩽ 400	0.956 (0.535-1.705)	0.878		
Tumor size (cm), > 5 *vs* ⩽ 5	2.154 (1.204-3.853)	0.010		
Tumor number, multiple *vs* single	1.048 (0.444-2.477)	0.915		
Portal vein thrombus: yes *vs* no	3.348 (1.492-7.512)	0.003		
Microvascular invasion: yes *vs* no	2.121 (1.151-3.911)	0.016	2.307 (1.217- 4.370)	0.010
Histological differentiation, poor *vs* well and moderate	2.888 (1.467-5.684)	0.002	2.375 (1.195-4.721)	0.014
BCLC stage, B + C *vs* 0 + A	2.110 (1.197-3.720)	0.010	2.155 (1.213-3.831)	0.009
Preoperative LMR, > 3.23 *vs* ⩽ 3.23	0.352 (0.199-0.623)	< 0.001	0.398 (0.219-0.725)	0.003

AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; BCLC: Barcelona Clinic Liver Cancer; LMR: Lymphocyte-to-monocyte ratio.

Table 3 Cox proportional hazards model of prognostic factors for recurrence-free survival in 210 patients with hepatocellular carcinoma after curative hepatectomy

Variable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Age(yr), ⩾ 60 *vs* < 60	1.319 (0.859-2.027)	0.206		
Gender, male *vs* female	0.855 (0.458-1.594)	0.621		
Liver cirrhosis: yes *vs* no	1.316 (0.831-2.086)	0.242		
ALT (U/L), > 75 *vs* ⩽ 75	1.709 (1.096-2.665)	0.018	1.510 (0.960-2.375)	0.074
Total bilirubin (μmol/L), > 34 *vs* ⩽ 34	1.471 (0.715-3.023)	0.294		
Albumin (g/L), ⩾ 35 *vs* < 35	0.279 (0.160-0.485)	< 0.001		
ALP (U/L), > 100 *vs* ⩽ 100	1.506 (0.964-2.354)	0.072		
AFP (ng/dL), > 400 *vs* ⩽ 400	0.934 (0.636-1.373)	0.730		
Tumor size (cm), > 5 *vs* ⩽ 5	2.020 (1.354-3.012)	0.001		
Tumor number, multiple *vs* single	1.599 (0.953-2.684)	0.075		
Portal vein thrombus, yes *vs* no	2.282 (1.150-4.529)	0.018		
Microvascular invasion, yes *vs* no	1.185 (0.742-1.892)	0.478		
Histological differentiation, poor *vs* well and moderate	2.628 (1.561-4.425)	< 0.001	2.610 (1.542-4.416)	< 0.001
BCLC stage, B + C *vs* 0 + A	1.724 (1.180-2.520)	0.005	1.645 (1.124-2.409)	0.010
Preoperative LMR, > 3.23 *vs* ⩽ 3.23	0.601 (0.410-0.883)	0.009	0.584 (0.398-0.859)	0.006

AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; BCLC: Barcelona Clinic Liver Cancer; LMR: Lymphocyte-to-monocyte ratio.

of serum albumin, large tumor size, the presence of portal vein thrombus, poor histological differentiation, and an advanced BCLC stage. Moreover, liver cirrhosis, an elevated level of serum alkaline phosphatase (ALP) and microvascular invasion were all associated with a shorter OS, whereas an elevated serum alanine aminotransferase (ALT) level was correlated with inferior RFS.

Variables showing statistical significance by univariate analysis were included in the multivariate Cox proportional hazard analysis (Tables 2 and 3). As tumor size, portal vein thrombus and serum albumin level were all associated with BCLC stage, we did not enter these variables into further multivariate models so as to avoid potential bias. The results revealed that a high preoperative LMR level was an independent predictor of favorable prognostic measures, including OS (HR = 0.398; 95%CI: 0.219-0.725, P = 0.003) and RFS (HR = 0.584; 95%CI: 0.398-0.859; P = 0.006). Among the remaining factors studied, poor histological differentiation and an advanced BCLC stage were identified as independent indicators for inferior RFS and OS. In addition, cirrhotic liver parenchyma, an elevated serum ALP level and microvascular invasion were independent factors for OS.

Comparisons of OS and RFS rates according to LMR level

Kaplan-Meier curve analysis revealed that a low LMR level was significantly associated with decreased

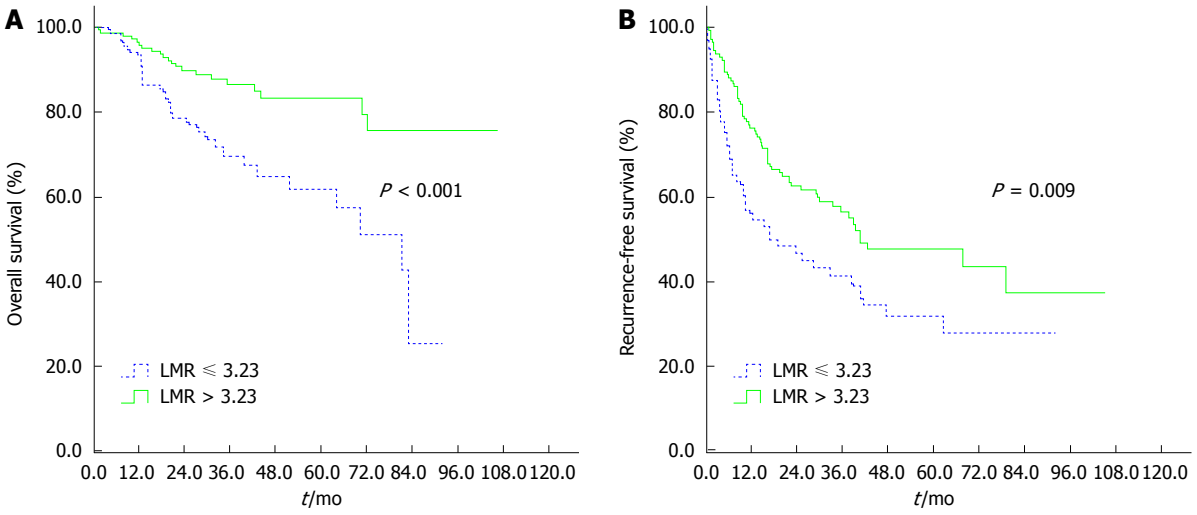


Figure 2 Kaplan-Meier survival analysis of preoperative lymphocyte-to-monocyte ratio in patients with hepatocellular carcinoma undergoing curative resection. A: Overall survival according to lymphocyte-to-monocyte ratio (LMR); B: Recurrence-free survival according to LMR.

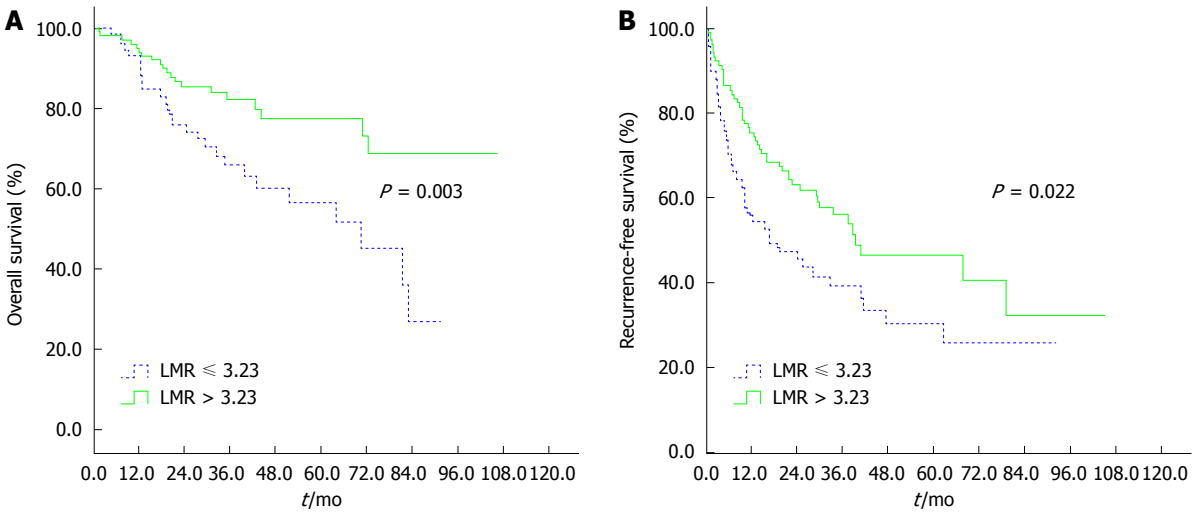


Figure 3 Kaplan-Meier survival analysis of preoperative lymphocyte-to-monocyte ratio in cirrhotic patients with hepatocellular carcinoma undergoing curative resection. A: Overall survival according to lymphocyte-to-monocyte ratio (LMR); B: Recurrence-free survival according to LMR.

OS and DFS. The 5-year OS and RFS rates were 61.9% and 27.8%, respectively, for patients with a preoperative LMR ⩽ 3.23 and were statistically lower than those for patients with a LMR > 3.23 (83.2% and 47.6%, respectively; $P < 0.001$ and $P = 0.009$, respectively; Figure 2). Subgroup analysis was performed according to underlying cirrhosis status (cirrhosis, $n = 161$; non-cirrhosis, $n = 49$). In cirrhotic patients with HCC, a low preoperative LMR level was associated with inferior OS and RFS ($P = 0.003$ and $P = 0.022$, respectively; Figure 3). However, there were no differences between low and high LMR levels for OS and RFS in non-cirrhotic patients ($P = 0.443$ and $P = 0.492$, respectively).

DISCUSSION

Accumulating studies have suggested that the infiltrating inflammatory microenvironment may represent an important determinant for the clinical outcome of HCC[7-12]. The imbalance of inflammatory immune cells, such as TAMs and TILs, in the tumor microenvironment, has been proven to be an important regulator of progression in HCC[11-16]. Systemic inflammatory response can be routinely determined by traditional hematological markers, such as C-reactive protein and neutrophil-to-lymphocyte ratio, which are considered to be valuable prognostic factors in patients with HCC[27-30]. Peripheral blood LMR, as a novel inflammatory biomarker, has been recently investigated and confirmed to be a predictor of clinical outcomes in lymphoma[19,20], colon cancer[21], non-small cell lung cancer[23], nasopharyngeal carcinoma[22], breast cancer[24] and gastric cancer[25].

To the best of our knowledge, this is the first study to investigate the preoperative LMR as a prognostic

marker in HCC patients initially treated by curative hepatectomy. Only HBV-related HCC was included to avoid potential confounding factors from different etiologies. An objective and reliable cutoff point for LMR was generated by employing ROC curve analysis. Univariate analysis revealed that patients with an LMR > 3.23 had significantly better OS and RFS than those with an LMR ⩽ 3.23. On multivariate analysis, LMR remained an independent prognostic marker for OS and RFS throughout the cohort. These results were consistent with previous findings on other types of tumors, in which a low pretreatment level of LMR was reported as an independent unfavorable prognostic factor[19-25]. However, the cutoff values were cancer-specific in the above studies, possibly reflecting the biologic differences among these studied malignancies.

The association between decreased LMR and poor oncologic outcome is complex and remains to be elucidated. There are several possible reasons accounting for this positive correlation. First, lymphocytes are the basic components of host antitumor immunity, which are important in the destruction of residual cancer cells and related micrometastases[20-22]. They infiltrate into tumor microenvironment and manifest as TILs, both the quantity and the phenotype of which may influence the effectiveness of antitumor immune reaction[8-10]. Unitt *et al*[8] found that reduced lymphocyte infiltration and a low $CD4^+/CD8^+$ T cell ratio were both significant independent predictors of HCC recurrence following liver transplantation. Two additional studies demonstrated that low intratumoral cytotoxic $CD8^+$ T and high intratumoral regulatory T cells were associated with a poorer prognosis in HCC patients after resection[9,10]. In general, peripheral blood lymphocyte count serves as a simple surrogate marker of the host immune status. In our study, an association between a low level of ALC and adverse OS was identified by univariate analysis. We also revealed that patients with a decreased LMR had relative lymphocytopenia, which might be responsible for an incompetent immune response against tumor[20-22].

Second, myeloid-lineage cells, including monocytes and their progeny, are known to have immune suppressive activity[31]. They can also promote tumor angiogenesis, tumor-cell invasion and metastasis[21,31]. Circulating monocytes are recruited to the tumor stroma and differentiate into TAMs. As a major component of tumor microenvironment in HCC, TAMs can interact with cancer cells to enhance tumor progression by producing various cytokines and chemokines[11-15]. Poor clinical outcomes associated with high infiltrations of TAMs have been indicated by Zhu *et al*[15] and Kong *et al*[16]. Peripheral blood monocytes may reflect the formation or existence of TAMs[23]. The pro-tumorigenic effect of monocytes on HCC has been associated with poor prognosis, as demonstrated by Sasaki *et al*[17] and Shen *et al*[18] and validated in the current study, which showed that monocytosis was associated with poor OS in patients with HCC after resection.

These data indicate that LMR might act as the surrogate marker which reflects the interaction between host immunity (*i.e*., ALC) and tumor microenvironment (*i.e*., AMC). The presence of preoperative lymphopenia and monocytosis both served as predictors of inferior OS in our study. However, as the combination of ALC and AMC, LMR provided a better prognostic value. A decreased LMR reflects an inflammatory status that favors tumor progression and impairs host immune surveillance, both of which are associated with poor oncologic outcome. Pretreatment LMR level was also inversely correlated with the presence of liver cirrhosis, and the poor outcome predicted by low LMR level was shown only in cirrhotic patients, not in non-cirrhotic ones. These results indicate that the association between cirrhosis and LMR may be an important mechanism for HCC progression.

LMR is a simple and easily assessable clinical biomarker for prognostic stratification of HBV-associated HCC patients after hepatectomy. However, findings of the current study should be interpreted within its possible limitations. First, formal investigations on the specific components of tumor microenvironment in this population were not performed. Second, due to the retrospective design of the study, selection bias was inevitable, which might have influenced the survival analysis. Third, as the study cohort was comprised of a small single-center sample, we were unable to divide the data set into a training set and a testing set for statistical validation.

In conclusion, our study is the first to demonstrate that preoperative LMR can serve as an independent prognostic factor for patients with HBV-associated HCC undergoing curative resection. As a simple and cost-effective biomarker, LMR could be used to identify HCC patients with a poorer survival, especially those with cirrhotic livers, which may guide postoperative treatment. Future biological studies should further correlate LMR with the tumor microenvironment. Prospective studies with larger cohorts are awaited to validate the clinical usage of LMR as a prognostic marker for HCC patients.

ACKNOWLEDGMENTS

The authors thank the surgeons and nurses in the Department of Liver Surgery, The Third Affiliated Hospital of Sun Yat-sen University for their help in treating the patients in this study.

COMMENTS

Background

Cumulative evidence has suggested that the inflammatory microenvironment may represent an important determinant for the clinical outcome of hepatocellular carcinoma (HCC). Peripheral blood lymphocyte-to-monocyte ratio (LMR), which is a novel inflammatory biomarker combining estimates of host immune homeostasis and tumor microenvironment, has been demonstrated

to serve as a predictor of clinical outcomes in various types of malignancies. However, the prognostic value of LMR in patients with HCC remains unknown.

Research frontiers

The prognostic value of LMR has been widely investigated in hematological malignancies such as diffuse large B-cell lymphoma and Hodgkin's lymphoma. However, data regarding the prognostic value of LMR in patients with solid tumors are spare. Recent published studies have shown that preoperative high level of LMR was a favorable prognostic factor in patients with operable lung cancer and colon cancer. Prior to this study, there have been no reports regarding the prognostic value of LMR in patients with HCC until now.

Innovations and breakthroughs

To date, this is the first study to investigate the preoperative LMR as a prognostic biomarker in HCC patients after curative resection. To avoid any potential confounding factors from different etiologies, the authors included only hepatitis B virus-associated HCC patients. They also calculated the optimal LMR cutoff for survival prediction. The results identified that a low LMR level ($\leqslant$ 3.23) was a significantly independent predictor of inferior survival in HCC patients who were initially treated by curative hepatectomy, suggesting that preoperative LMR represents a promising prognostic marker for HCC.

Applications

The study indicated that a low preoperative LMR level was an independent unfavorable prognostic factor for HCC patients who underwent curative hepatectomy. As a simple and cost-effective biomarker, LMR can be used to identify HCC patients with a poorer survival, especially those with cirrhotic livers, which may guide postoperative treatment.

Terminology

LMR is calculated by dividing the lymphocyte count by the monocyte count in peripheral blood.

Peer-review

This is an interesting study with sound methodology and statistical analyses, in which the authors investigated the prognostic value of preoperative LMR in HCC patients undergoing curative hepatectomy. The results suggest that a low preoperative LMR level was an independent unfavorable prognostic factor.

REFERENCES

1 **Sherman M**. Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis. *Semin Liver Dis* 2010; **30**: 3-16 [PMID: 20175029 DOI: 10.1055/s-0030-1247128]

2 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

3 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207 [PMID: 12547409 DOI: 10.1016/S0168-8278(02)00360-4]

4 **Gluer AM**, Cocco N, Laurence JM, Johnston ES, Hollands MJ, Pleass HC, Richardson AJ, Lam VW. Systematic review of actual 10-year survival following resection for hepatocellular carcinoma. *HPB* (Oxford) 2012; **14**: 285-290 [PMID: 22487065 DOI: 10.1111/j.1477-2574.2012.00446.x]

5 **Shah SA**, Cleary SP, Wei AC, Yang I, Taylor BR, Hemming AW, Langer B, Grant DR, Greig PD, Gallinger S. Recurrence after liver resection for hepatocellular carcinoma: risk factors, treatment, and outcomes. *Surgery* 2007; **141**: 330-339 [PMID: 17349844 DOI: 10.1016/j.surg.2006.06.028]

6 **Sumie S**, Kuromatsu R, Okuda K, Ando E, Takata A, Fukushima N, Watanabe Y, Kojiro M, Sata M. Microvascular invasion in patients with hepatocellular carcinoma and its predictable clinicopathological factors. *Ann Surg Oncol* 2008; **15**: 1375-1382 [PMID: 18324443 DOI: 10.1245/s10434-008-9846-9]

7 **Allavena P**, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol* 2008; **66**: 1-9 [PMID: 17913510 DOI: 10.1016/j.critrevonc.2007.07.004]

8 **Unitt E**, Marshall A, Gelson W, Rushbrook SM, Davies S, Vowler SL, Morris LS, Coleman N, Alexander GJ. Tumour lymphocytic infiltrate and recurrence of hepatocellular carcinoma following liver transplantation. *J Hepatol* 2006; **45**: 246-253 [PMID: 16580084 DOI: 10.1016/j.jhep.2005.12.027]

9 **Gao Q**, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, Xu Y, Li YW, Tang ZY. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007; **25**: 2586-2593 [PMID: 17577038 DOI: 10.1200/JCO.2006.09.4565]

10 **Chen KJ**, Zhou L, Xie HY, Ahmed TE, Feng XW, Zheng SS. Intratumoral regulatory T cells alone or in combination with cytotoxic T cells predict prognosis of hepatocellular carcinoma after resection. *Med Oncol* 2012; **29**: 1817-1826 [PMID: 21678026 DOI: 10.1007/s12032-011-0006-x]

11 **Peng SH**, Deng H, Yang JF, Xie PP, Li C, Li H, Feng DY. Significance and relationship between infiltrating inflammatory cell and tumor angiogenesis in hepatocellular carcinoma tissues. *World J Gastroenterol* 2005; **11**: 6521-6524 [PMID: 16425427]

12 **Wu K**, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res* 2009; **69**: 8067-8075 [PMID: 19826049 DOI: 10.1158/0008-5472.CAN-09-0901]

13 **Zhou J**, Ding T, Pan W, Zhu LY, Li L, Zheng L. Increased intratumoral regulatory T cells are related to intratumoral macrophages and poor prognosis in hepatocellular carcinoma patients. *Int J Cancer* 2009; **125**: 1640-1648 [PMID: 19569243 DOI: 10.1002/ijc.24556]

14 **Mano Y**, Aishima S, Fujita N, Tanaka Y, Kubo Y, Motomura T, Taketomi A, Shirabe K, Maehara Y, Oda Y. Tumor-associated macrophage promotes tumor progression via STAT3 signaling in hepatocellular carcinoma. *Pathobiology* 2013; **80**: 146-154 [PMID: 23364389 DOI: 10.1159/000346196]

15 **Zhu XD**, Zhang JB, Zhuang PY, Zhu HG, Zhang W, Xiong YQ, Wu WZ, Wang L, Tang ZY, Sun HC. High expression of macrophage colony-stimulating factor in peritumoral liver tissue is associated with poor survival after curative resection of hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 2707-2716 [PMID: 18509183 DOI: 10.1200/JCO.2007.15.6521]

16 **Kong LQ**, Zhu XD, Xu HX, Zhang JB, Lu L, Wang WQ, Zhang QB, Wu WZ, Wang L, Fan J, Tang ZY, Sun HC. The clinical significance of the CD163+ and CD68+ macrophages in patients with hepatocellular carcinoma. *PLoS One* 2013; **8**: e59771 [PMID: 23555776 DOI: 10.1371/journal.pone.0059771]

17 **Sasaki A**, Iwashita Y, Shibata K, Matsumoto T, Ohta M, Kitano S. Prognostic value of preoperative peripheral blood monocyte count in patients with hepatocellular carcinoma. *Surgery* 2006; **139**: 755-764 [PMID: 16782430]

18 **Shen SL**, Fu SJ, Huang XQ, Chen B, Kuang M, Li SQ, Hua YP, Liang LJ, Peng BG. Elevated preoperative peripheral blood monocyte count predicts poor prognosis for hepatocellular carcinoma after curative resection. *BMC Cancer* 2014; **14**: 744 [PMID: 25280428 DOI: 10.1186/1471-2407-14-744]

19 **Porrata LF**, Ristow K, Colgan JP, Habermann TM, Witzig TE, Inwards DJ, Ansell SM, Micallef IN, Johnston PB, Nowakowski GS, Thompson C, Markovic SN. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma. *Haematologica* 2012; **97**: 262-269 [PMID: 21993683 DOI: 10.3324/haematol.2011.050138]

20 **Li ZM**, Huang JJ, Xia Y, Sun J, Huang Y, Wang Y, Zhu YJ, Li YJ, Zhao W, Wei WX, Lin TY, Huang HQ, Jiang WQ. Blood lymphocyte-to-monocyte ratio identifies high-risk patients in diffuse large B-cell lymphoma treated with R-CHOP. *PLoS One* 2012; **7**: e41658 [PMID: 22911837 DOI: 10.1371/journal.

pone.0041658]
21 **Stotz M**, Pichler M, Absenger G, Szkandera J, Arminger F, Schaberl-Moser R, Samonigg H, Stojakovic T, Gerger A. The preoperative lymphocyte to monocyte ratio predicts clinical outcome in patients with stage III colon cancer. *Br J Cancer* 2014; **110**: 435-440 [PMID: 24357796 DOI: 10.1038/bjc.2013.785]
22 **Li J**, Jiang R, Liu WS, Liu Q, Xu M, Feng QS, Chen LZ, Bei JX, Chen MY, Zeng YX. A large cohort study reveals the association of elevated peripheral blood lymphocyte-to-monocyte ratio with favorable prognosis in nasopharyngeal carcinoma. *PLoS One* 2013; **8**: e83069 [PMID: 24386144 DOI: 10.1371/journal.pone.0083069]
23 **Hu P**, Shen H, Wang G, Zhang P, Liu Q, Du J. Prognostic significance of systemic inflammation-based lymphocyte-monocyte ratio in patients with lung cancer: based on a large cohort study. *PLoS One* 2014; **9**: e108062 [PMID: 25275631 DOI: 10.1371/journal.pone.0108062]
24 **Ni XJ**, Zhang XL, Ou-Yang QW, Qian GW, Wang L, Chen S, Jiang YZ, Zuo WJ, Wu J, Hu X, Shao ZM. An elevated peripheral blood lymphocyte-to-monocyte ratio predicts favorable response and prognosis in locally advanced breast cancer following neoadjuvant chemotherapy. *PLoS One* 2014; **9**: e111886 [PMID: 25372468 DOI: 10.1371/journal.pone.0111886]
25 **Zhou X**, Du Y, Xu J, Huang Z, Qiu T, Wang X, Qian J, Zhu W, Liu P. The preoperative lymphocyte to monocyte ratio predicts clinical outcomes in patients with stage II/III gastric cancer. *Tumour Biol* 2014; **35**: 11659-11666 [PMID: 25139101 DOI: 10.1007/s13277-014-2504-x]
26 **Omata M**, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, Kudo M, Lee JM, Choi BI, Poon RT, Shiina S, Cheng AL, Jia JD, Obi S, Han KH, Jafri W, Chow P, Lim SG, Chawla YK, Budihusodo U, Gani RA, Lesmana CR, Putranto TA, Liaw YF, Sarin SK. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int* 2010; **4**: 439-474 [PMID: 20827404 DOI: 10.1007/s12072-010-9165-7]
27 **Oh BS**, Jang JW, Kwon JH, You CR, Chung KW, Kay CS, Jung HS, Lee S. Prognostic value of C-reactive protein and neutrophil-to-lymphocyte ratio in patients with hepatocellular carcinoma. *BMC Cancer* 2013; **13**: 78 [PMID: 23409924 DOI: 10.1186/1471-2407-13-7]
28 **Mano Y**, Shirabe K, Yamashita Y, Harimoto N, Tsujita E, Takeishi K, Aishima S, Ikegami T, Yoshizumi T, Yamanaka T, Maehara Y. Preoperative neutrophil-to-lymphocyte ratio is a predictor of survival after hepatectomy for hepatocellular carcinoma: a retrospective analysis. *Ann Surg* 2013; **258**: 301-305 [PMID: 23774313 DOI: 10.1097/SLA.0b013e318297ad6b]
29 **Fu SJ**, Shen SL, Li SQ, Hua YP, Hu WJ, Liang LJ, Peng BG. Prognostic value of preoperative peripheral neutrophil-to-lymphocyte ratio in patients with HBV-associated hepatocellular carcinoma after radical hepatectomy. *Med Oncol* 2013; **30**: 721 [PMID: 24026659 DOI: 10.1007/s12032-013-0721-6]
30 **Templeton AJ**, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B, Tannock IF, Amir E. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014; **106**: dju124 [PMID: 24875653 DOI: 10.1093/jnci/dju124]
31 **Gabrilovich DI**, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 2009; **9**: 162-174 [PMID: 19197294 DOI: 10.1038/nri2506]

P- Reviewer: Montasser Iman F, Peng BG, Sturesson C
S- Editor: Yu J **L- Editor**: Wang TQ **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10907

World J Gastroenterol 2015 October 14; 21(38): 10907-10914
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Gastroenterologist perceptions of faecal microbiota transplantation

Sudarshan Paramsothy, Alissa J Walsh, Thomas Borody, Douglas Samuel, Johan van den Bogaerde, Rupert WL Leong, Susan Connor, Watson Ng, Hazel M Mitchell, Nadeem O Kaakoush, Michael A Kamm

Sudarshan Paramsothy, Alissa J Walsh, Gastroenterology and Hepatology Department, St Vincent's Hospital, Sydney, Darlinghurst NSW 2010, Australia

Thomas Borody, Centre for Digestive Diseases, Sydney, Five Dock NSW 2046, Australia

Douglas Samuel, Rupert WL Leong, Gastroenterology and Hepatology Department, Bankstown-Lidcombe Hospital, Sydney, Bankstown, NSW 2200, Australia

Johan van den Bogaerde, Gastroenterology and Hepatology Department, Nambour General Hospital, Nambour, QLD 4560, Australia

Susan Connor, Watson Ng, Gastroenterology and Hepatology Department, Liverpool Hospital, Sydney, Liverpool NSW 2170, Australia

Hazel M Mitchell, Nadeem O Kaakoush, University of New South Wales, School of Biotechnology and Biomolecular Sciences, Kensington, NSW 2052, Australia

Michael A Kamm, Departments of Gastroenterology and Medicine, St Vincent's Hospital and University of Melbourne, Melbourne, Fitzroy VIC 3065, Australia

Author contributions: Paramsothy S, Walsh AJ, Borody T, Samuel D, van den Bogaerde J, Leong RWL, Connor S, Ng W, Mitchell HM, Kaakoush NO and Kamm MA designed the research and devised the research questionnaire; Paramsothy S and Walsh AJ participated in the acquisition of the data; Paramsothy S, Borody T and Kamm MA analyzed and interpreted the data; and Paramsothy S, Walsh AJ, Borody T, Mitchell HM and Kamm MA drafted the manuscript.

Supported by The Clinical trial (ClinicalTrials.gov Identifier: NCT01896635) this study was performed in conjunction with has received funding from the Broad Medical Research Program at CCFA and the GESA IBD Clinical Research Grant.

Institutional review board statement: The clinical trial (ClinicalTrials.gov Identifier: NCT01896635) this study (involving a voluntary anonymous survey of gastroenterology colleagues) was performed in conjunction with was reviewed and approved by the St Vincent's Hospital Human Research Ethical Committee.

Informed consent statement: All respondents provided written informed consent acknowledged by completion and return of the anonymous survey.

Conflict-of-interest statement: Paramsothy S, Walsh AJ, Samuel D, van den Bogaerde J, Leong RWL, Connor S, Ng W, Mitchell HM, Kaakoush NO and Kamm MA have no financial or potential competing interest or affiliation with any institution, organization, or company relating to the manuscript. Borody T has an interest in the Centre for Digestive Diseases, where faecal microbiota transplantation is a treatment option for patients and has filed patents in this field.

Data sharing statement: No additional data are available.

Correspondence to: Michael A Kamm, Professor, Departments of Gastroenterology and Medicine, St Vincent's Hospital and University of Melbourne, Victoria Parade, Melbourne, Fitzroy VIC 3065, Australia. mkamm@unimelb.edu.au
Telephone: +61-3-94175064
Fax: +61-3-94162485

Received: March 30, 2015
Peer-review started: March 31, 2015
First decision: June 19, 2015
Revised: July 6, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015

Published online: October 14, 2015

Abstract

AIM: To explore gastroenterologist perceptions towards and experience with faecal microbiota transplantation (FMT).

METHODS: A questionnaire survey consisting of 17 questions was created to assess gastroenterologists' attitude towards and experience with FMT. This was anonymously distributed in hard copy format amongst attendees at gastroenterology meetings in Australia between October 2013 and April 2014. Basic descriptive statistical analyses were performed.

RESULTS: Fifty-two clinicians participated. Twenty one percent had previously referred patients for FMT, 8% more than once. Ninety percent would refer patients with *Clostridium difficile* infection (CDI) for FMT if easily available, 37% for ulcerative colitis, 13% for Crohn's disease and 6% for irritable bowel syndrome. Six percent would not refer any indication, including recurrent CDI. Eighty-six percent would enroll patients in FMT clinical trials. Thirty-seven percent considered the optimal mode of FMT administration transcolonoscopic, 17% nasoduodenal, 13% enema and 8% oral capsule. The greatest concerns regarding FMT were: 42% lack of evidence, 12% infection risk, 10% non infectious adverse effects/lack of safety data, 10% aesthetic, 10% lack of efficacy, 4% disease exacerbation, and 2% inappropriate use; 6% had no concerns. Seventy seven percent believed there is a lack of accessibility while 52% had an interest in learning how to provide FMT. Only 6% offered FMT at their institution.

CONCLUSION: Despite general enthusiasm, most gastroenterologists have limited experience with, or access to, FMT. The greatest concerns were lack of supportive evidence and safety issues. However a significant proportion would refer indications other than CDI for FMT despite insufficient evidence. These data provide guidance on where education and training are required.

Key words: Perceptions; Gastroenterologist; *Clostridium difficile*; Inflammatory bowel disease; Faecal microbiota transplantation

Core tip: This is the first study assessing the experiences, attitudes and practice of gastroenterologists towards faecal microbiota transplantation (FMT) across a range of indications other than just *Clostridium difficile* infection. Despite general enthusiasm, most gastroenterologists have limited experience with, or access to, FMT. Views differ widely regarding the potential therapeutic role of FMT in various gastrointestinal diseases. Major concerns include lack of evidence and safety data, infection risk, aesthetic factors and possible lack of efficacy. There is limited familiarity with the current evidence base and appropriate indications for FMT highlighting the need for education on where FMT fits in to current clinical practice.

Paramsothy S, Walsh AJ, Borody T, Samuel D, van den Bogaerde J, Leong RWL, Connor S, Ng W, Mitchell HM, Kaakoush NO, Kamm MA. Gastroenterologist perceptions of faecal microbiota transplantation. *World J Gastroenterol* 2015; 21(38): 10907-10914 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10907.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10907

INTRODUCTION

The last few years has seen a surge in interest in faecal microbiota transplantation (FMT)[1]. While not a new treatment, until recently it was regarded as an "alternative" therapy with little scientific basis, outside the realm of mainstream medical practice and offered by only a handful of centres worldwide. The dramatic change is largely attributable to the remarkable efficacy of FMT in recurrent *Clostridium difficile* infection (rCDI) at a time of a global CDI epidemic[2,3]. The cure rate of approximately 90% for FMT in rCDI[4-6] is much superior to the 20%-30% success rates associated with prolonged anti-microbial therapy.

The rapid advancements in gastrointestinal microbiota research including the work of large national and multinational collaborative projects such as the Human Microbiome Project[7,8] have further fueled interest in the role of the gastrointestinal microbiota in health and disease, and the therapeutic potential of FMT. Such research has linked gastrointestinal dysbiosis to enteric[9] conditions as varied as inflammatory bowel disease (IBD)[10,11], irritable bowel syndrome (IBS), and colorectal cancer[12], and to systemic conditions including obesity and metabolic syndrome[13,14], cardiovascular disease[15], and liver disease[16]. Clinical trials are currently underway in several of these conditions.

The role of the gastrointestinal microbiota in health and disease and the "promise" of FMT has captured the attention of patients, the general community and mainstream media. Patients are attracted to FMT as they perceive it as a "natural" and "holistic" therapy which seems safer than long term medications and their associated side effects[17]. This is despite a lack of long term safety data and initial reports of potential far reaching complications[18]. Studies have demonstrated that the aesthetics of using faecal material is not as significant a deterrent for patients as previously expected[19,20]. There appears to be patient

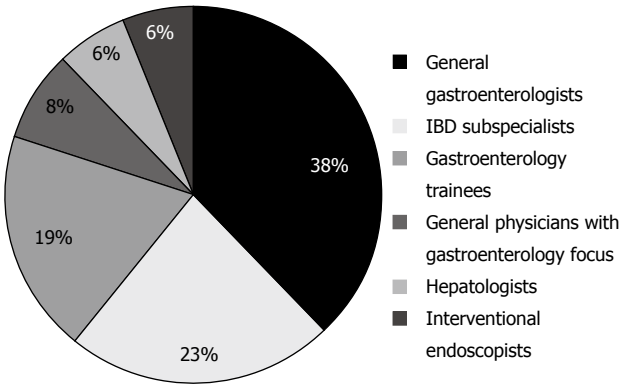


Figure 1 Subspecialty characteristics of respondents.

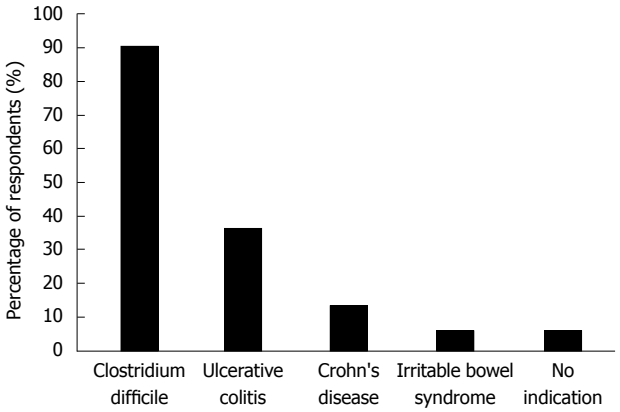


Figure 2 Perceived faecal microbiota transplant indications.

enthusiasm to make this therapy available for a range of conditions, despite the paucity of evidence outside the setting of CDI. This is reflected in the number of patient FMT self-help and do it yourself websites and forums.

The view of gastroenterologists towards FMT is less clear. While there is increasing research in the field of FMT, this appears to be tempered by concerns about lack of efficacy and safety data, and ongoing skepticism regarding the mechanism of action of FMT therapy[20]. There are only a few reports assessing the sentiments of gastroenterologists and other physicians with regards to FMT in CDI[21,22]. To our knowledge, the perceptions of gastroenterologists towards FMT for indications other than CDI has not been assessed. This survey of Australian gastroenterologists aimed to determine the wider gastroenterology community attitudes towards, and experience with, FMT.

MATERIALS AND METHODS

A questionnaire survey was created to assess gastroenterologists' attitude towards and experience with FMT (Table 1). It consisted of 17 questions. This was anonymously distributed in hard copy format amongst attendees at gastroenterology meetings in Australia between October 2013 and April 2014. Basic descriptive statistical analyses were performed using SPSS Statistics Version 22.0.

RESULTS

Respondents

52 clinicians participated in the survey. Subspecialty breakdown of respondents is shown in Figure 1. The general physicians included in the data set are those with dual training or a specialty interest in gastroenterology. With regards to nature of practice, 14 (27%) were public hospital staff specialists, 13 (25%) visiting medical officers, 11 (21%) solely in private practice, 10 (19%) trainee gastroenterologists in the public hospital system, 3 (6%) public hospital staff specialists with associated private practice, and 1 (2%) a predominantly research-based gastroenterologist.

Experience with FMT

Twenty-seven respondents (52%) had never been consulted by a patient who had received FMT before. Eleven (21%) reported having referred a patient for FMT: 7 respondents (13%) had referred a patient for FMT once, 1 respondent (2%) three times, 1 respondent (2%) four times, 1 respondent (2%) six times and 1 respondent (2%) over one hundred times. Three respondents (6%) were offering FMT as a therapeutic option at their practice or institution.

Current stance on FMT indications

The current stance of respondents towards various FMT indications is shown in Figure 2. Forty-seven respondents (90%) would refer patients with CDI for FMT if it were easily available. Regarding other indications, 19 (37%) would refer patients with ulcerative colitis, 7 (13%) for Crohn's disease and 3 (6%) for IBS. Three (6%) would not consider referring for FMT for any indication. No respondent reported that they would advise against FMT if approached by a patient interested in undergoing such treatment; 3 (6%) reported they were ambivalent, 15 (29%) stated they would acknowledge the patient's interest and refer for FMT, 26 (50%) would only refer for FMT for the indication of rCDI while 21 (40%) would suggest patients only participate in clinical trials of FMT. Forty five respondents (86%) would be willing to enroll their patients in clinical trials assessing FMT, three (6%) were unsure and 1 (2%) was not willing [3 (6%) non respondents]. Twenty-six (50%) would consider FMT as a last resort therapy for a medical condition where FMT was speculated to have benefit if their patient had refractory disease and was facing surgery while 12 (23%) said they would only do so in the context of a clinical trial.

Perceived efficacy of FMT

Regarding the statement "I don't believe in FMT and I don't think it is an effective therapy": 1 (2%)

Table 1 Gastroenterologist "faecal microbiota transplantation" perceptions survey

Gastroenterologist "faecal microbiota transplantation" (fmt) perceptions survey
1: How would you best describe yourself? (may select more than one option)
a: General Gastroenterologist
b: Hepatology subspecialist
c: Inflammatory Bowel Disease subspecialist
d: Advanced/Therapeutic endoscopy subspecialist
e: Gastroenterology trainee
f: Other; please describe in space below
2: What is the nature of your practice/work? (may select more than one option)
a: Staff Specialist
b: Public Hospital Visiting Medical Officer
c: Private Practice
d: > 40% Medical Research
e: Other; please describe in space below
3: Have you been consulted by a patient who has had FMT before? If yes please circle the indication for the FMT (may select more than one option)
a. No
b: *Clostridium difficile*
c: Ulcerative Colitis
d: Crohn's disease
e: Irritable bowel syndrome
f: Other; please describe in space below
4: Have you ever referred a patient for FMT before?
a: Yes – please elaborate in space below (indication, number of referrals, outcome)
b: No
5: Please select which of the following indications, if any, you would consider referring for FMT if easily available (may select more than one option)
a: Clostridium difficile
b: Ulcerative Colitis
c: Crohn's disease
d: Irritable bowel syndrome
e: Other; please list in space below
f: I would not consider referring for FMT for any indication
6: If a patient saw you and expressed interest in undergoing FMT would you (you may select more than one option)
a: Advise against it
b: Remain ambivalent
c: Acknowledge their interest and refer them for FMT
d: Only refer them for FMT for the indication of recurrent *Clostridium difficile*
e: Suggest they only participate in clinical trials involving FMT
f: Other; please describe in space below
7: Please select your response in answer to each of the following potential concerns with FMT
a: I don't believe in FMT and I don't think it is an effective therapy
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
b: While FMT may work at present there is inadequate evidence for efficacy
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
c: There is a significant infection risk from donor stool despite screening
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
d: I have other safety concerns regarding non-infectious adverse reactions with FMT
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
e: There is a risk of disease exacerbation with FMT
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
f: I don't think my patients would contemplate or consent to FMT
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
g: "Yuck" factor (Aesthetics)
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
h: Lack of availability/accessibility to FMT
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
i: Other; please describe in space below
8: What is your greatest concern, if any, regarding FMT? Please select only one
a: Lack of efficacy
b: Lack of evidence
c: Infection risk from donor stool despite screening
d: Non infectious adverse reaction and lack of safety data
e: Possible disease exacerbation
f: "Yuck" factor of donor stool
g: None; I have no concerns regarding FMT
h: Other; please list in space below
9: How do you feel the potential risks of FMT compare with blood transfusion or other biologic product administration?
a: More risk with blood transfusion than FMT
b: More risk with FMT than blood transfusion

c: Not sure
d: Other; please describe in space below
10: What do you think is the optimal modality through which to deliver FMT?
a: Transcolonoscopic
b: Enema based
c: Nasoduodenal/jejunal
d: Other; please list in space below
e: I don't have an opinion
11: If your patient had exhausted all other medical options and was facing surgery for refractory disease in which FMT has been suggested as a potential therapeutic option, would you consider FMT as a last resort therapy?
a: Yes
b: Yes but only for *Clostridium difficile*
c: Yes but only in a clinical trial
d: Not sure
e: No
f: Other; please describe in space below
12: Do you think FMT holds promise as a potential future therapy for certain gastrointestinal diseases?
a: Yes
b: No
c: Not Sure
d: Other; please describe in space below
13: Would you be willing to enroll your patients in clinical trials assessing FMT?
a: Yes
b: No
c: Not Sure
d: Other; please describe in space below
14: In the next 3 yr, do you foresee a situation where you would consider referring a patient for FMT outside a clinical trial if a trusted service was available? Please select your answer for each of the following indications
a. No, I would not consider referring for FMT for any indication
b: Recurrent Clostridium difficile infection
Highly Likely Somewhat Likely Somewhat Unlikely Highly unlikely
c: Ulcerative Colitis
Highly Likely Somewhat Likely Somewhat Unlikely Highly unlikely
d: Crohn's disease
Highly Likely Somewhat Likely Somewhat Unlikely Highly unlikely
e: Irritable bowel syndrome or other functional gut disorder
Highly Likely Somewhat Likely Somewhat Unlikely Highly unlikely
15: With regards to FMT, please select your response to the following statements
a: I already offer FMT as a therapeutic option in my practice
b: I have an interest in learning how to process and administer FMT so that I or my institution can arrange such therapy for our patients independently
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
c: I believe a few select centres that satisfy appropriate regulatory requirements should be available in my city to offer FMT
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
d: I don't believe the therapy should be available for routine clinical use
Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree
16: After reviewing the attached FOCUS study letter of invitation, protocol summary and selection criteria
a: Are you likely to refer patients who meet selection criteria to this study?
Highly Likely Somewhat Likely Somewhat Unlikely Highly unlikely
b: Do you have any actual patients in mind that you would consider referring to this study?
Highly Likely Somewhat Likely Somewhat Unlikely Highly unlikely
17: Any other comments regarding FMT that you wish to make?

strongly agreed, 7 (14%) somewhat agreed, 20 (38%) somewhat disagreed and 22 (42%) strongly disagreed [2 (4%) non respondents]. Regarding the statement "While FMT may work at present there is inadequate evidence for efficacy": 6 (12%) strongly agreed, 25 (48%) somewhat agreed, 13 (25%) somewhat disagreed and 6 (12%) strongly disagreed [2 (4%) non respondents].

Perceived safety of FMT

Thirteen respondents (25%) somewhat agreed that there was a significant infection risk from donor stool despite screening, while 27 (52%) somewhat disagreed and 10 (19%) strongly disagreed [2 (4%) non respondents]. Regarding safety concerns pertaining to non infectious adverse reactions with FMT, 1 (2%) strongly agreed, 18 (34%) somewhat agreed, 26 (50%) somewhat disagreed and 6 (12%) strongly disagreed [1 (2%) non respondents]. 21 respondents (40%) somewhat agreed there was a risk of disease exacerbation with FMT, 26 (50%) somewhat disagreed and 3 (6%) strongly disagreed [2 (4%) non respondents]. Twenty four respondents (46%) felt the potential risks of FMT were less than for a blood transfusion or other biologic product administration, 24 (46%) were unsure and 2 (4%) felt FMT was more

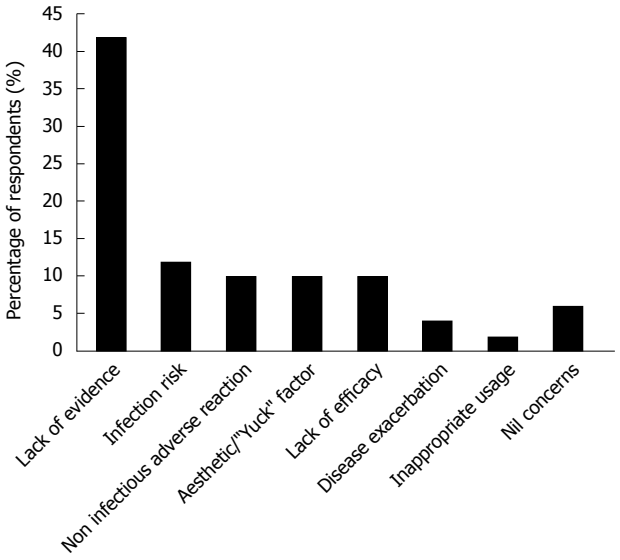


Figure 3 Greatest concerns about faecal microbiota transplant.

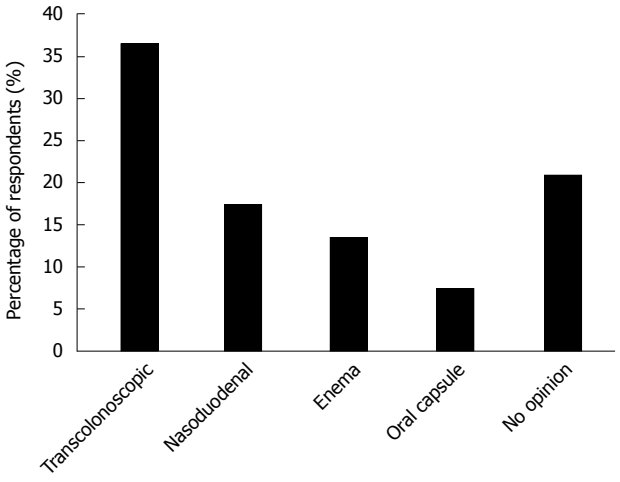


Figure 4 Optimal modality of administration.

risky than a blood transfusion or other biologic product administration [2 (4%) non respondents].

Perceived patient acceptance of FMT

One respondent (2%) strongly believed that their patients would not contemplate or consent to FMT, 13 (25%) somewhat agreed, 28 (54%) somewhat disagreed and 9 (17%) strongly disagreed [1 (2%) non respondent]. Nine respondents (17%) strongly believed their patients would be put off by the aesthetics of FMT, 24 (46%) somewhat agreed, 12 (23%) somewhat disagreed while 6 (12%) strongly disagreed [1 (2%) non respondent].

Greatest concerns regarding FMT

The greatest concerns regarding FMT are shown in Figure 3. Lack of evidence was the most commonly cited concern (42%) with safety/adverse events (infectious and non infectious), lack of efficacy and aesthetic factors also reported frequently.

Availability and accessibility of FMT

Seventy-seven percent of respondents agreed that there is a lack of availability or accessibility to FMT. Fifty two percent had an interest in learning how to process and administer FMT so their institution could offer the therapy. Seventy-nine percent agreed (35% strongly agreed, 44% somewhat agreed) with the statement that a few centres that satisfy appropriate regulatory requirements should be available in any area or region to offer FMT. Regarding the statement that FMT should not be available for routine clinical use, 3 (6%) strongly agreed, 14 (27%) somewhat agreed, 22 (42%) somewhat disagreed and 8 (15%) strongly disagreed [5 (10%) non respondents].

Route of administration and future of FMT

Figure 4 shows the perceived optimal modality of FMT administration with the transcolonoscopic route most popular (37%) followed by nasoduodenal (17%), while a significant proportion had no opinion. When asked if they thought FMT held promise as a future therapy for certain gastrointestinal diseases, 77% said yes, 15% were unsure and 4% said no. (4% non respondent). When asked whether in the next 3 years they could foresee referring for FMT outside a clinical trial if a trusted service was available, none stated no for all indications, 60% said highly likely and 29% somewhat likely for rCDI, 13% said highly likely and 50% somewhat likely for UC, 4% said highly likely and 44% somewhat likely for Crohn's disease, 31% said somewhat likely, 33% somewhat unlikely and 31% said highly unlikely for IBS.

DISCUSSION

To our knowledge this is the first report assessing the perception and practice of gastroenterologists towards FMT across a range of indications other than rCDI. This study suggests that views vary widely amongst gastroenterologists regarding the role of FMT. Despite general enthusiasm, experience with FMT remains limited and lack of accessibility appears to be a contributing factor.

The most commonly reported concern by gastroenterologists regarding FMT was the lack of evidence about efficacy. Almost 60% felt that while FMT may be effective, at present there is inadequate supportive evidence and this was the major concern cited by almost half of respondents. However at the same time, despite a limited evidence base many gastroenterologists advocated FMT for indications other than rCDI. Over a third reported they would refer their UC patients for FMT if easily available, 10% would refer Crohn's disease, and 6% for IBS. Almost a third were happy to refer patients with non CDI indications for FMT outside a clinical trial setting. At the other extreme, around 15% did not believe FMT was an effective therapy and a small proportion would not

refer any patient for FMT, even in the setting of CDI, despite the growing body of evidence demonstrating efficacy and short term safety of this therapy in a condition with significant morbidity and mortality.

The majority of surveyed gastroenterologists did not express reservations regarding the safety of FMT from infection transmission, other non infectious adverse events or disease exacerbation, despite relatively limited short term data and negligible long term data.

Almost three quarters of gastroenterologists surveyed believed their patients would contemplate or consent to FMT, though almost two thirds believed they would be concerned by the aesthetic factor. Published studies on patient perception towards FMT have found a majority would consider such therapy and that the aesthetic factor is not a major issue, suggesting the perception of gastroenterologists are only partly consistent with those of their patients[17,19]. However there appears to be improvement in gastroenterologist awareness of patient attitudes towards FMT compared to the earliest report assessing gastroenterologist perceptions of FMT in which 71% cited lack of patient acceptance and tolerability as the main barrier to FMT for CDI[21].

Half the respondents felt a lower gastrointestinal route was the optimal mode of FMT administration, with only a quarter advocating an upper gastrointestinal route and the remainder not having an opinion. These findings may be influenced to some degree by the ease of endoscopic access and administration available to gastroenterologists. A small number volunteered that oral capsule would be the optimal method despite this not being listed as a pre-specified choice on the questionnaire and minimal publications at the time of survey distribution reporting its use; the evidence for such a mode of delivery in rCDI is only just appearing in clinical trials.

Over three quarters of respondent gastroenterologists believe FMT holds promise as a potential therapy for certain gastrointestinal diseases, and would be willing to enroll their patients in FMT clinical trials. In the next 3 years, the majority expected they would be referring patients for FMT outside a clinical trial setting for both rCDI and UC if a trusted service was available, almost 50% for Crohn's disease and one third for IBS. This represents a significant shift in the last few years from when less than half of respondent gastroenterologists would consider FMT in the setting of CDI[21], despite arguably more convincing evidence at that stage for FMT in CDI than currently exists for FMT in non CDI settings.

A limitation of this study is the relatively small total respondent number. Furthermore, it was not possible to determine the response rate as the method of survey distribution involved circulating hard copies of the questionnaire at gastroenterology meetings rather than formal mailbox or email distribution. Finally, all respondents were Australian gastroenterologists, the majority from Sydney, potentially limiting the generalisability of the responses.

This study is the first report of gastroenterologist practice and perceptions regarding the use of FMT to include indications beyond rCDI. It highlights that while there is a large degree of interest in FMT amongst the profession, experience remains limited and opinions conflicting regarding its therapeutic potential and safety, sometimes inconsistent with the current medical evidence base. It indicates areas of educational need, and the need to address patients' expectations.

COMMENTS

Background

Faecal microbiota transplantation (FMT) has attracted substantial interest over recent years from researchers, clinicians, patients and mainstream media due to its extraordinary efficacy in the treatment of recurrent Clostridium difficile infection (rCDI), a condition with significant morbidity and mortality. As a result, there is growing interest in exploring the potential for FMT in the treatment of other disease states where pathogenesis is presumed to be secondary to dysbiosis. However concerns have been raised about the lack of efficacy and safety data along with limited accessibility and experience outside specialized centres.

Research frontiers

While interest in FMT is growing, controversy exists regarding potential indications, efficacy and safety for FMT. While patient perceptions of FMT have been reported and suggest widespread interest and enthusiasm, the overall opinions and experience of gastroenterologists related to FMT are not clear and have not been studied for conditions other than just CDI. The research hotspot this study addresses is to explore gastroenterologist attitudes towards, and experience with, FMT in general.

Innovations and breakthroughs

In recent years, uncontrolled and controlled studies have demonstrated that FMT is highly effective in the treatment of CDI. However data is still lacking regarding long term safety and non infectious adverse events. Controlled efficacy data for other potential indications are required though several clinical trials are currently underway.

Applications

This study suggests that while there is general interest in FMT, experience and accessibility are major limiting factors for most gastroenterologists that need to be addressed. Knowledge of current evidence based indications was suboptimal suggesting the need for further education and training. The greatest concerns were lack of supportive evidence and safety issues, highlighting areas for future research.

Terminology

FMT involves the transfer of faecal material (and associated microbiota) from a healthy donor to a recipient for the purpose of treating an underlying disease. The mechanism of action is generally believed to be *via* correction of underlying disease dysbiosis. Dysbiosis is a disturbance in the natural balance of the microbial ecology of a part of the body.

Peer-review

An informative paper, suitable for educational purposes and with potential to be of general interest because the topic is controversial and current.

REFERENCES

1 **Smith MB**, Kelly C, Alm EJ. Policy: How to regulate faecal transplants. *Nature* 2014; **506**: 290-291 [PMID: 24558658 DOI:

10.1038/506290a]
2 **Lessa FC**, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC. Burden of Clostridium difficile infection in the United States. *N Engl J Med* 2015; **372**: 825-834 [PMID: 25714160 DOI: 10.1056/NEJMoa1408913]
3 **Leffler DA**, Lamont JT. Clostridium difficile infection. *N Engl J Med* 2015; **372**: 1539-1548 [PMID: 25875259 DOI: 10.1056/NEJMra1403772]
4 **Gough E**, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. *Clin Infect Dis* 2011; **53**: 994-1002 [PMID: 22002980 DOI: 10.1093/cid/cir632]
5 **Kassam Z**, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. *Am J Gastroenterol* 2013; **108**: 500-508 [PMID: 23511459 DOI: 10.1038/ajg.2013.59]
6 **van Nood E**, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013; **368**: 407-415 [PMID: 23323867 DOI: 10.1056/NEJMoa1205037]
7 **Turnbaugh PJ**, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature* 2007; **449**: 804-810 [PMID: 17943116 DOI: 10.1038/nature06244]
8 **Peterson J**, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, Bonazzi V, McEwen JE, Wetterstrand KA, Deal C, Baker CC, Di Francesco V, Howcroft TK, Karp RW, Lunsford RD, Wellington CR, Belachew T, Wright M, Giblin C, David H, Mills M, Salomon R, Mullins C, Akolkar B, Begg L, Davis C, Grandison L, Humble M, Khalsa J, Little AR, Peavy H, Pontzer C, Portnoy M, Sayre MH, Starke-Reed P, Zakhari S, Read J, Watson B, Guyer M. The NIH Human Microbiome Project. *Genome Res* 2009; **19**: 2317-2323 [PMID: 19819907 DOI: 10.1101/gr.096651.109]
9 **Neish AS**. Microbes in gastrointestinal health and disease. *Gastroenterology* 2009; **136**: 65-80 [PMID: 19026645 DOI: 10.1053/j.gastro.2008.10.080]
10 **Gevers D**, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M, Morgan XC, Kostic AD, Luo C, González A, McDonald D, Haberman Y, Walters T, Baker S, Rosh J, Stephens M, Heyman M, Markowitz J, Baldassano R, Griffiths A, Sylvester F, Mack D, Kim S, Crandall W, Hyams J, Huttenhower C, Knight R, Xavier RJ. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014; **15**: 382-392 [PMID: 24629344 DOI: 10.1016/j.chom.2014.02.005]
11 **Manichanh C**, Borruel N, Casellas F, Guarner F. The gut microbiota in IBD. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 599-608 [PMID: 22907164 DOI: 10.1038/nrgastro.2012.152]
12 **Sears CL**, Garrett WS. Microbes, microbiota, and colon cancer. *Cell Host Microbe* 2014; **15**: 317-328 [PMID: 24629338 DOI: 10.1016/j.chom.2014.02.007]
13 **Le Chatelier E**, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clément K, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T, Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**: 541-546 [PMID: 23985870 DOI: 10.1038/nature12506]
14 **Ridaura VK**, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013; **341**: 1241214 [PMID: 24009397 DOI: 10.1126/science.1241214]
15 **Tang WH**, Wang Z, Fan Y, Levison B, Hazen JE, Donahue LM, Wu Y, Hazen SL. Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. *J Am Coll Cardiol* 2014; **64**: 1908-1914 [PMID: 25444145 DOI: 10.1016/j.jacc.2014.02.617]
16 **Schnabl B**, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 2014; **146**: 1513-1524 [PMID: 24440671 DOI: 10.1053/j.gastro.2014.01.020]
17 **Kahn SA**, Vachon A, Rodriquez D, Goeppinger SR, Surma B, Marks J, Rubin DT. Patient perceptions of fecal microbiota transplantation for ulcerative colitis. *Inflamm Bowel Dis* 2013; **19**: 1506-1513 [PMID: 23624888 DOI: 10.1097/MIB.0b013e318281f520]
18 **Alang N**, Kelly CR. Weight gain after fecal microbiota transplantation. *Open Forum Infect Dis* 2015; **2**: ofv004 [PMID: 26034755 DOI: 10.1093/ofid/ofv004]
19 **Zipursky JS**, Sidorsky TI, Freedman CA, Sidorsky MN, Kirkland KB. Patient attitudes toward the use of fecal microbiota transplantation in the treatment of recurrent Clostridium difficile infection. *Clin Infect Dis* 2012; **55**: 1652-1658 [PMID: 22990849 DOI: 10.1093/cid/cis809]
20 **Brandt LJ**. Editorial commentary: fecal microbiota transplantation: patient and physician attitudes. *Clin Infect Dis* 2012; **55**: 1659-1660 [PMID: 22990845 DOI: 10.1093/cid/cis812]
21 **Kelly C**, de Leon L, Kerstetter D, Okpara N. Barriers to Greater Utilization of Fecal Bacteriotherapy for Chronic Clostridium difficile Infection (abstract). American College of Gastroenterology annual meeting; San Antonio, Texas; 15-20 October, 2010
22 **Sofi AA**, Georgescu C, Sodeman T, Nawras A. Physician outlook toward fecal microbiota transplantation in the treatment of Clostridium difficile infection. *Am J Gastroenterol* 2013; **108**: 1661-1662 [PMID: 24091517 DOI: 10.1038/ajg.2013.207]

P- Reviewer: Zuluaga AF **S- Editor**: Ma YJ **L- Editor**: A
E- Editor: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10915

World J Gastroenterol 2015 October 14; 21(38): 10915-10925
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Systematic analysis of the safety and benefits of transvaginal hybrid-NOTES cholecystectomy

Dirk R Bulian, Jurgen Knuth, Kai S Lehmann, Axel Sauerwald, Markus M Heiss

Dirk R Bulian, Jurgen Knuth, Markus M Heiss, Department of Abdominal, Vascular and Transplant Surgery, Cologne-Merheim Medical Center, Witten/Herdecke University, 51109 Cologne, Germany

Jurgen Knuth, Department of General, Visceral, Vascular and Thoracic Surgery, Hospital of Kempten, 87439 Kempten, Germany

Kai S Lehmann, Department of General, Visceral and Vascular Surgery, Charité University Medical Center Berlin, Campus Benjamin Franklin, 12200 Berlin, Germany

Axel Sauerwald, Department for Obstetrics and Gynaecology Holweide, Holweide Hospital, 51067 Cologne, Germany

Author contributions: Bulian DR and Lehmann KS designed research; Bulian DR, Knuth J, Lehmann KS and Sauerwald A performed research; Bulian DR and Heiss MM checked the data analysis and reviewed the article; Bulian DR and Knuth J wrote the paper.

Conflict-of-interest statement: Bulian DR, Knuth J, Lehmann KS, Sauerwald A and Heiss MM do not report any conflict of interest.

Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at buliand@kliniken-koeln.de. Participants gave informed consent for data sharing. No additional data are available.

Correspondence to: Dirk R Bulian, MD, Department of Abdominal, Vascular and Transplant Surgery, Cologne-Merheim Medical Center, Witten/Herdecke University, Ostmerheimer Strasse 200, 51109 Cologne, Germany. buliand@kliniken-koeln.de
Telephone: +49-221-89073770
Fax: +49-221-89078561

Received: April 26, 2015
Peer-review started: April 27, 2015
First decision: June 23, 2015
Revised: July 21, 2015
Accepted: September 14, 2015
Article in press: September 14, 2015
Published online: October 14, 2015

Abstract

AIM: To evaluate transvaginal hybrid-NOTES cholecystectomy (TVC) during its clinical establishment and compare it with the traditional laparoscopic technique (LC).

METHODS: The specific problems and benefits of TVC were reviewed using a registry analysis, a comparative cohort study and a randomized clinical trial. At first, feasibility, safety and specific complications of the TVC were analyzed based on the first 488 data sets of the German NOTES Registry (GNR). Hereafter, we compared the early postoperative results of our first 50 TVC-patients with those of 50 female LC-patients matched by age, BMI and ASA classification. The same cohort was contacted an average of two years later to evaluate long-term results concerning pain and satisfaction with the aesthetic results and the overall postoperative results as well as sexual intercourse by means of two domains of the German version of the Female Sexual Function Index (FSFI-d). Consequently, we performed a randomized clinical trial comparing 20 TVC-patients with 20 needlescopic/3-trocar cholecystectomies (NC) also concerning the early postoperative results as well as pain, satisfaction and quality of life by means of the Eypasch Gastrointestinal Quality of Life Index (GIQLI) in the later course. Finally,

we discussed the results in accordance with other published studies.

RESULTS: The complication (3.5%) and conversion rates (4.1%) for TVC were low in the GNR and comparable to those of the LC. Access related intraoperative complications included injuries to the bladder (n = 4; 0.8%) and bowel (n = 3; 0.6%). The study cohort revealed less postoperative pain after TVC comparing to the LC-patients on the day of surgery (NRS, 1.5/10 *vs* 3.1/10, P = 0.003), in the morning (NRS, 1.9/10 *vs* 2.8/10, P = 0.047) and in the evening (NRS, 1.1/10 *vs* 1.8/10, P = 0.025) of postoperative day (POD) one. The randomized clinical trial consistently found less cumulative pain until POD 2 (NRS, 8/40 *vs* 14/40, P = 0.043), as well as until POD 10 (NRS, 22/190 *vs* 41/190, P = 0.010). Furthermore, the TVC-patients had a better quality of life on POD 10 than did the LC-patients (GIQLI, 124/144 *vs* 107/144, P = 0.028). The complication rates were comparable and no specific problems were detected in the long-term follow-up for sexual intercourse for either group. The TVC-patients were more satisfied with the aesthetic result in the long-term course in the matched cohort analysis (1.00 *vs* 1.88, P < 0.001) as well as in the randomized clinical trial (1.00 *vs* 1.70, P < 0.001) when compared with the LC-patients.

CONCLUSION: TVC is a feasible procedure with a high safety profile and has advantages in regard to postoperative pain and aesthetic results when compared with LC or NC.

Key words: NOTES; Cholecystolithiasis; Postoperative complications; Postoperative pain; Transvaginal hybrid-NOTES cholecystectomy

Core tip: Transvaginal hybrid-NOTES cholecystectomy (TVC) increased in popularity after its introduction in 2007. We systematically evaluated this new technique with regards to its specific complications and advantages compared with those of the laparoscopic technique (LC) using a registry analysis, a matched cohort analysis and a randomized clinical trial. TVC had a low conversion rate and complication rate. Injuries to the bladder and urinary tract infections were rare but access-specific complications. TVC-patients showed less postoperative pain and a better quality of life in the short-term course than did the LC-patients. TVC led to an improved satisfaction with the aesthetic results also in long-term course. No specific problems, not even for sexual intercourse, were detected.

Bulian DR, Knuth J, Lehmann KS, Sauerwald A, Heiss MM. Systematic analysis of the safety and benefits of transvaginal hybrid-NOTES cholecystectomy. *World J Gastroenterol* 2015; 21(38): 10915-10925 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10915.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10915

INTRODUCTION

On one hand, operative procedures serve to heal or at least alleviate disease or disease-related complaints. On the other hand, these methods hold general and specific risks and obviously harm the physical integrity of the patient. Operative risks result not only from the actual procedure on the target organ but also from the necessary access. The consequences and risks of long-term common abdominal access, namely laparotomy, are pain at the incision site[1], wound infection[2,3], burst abdomen[4-6], incisional hernias and scarring. They may result in a prolonged hospital stay, increased lethality, and possibly further procedures to correct associated complications[7-9]. To avoid or at least reduce the access-related problems mentioned above by reducing the access inherent trauma, laparoscopy was developed[10,11]. However, the completely unaccustomed instrument handling demanded new, initially unfamiliar skills. This resulted in an increased initial complication rate, *e.g.*, bile duct injuries during cholecystectomy[12].

For many decades, gynecological intraperitoneal procedures have been performed by avoiding the abdominal wall and instead utilizing a natural orifice, namely the transvaginal access point[13-15]. In 2004, Kalloo *et al*[16] introduced the NOTES-concept for gastroenterologic disease treatment. As a result, cholecystectomy was performed transvaginally by several groups in 2007[17-21]. Even prior to that, the retrieval of larger specimens, such as the spleen[22], and even the gallbladder during cholecystectomies in cases of large stones[23], were performed transvaginally but without attracting much attention in the abdominal surgery field. It was not until the aim of performing an operation completely through a natural orifice with a flexible endoscope became desirable that the development of several techniques and the distribution of the NOTES-concept itself gained momentum. However, intraabdominal procedures are only performed with great difficulty when employing the currently available standard endoscopes. Thus, several clinics[24] established transvaginal/transumbilical Hybrid-NOTES cholecystectomy (TVC) using rigid instruments as described by Zornig *et al*[17].

Based on experiences with the clinical implementation of the traditional laparoscopic cholecystectomy (LC) at the end of the 1980's including the already mentioned higher rate of complications, a concomitant evaluation of the clinical implementation of TVC as a new method in surgery seemed obligatory. Apart from its safety, investigations into additional benefits for the patients, such as reduced postoperative pain, required clarification.

Table 1 Overview of the studies that were included for the analysis

Study	Patients	Study aim
Registry analysis[24]	551	Technical and clinical feasibility
	(488 TVC)	Safety (particularly regarding access related complications)
Cohort study A[25]	100	Benefit (postoperative pain)
(Early postoperatively)	(50 TVC; 50 LC)	Disadvantages (specific complications)
Cohort study B[26]	88[1]	Long-term problems (particularly regarding sexual intercourse)
(24 mo postoperatively)	(46 TVC; 42 LC)	Patient satisfaction
NATCH-study[29]	40	Benefit (postoperative pain)
(Early postoperatively)	(20 TVC; 20 NC)	Disadvantages (specific complications)
NATCH-study[30]	40	Long-term problems (particularly regarding sexual intercourse)
(3 and 6 mo postoperatively)	(20 TVC; 20 NC)	Patient satisfaction

[1]From the collective of cohort study A. TVC: Transvaginal cholecystectomy; LC: Traditional laparoscopic cholecystectomy; NC: Needlescopic 3-trocar-cholecystectomy.

MATERIALS AND METHODS

The following aspects were evaluated by our study group in different trials/studies and will be discussed in this review: feasibility of TVC in clinical routine, safety of the method, possible access-related complications, potential for less postoperative pain when compared with LC, negative influence on sexual life due to transvaginal access, higher patient satisfaction with the esthetic result compared with LC and evaluation of morphological changes as a result of the transvaginal access.

For clarification of these problems, five analyses were used on three different studies with a different conceptual design (Table 1).

On the basis of a national registry[24], feasibility and safety, including access-related complications, were analyzed.

On the basis of a comparative cohort study for our first 50 TVCs[25], the expected advantage regarding reduced postoperative pain and other early postoperative parameters were evaluated in comparison with LC. To record more than just short-term complications, a two-year follow-up exam of the same patients was conducted in order to detect and compare problems concerning sexual life and satisfaction with the esthetic result[26].

Because there is evidence for the usage of mini-instruments to reduce postoperative pain in LC[27,28] and a reduction of the number of trocars should have a similar effect, a prospective/randomized, unblinded, single center trial was initiated to compare TVC with the allegedly least traumatizing technique for the abdominal wall, which is the needlescopic 3-trokar-cholecystectomy (NC)[29].

Apart from short-term advantages, long-term safety was also assessed in the prospective/randomized trial. Thus, parameters regarding sexual life and patient satisfaction were also registered at three and six months postoperatively[30].

Step 1: Registry analysis

The German registry for natural orifice translumenal endoscopic surgery: report of the first 551 patients[24].

The German NOTES Registry (GNR) was implemented in March 2008. All surgical departments in Germany were invited *via* mail, email, and conference participation, to document all NOTES-related procedures in the GNR *via* an online portal that was especially programmed for this purpose. Participation was voluntary, pseudonymized, not linked to any membership and free of charge after creating an account for the respective department. Technical realization was carried out by the DGAV. In our clinic, all NOTES-related procedures were registered in the GNR.

Registered data included general and patient related data, target organ, therapy and postoperative course. The first published analysis after documentation of more than 550 cases was analyzed using SPSS, version 16 (IBM Germany, Ehningen) and SAS, version 9.2 (SAS Institute, United States, North Carolina). Apart from descriptive statistics of all procedures, a sub group analysis was planned for those target organs that made up for more than 15% of the overall procedures or an absolute number of more than 50 procedures. Eventually, this criterion applied to the target organ "gallbladder" only.

Step 2: Matched cohort analysis; short-term results

Less pain after transvaginal/transumbilical cholecystectomy than after the classical LC: short-term results of a matched-cohort study[25].

In our clinic, the first TVC was performed on December 8, 2008. Since then, for all patients with an indication for elective cholecystectomy (CHE) due to symptomatic cholecystolithiasis, the following pre-, intra- and postoperative parameters were recorded and documented in a prospective data base: age, body mass index (BMI), comorbidities, previous abdominal surgery, American Society for Anesthesiologists (ASA)-classification, history of cholecystitis or cholestasis, date of the operation, procedural time, amount of percutaneous trocars, conversion, pre- and postoperative leukocytes, CRP and bilirubin, intra- and postoperative complications, histopathological results, pain [in the recovery room and on the morning and

evening of the first postoperative day as measured blindly using the numeric rating scale (NRS-11)[31] from 0 (no pain) to 10 (worst imaginable pain) by nurses especially trained for this purpose], analgesic consumption, time of first solid food intake and postoperative hospital stay.

The choice of technique was made by the patients after informed consent about the possible advantages and disadvantages of both TVC and LC was obtained. Additionally, all TVC-patients were preoperatively examined by a gynecologist to exclude contraindications for a transvaginal procedure. Perioperative treatment was the same for both groups. TVC-patients were advised to abstain from penetrating sexual intercourse for two weeks postoperatively. Furthermore, there was a gynecological re-examination after ten to twelve days.

TVC was performed as described by Zornig *et al*[17]. For LC, four trocars were used: two 11 mm- and two 6 mm-trocars. The gallbladder was retrieved through the umbilical trocar access, which was increased in size in cases of multiple or large stones.

A comparative analysis of the first 50 TVC-patients with 50 traditional laparoscopic patients (LC-group) from the same time period matched according to age, BMI and ASA classification, was conducted.

Step 3: Matched cohort analysis; long-term results

Long-term results of transvaginal/transumbilical *vs* classical laparoscopic cholecystectomy - an analysis of 88 patients[26].

All 100 patients of the previous cohort study[25] were contacted *via* telephone after an average of 2.05 years (1.04-3.14) postoperatively. They were asked to answer a questionnaire about the postoperative course and their satisfaction. A standardized questionnaire was sampled *via* telephone. Alternatively, patients were offered to have the questionnaire sent to them with a self-addressed stamped envelope.

All telephone interviews were conducted by the same female interviewer to avoid not only interpersonal variability but also patients' reservations toward a male interviewer especially in regard to items dealing with sexuality. Evaluation was anonymous. The questionnaire was comprised of 48 items including five items about previous abdominal surgery, five items about previous deliveries, 13 items about the postoperative course, three general items about sexual intercourse, three items each of the domains "satisfaction" and "pain" of the German version of the Female Sexual Function Index (FSFI-d; items 14-16 and 17-19)[32,33] related to three different points in time (cooperatively, early postoperatively and during the last four weeks before the interview), two items each about satisfaction with the esthetic result and the overall surgical result (very satisfied/satisfied/ambiguous/dissatisfied/very dissatisfied) as well as two items about the patient's choice of technique in the hypothetical case of a new necessary cholecystectomy and whether or she would recommend the technique to friends or relatives. Data processing and statistical analysis was conducted using SPSS, Version 19 (IBM Germany, Ehningen).

Step 4: Randomized clinical trial; short-term results

Transvaginal/transumbilical hybrid-NOTES *vs* 3-trocar needlescopic cholecystectomy: short-term results of a randomized clinical trial[29]

After preparation of a study protocol, calculation of sample size, approval by the ethics committee and registration of the study, 40 patients were included in a randomized, prospective, single center and unblinded clinical trial between February 2010 and June 2012. Randomization was 1:1 for TVC and NC. All procedures were conducted by the same surgeon in order to avoid not only interindividual differences but also the influence of a learning curve, which was already completed for the performing surgeon at the beginning of the trial.

Postoperative pain medication, return to food intake and dismissal criteria were standardized and identical for both groups. On postoperative day ten, the first post-dismissal examination took place and included a clinical exam and inquiry of the esthetic result both by the study-physician and the patient using a satisfaction-scale (ordinal scale). Until that assessment point, the patients had documented pain intensity, pain localization and analgesic consumption three times daily. All TVC-patients were re-examined by a gynecologist on postoperative day 12-14.

The primary outcome measure was the cumulative early postoperative pain intensity during movement within the first 48 h following surgery and was comprised of four measurements (pain intensity six hours postoperatively, on the morning and the evening of the first postoperative day and the morning of the second postoperative day) measured on the Numeric Rating-Scale (NRS-11)[31]. Secondary short-term outcome measures, documented prospectively, were satisfaction of both the patient and the examiner with the esthetic results of the operation regarding the abdominal wall on postoperative day 10, intra- and postoperative complication rates, conversion rate to traditional laparoscopic or conventional technique, procedural time, evaluation of the operative handling (instrument handling, camera handling, dissection and gallbladder retrieval) by the first and the second surgeon, cumulative postoperative pain intensity during movement until postoperative day ten as measured in the morning and in the evening with the NRS-11, cumulative consumption of peripheral and central analgesics during the first ten days, re-operation rate, time to return to daily, professional and leisure activity as well as quality of life, measured on postoperative day 10 using the Eypasch Gastrointestinal Index for Quality of Life (GIQLI)[34]. Furthermore, age, BMI,

ASA classification, number of gallstones, size of the largest stone, history of previous cholecystitis, pre- and postoperative lab results (leukocytes, CRP), number of percutaneous trocars, histological result and postoperative hospital stay were prospectively documented.

SPSS, version 19 (IBM Germany, Ehningen) was used for data processing and statistical analysis. An intention-to-treat-analysis was conducted for all calculations.

Step 5: Randomized clinical trial; long-term results

Transvaginal hybrid NOTES cholecystectomy - results of a randomized clinical trial after 6 mo[30].

This prospective/randomized long-term analysis was conducted with the same 40 patients who had been randomized for TVC or NC in the study mentioned above. Satisfaction with the esthetic result and overall satisfaction, abdominal pain during movement according to the NRS-11 and occurrence of trocar hernias were documented according to a structured questionnaire three and six months postoperatively in a telephone. In cases of a positive or unclear answer for the item "trocar hernia", patients were examined clinically and with sonography. Furthermore, three months postoperatively, satisfaction and occurrence of pain during after sexual intercourse were evaluated using the domains "satisfaction" and "pain" from the German FSFI-d. Naturally, only patients who had had sexual intercourse during the mentioned time frame could be questioned about this matter. Both domains were evaluated separately and together. Moreover, there was a gynecological exam six months postoperatively to detect morphological long-term changes at the point of access. Apart from taking the history, the gynecologist performed a palpation and speculum-inspection, especially of the posterior vault of the vagina, and a transvaginal sonography. The gynecologist also documented complaints, whether the patients already had postoperative sexual intercourse, and if a further follow-up examination was necessary.

Data processing and statistical analysis (intention-to-treat-analysis) was conducted using SPSS, version 21 (IBM Germany, Ehningen).

RESULTS

Step 1: Registry analysis

The German registry for natural orifice translumenal endoscopic surgery: report of the first 551 patients[24].

Of the 64 accounts that were generated, 28 were active and resulted in 551 patient data sets with 572 target organs. The most frequent target organ was the gallbladder (85.3%), and the most frequent indication was symptomatic gallstones (73.6%).

Only female patients underwent NOTES procedures. Complications occurred in 3.3% of all cholecystectomies. Hospital stays were significantly longer in cases with a complication compared with those who did not experience complications (6.7 d *vs* 2.6 d, $P < 0.001$). All procedures, except two that lacked further specifications, were carried out transvaginally. Nearly all patients (99.3%) were underwent procedures in which the Hybrid-NOTES techniques were applied with one or more percutaneous trocars for cholecystectomies with an average of 1.2 ± 0.5 trocars. Most procedures ($n = 534$) used a rigid laparoscope, and 96.6% of the procedures were performed by a general or abdominal surgeon. Sixty-four percent of the procedures were assisted by a gynecologist.

Intraoperative complications occurred in seven cases, and postoperative complications occurred in ten cases. The most frequent complication was injury to the bladder, which only occurred in obese patients (BMI $\geqslant$ 30.0 kg/m^2) and older patients ($\geqslant$ 59 years). Postoperative complications included bleeding and vaginal or urinary tract infections. Twenty procedures (3.6%) were converted to traditional laparoscopy and seven (1.3%) to laparotomy. In seven cases, a planned NOTES access was not performed due to technical problems with the transvaginal access. No difference was detected with regards to complication and conversion rates in the presence or absence of a gynecologist. All patients studied survived the entire study period.

Multivariate analysis of the cholecystectomies revealed a significant correlation between conversion rate and hospital stay with BMI and age, but not with case load of the operating clinics. Procedural time was dependent on BMI and case load, and the amount of percutaneous trocars was dependent on patient age and case load, and these trends were all significant. In "high-volume"-clinics, procedural time was shorter and the amount of percutaneous trocars lower. The rate of complications showed no significant dependence on any factor.

Step 2: Matched cohort analysis; short-term results

Less pain after transvaginal/transumbilical cholecystectomy than after the classical LC: short-term results of a matched-cohort study[25].

Age, BMI, ASA classification, preoperative leukocytes and CRP did not differ significantly between both groups. Only previous gynecological conditions or previous gynecological procedures were significantly more frequent in the TVC-group (18 x *vs* 6 x, $P = 0.009$). Average procedural time was identical in both groups (77.8 min). For the first 25 TVC-procedures, the average procedural time was 82.3 min, and for the second group of 25 procedures it was 73.3 min. In the LC-group, the times were 76.9 and 78.6 min, respectively, with no significant differences between the TVC- and LC-groups. In the TVC-group, there was no conversion, and in the LC-group one patient (2%) needed conversion due to a bile duct injury. This was also the only intraoperative complication

in the LC-group (2%). In the TVC-group, there was one intraoperative injury to the urinary bladder (2%) that did not require conversion. On the day of the operation, the TVC-patients had significantly less pain than did the LC-patients (NRS: 1.5 *vs* 3.1, $P = 0.003$), although at the same time these patients received significantly less analgesic medication in the recovery room than did the LC-patients (19 x *vs* 32 x, $P = 0.016$). Notably, TVC-patients received significantly less opiates (16 x *vs* 31 x, $P = 0.005$). Also on the morning and on the evening of the first postoperative day, there was a significant reduction in pain in the TVC-group (NRS: 1.9 *vs* 2.8, $P = 0.047$; 1.1 *vs* 1.8, $P = 0.025$). Inflammation, as measured by CRP, was significantly lower during the first two days after TVC when compared with LC (25.5 mg/L *vs* 39.1 mg/L, $P = 0.015$). Additionally, the individual increase in CRP (difference between pre- and postoperative value) was significantly lower following TVC compared to LC (21.1 mg/L *vs* 33.1 mg/L, $P = 0.003$). The postoperative leukocyte count was not different between both groups (7.8/nL *vs* 8.0/nL, $P = 0.4$). After TVC, the postoperative hospital stay was significantly shorter (2.7 instead of 3.4 d, $P < 0.001$). First solid food intake happened significantly earlier in the TVC than in the LC group (1.02 d *vs* 1.40 d, $P < 0.001$) and took place significantly more often on the first postoperative day (49 x *vs* 30 x, $P < 0.001$).

Step 3: Matched cohort analysis; long-term results

Long-term results of transvaginal/transumbilical *vs* classical laparoscopic cholecystectomy - an analysis of 88 patients[26].

All 88 patients who could be readily reached answered the questionnaire (TVC: 46; LC: 42; return quota 92% and 84%, $P = 0.357$). Those patients, as in the original 100, did not significantly differ in terms of age, BMI, ASA classification, precious abdominal surgery or amount of previous deliveries. The majority (76.1%) of the TVC-patients and 61.0% of the LC-patients had sexual intercourse in the six months before the CHE. After the CHE the same frequencies were 78.3% and 61.0%, respectively ($P = 0.165$ and 0.102). Additionally, the point of first sexual intercourse following CHE was not significantly different. Neither the domains of the FSFI-d, nor their sum were significantly different for all three examined time-points. The TVC-patients were significantly more satisfied with the esthetic results of the CHE and with the overall result ($P < 0.001$ and $P = 0.001$). All TVC-patients would hypothetically opt for the same technique again, and only 80.5% ($P = 0.002$) would do so after LC. Furthermore, all TVC-patients would recommend the technique applied on them to friends or relatives, whereas only 69.2% would do so in the LC-group ($P < 0.001$). Both general postoperative complications as well as wound infections were less frequent after TVC, but not significantly so (17.8% *vs* 35.7%, $P = 0.088$ and 2.2% *vs* 7.1%, $P = 0.344$). CHE related incisional hernias occurred in two out of the 42 LC-patients, but not in the TVC-group (4.8% and 0%, $P = 0.225$). Significantly less TVC-patients felt impaired by the CHE (6.5% *vs* 23.8%, $P = 0.034$). Some of the TVC-patients (19.6%) and the LC-patients (28.6%) reported epigastric pain, and 6.5% and 4.8% had had pain in the lower abdomen and/or pubic area, respectively, during the four weeks before the inquiry ($P = 0.453$ and $P = 1$). Pain intensity was not significantly different between the two groups ($P = 0.451$ and $P = 0.700$). After the procedure, the TVC-patients could resume everyday life activities (work, school, household, *etc.*) significantly earlier than could the LC-patients (after 5.4 d *vs* 14.4 d, $P < 0.001$). In each group, one patient noticed a change in her menstruation after the CHE. Four TVC-patients and no LC-patients noticed vaginal discharge after the CHE outside of the menstruation cycle (8.7% and 0%, $P = 0.118$).

Step 4: Randomized clinical trial; short-term results

Transvaginal/transumbilical hybrid-NOTES *vs* 3-trocar needlescopic cholecystectomy: short-term results of a randomized clinical trial[29].

All patients were treated per protocol, and no conversions or additional trocars were necessary. In addition, no intraoperative complications occurred. Patient-derived parameters were comparable between the two groups. One patient from each group did not fill out the pain- and analgesics-documentation log prospectively, so these had to be excluded from the respective analysis. The primary outcome parameter was cumulative pain intensity during movement within the first 48 h following surgery. It was significantly lower in the TVC-group ($P = 0.043$). Furthermore, the cumulative postoperative pain intensity during movement until postoperative day 10 was significantly lower in the TVC-group ($P = 0.010$), although TVC-patients had a significantly lower consumption of analgesics ($P = 0.019$). TVC-patients had a significantly better GIQLI-score ($P = 0.028$) and were significantly more satisfied with the esthetic results of the operation ($P < 0.001$). Both surgeons considered the retrieval of the gallbladder in TVC to be significantly easier ($P < 0.001$ and $P = 0.010$), whereas instrument handling was found to be significantly more difficult for TVC by the second surgeon ($P = 0.020$). The remaining outcome parameters did not significantly differ between the two groups. All gynecologic follow-up exams had a regular postoperative findings.

Step 5: Randomized clinical trial; long-term results

Transvaginal hybrid NOTES cholecystectomy - results of a randomized clinical trial after 6 mo[30].

All 20 patients of the TVC-group were "very satisfied" with the postoperative result of the scars both three and six months postoperatively, which was significantly better than results in the TC-group ($P = 0.004$ and $P < 0.001$). However, the overall

satisfaction with the procedural result was not significantly different between both groups. One TVC-patient complained about lower abdominal pain three months postoperatively with a value of three on the NRS-11, one NC-patient had pain in the right upper quadrant after six months with a value of six on the NRS-11. Hernias were not detected, although two NC-patients were clinically evaluated after three and six months due to respective results in the telephone interview. Because three TVC- and six NC-patients had no penetrating sexual intercourse during the six pre- and postoperative months, only 31 patients could answer the FSFI-d questions. All preoperatively active patients also had postoperative sexual intercourse. The analysis of the FSFI-d-items revealed no significant difference between both techniques, neither for each domain separately, nor for evaluation of both domains together. None of the gynecologic follow-up exams showed pathological findings.

DISCUSSION

The aim of our studies was to evaluate TVC, a new surgical technique that previously had not been systematically evaluated. After Kalloo *et al*[16] introduced the NOTES-principle in 2004 by description of the perioral, transgastral access, that principle was adopted by several study groups for different intraperitoneal interventions in both experimental settings and clinical implementation. In 2007, a variety of transvaginal techniques for cholecystectomy were published by several study groups[17-21].

First and foremost, the distribution and advancements of the new technique, including possible procedure-related complications, had to be transparent in order to facilitate timely intervention if necessary, bearing in mind the rapid increase of bile duct injuries following the introduction of minimally invasive surgery in form of LC at the end of the 80's[35,36]. For this reason, the appropriate scientific surgical society, the DGAV with its political and social responsibility, created a national NOTES-registry (GNR) order to achieve nationwide documentation of respective procedures to facilitate early detection of possible problems.

After 14 mo, the first analysis of the GNR, including 551 patients showed that more than 85% of the documented entirely transvaginal procedures, 99% of which utilized an additional umbilical trocar, (Hybrid-NOTES-technique) were cholecystectomies[24]. Furthermore, most cholecystectomies applied the Hybrid-NOTES-technique as described by Zornig *et al*[17] with rigid instruments. Complications occurred in 3.1% of all patients, which is comparable to the results of LC in a large meta-analysis[37]. The most frequent intraoperative complication was a urinary bladder injury, which occurred in four patients with previous hysterectomy during establishment of the transvaginal access. This certainly accounts for an access-related complication. Otherwise, three intraoperative bowel injuries were documented. Postoperatively, there were two cases of vaginal bleeding, one abscess in the Douglas-cavity and two vaginal infections in terms of access related complications. Some patients (3.6%) needed conversion to LC, and 1.3% needed conversion open cholecystectomy. Multivariate analysis of the documented cholecystectomies revealed several significant influences: the case load of the performing clinics on procedural time and the amount of trocars; BMI on conversion rate, procedural time and hospital stay; age on conversion rate, hospital stay and number of trocars. None of the analyzed parameters had a significant influence on the complication rate. Thus, at least for the clinics taking part in the registry, a responsible handling of the clinical implementation of this new technique became evident.

However, these results are based on the analysis of a voluntary registry and strongly depend on the quality of documentation as entered by the participating clinics. The data were not monitored, and neither were there audits. The logistic and financial effort would have necessitated membership fees or industrial sponsors. Furthermore, it is not guaranteed that all clinics that perform NOTES procedures actually took part in the registry. On the other hand, the robust results of the registry analysis might partly be explained by patient selection but also by the fact that the NOTES procedures were only performed by few surgeons with substantial experience and high expertise in the field of minimally invasive surgery. This fact certainly reflects the responsible clinical implementation of the new technique. Therefore, the validity of a comparison with LC-data derived from health services research is limited.

These results were recently confirmed in the latest analysis of the GNR, where 2992 data sets from March 2008 until November 2013 were analyzed[38]. With more than 88%, TVC was still the most frequent procedure by far, followed by appendectomy and colon resection. The recent rate of intraoperative complications was 1.6%, and that of postoperative complications was 3.6%. In this analysis, too, the main intraoperative complication was urinary bladder injury, and bowel injuries were still very rare but potentially serious complications. Postoperatively, urinary tract infections were classified as access related. Rarely (1.1%), procedures were converted to LC or the open procedure (0.4%).

Thus, our first analysis of the GNR, representing the worldwide largest analysis of NOTES procedures at that point, was the first step to proving the feasibility of Hybrid-NOTES procedures, particularly TVC.

The next step was the comparative analysis of TVC *vs* LC in order to prove the advantages of reducing access related trauma in the abdominal wall. For this purpose, the first 50 TVC-patients in our clinic in 2008 were compared to 50 matched LC-patients from the same time frame[25]. Here, when compared to LC, TVC significantly decreased not only postoperative

pain on the day of the operation and on the first postoperative Tag but also the hospital stay. At the same time, the frequency of analgesic consumption in the recovery room, especially of opioids, as well as the postoperative rise in CRP were significantly lower after TVC. Furthermore, TVC-patients had significantly earlier intake of solid food.

The decrease in postoperative pain by the Hybrid-NOTES technique was confirmed in other comparative non-randomized studies. The retrospective case-control-study by Hensel *et al*[39] compared 47 TVC with 46 LC-3-trocar technique patients. Apart from less postoperative pain and less analgesic consumption, there were less nausea and vomiting following TVC. Additionally, TVC-patients could drink earlier, were mobilized quicker and had a shorter hospital stay than did the LC-3-trocar-patients. In a prospective, non-randomized observational study, Kilian *et al*[40] compared 20 LC *vs* 15 TVC and 16 single-port cholecystectomies. Despite the small case number, postoperative pain and hospital stay were significantly shorter after TVC than after LC. Another study with three branches and a small case number by Solomon *et al*[41] again found less postoperative pain after TVC when compared with LC and single-incision-CHE. Two further studies confirmed the reduced pain intensity after TVC[42,43]. However, Zornig *et al*[44], with their analysis of the highest number of cases comparing 100 TVC with 100 LC, found no difference for the analyzed parameters including analgesic consumption and hospital stay. Both Zornig *et al*[44] and Noguera *et al*[45] reported a significantly longer procedural time for TVC, while Hensel *et al*[39], Kilian *et al*[40] and our analysis found no significant difference. Notably, in the study by Zornig *et al*[44], all procedures from both groups were performed by the chief of department or a senior surgeon, whereas in our LC-group, more than half of the procedures were performed by residents under the supervision of a senior surgeon. Thus a bias results, which is due to the fact that LC is generally considered a teaching procedure. Furthermore, with the analysis of procedural times, we found a learning curve for TVC because the second cluster of TVC procedures was nine minutes shorter than the first 25 TVC procedures. None of the mentioned studies found a significant difference in intra- and early postoperative complications between TVC and LC, which strengthens the favorable results of the GNR-analysis. However, one must consider that probably all of the registered centers are clinics with an above-average expertise in minimally invasive surgery. Therefore, a transferal of the result demands caution. We assume that the proven pain reduction is a result of the missing abdominal wall trauma due to retrieval of the gall bladder in TVC because this has the most relevant technical difference of both techniques. Especially in cases with large gallstones, a great amount of gallstones or a thickened organ wall manipulation at the abdominal wall requires a greater traumatization.

Because a benefit of TVC in regard to less early postoperative pain was evident without increased intra- or early postoperative complication rates, a long-term follow-up and the analysis of long-term parameters was the aim of further studies. This was not because mainly young, sexually active women who took part in respective surveys were concerned about long-term impairment of sexual activity following a transvaginal procedure[46-48]. Additionally, according to Kobiela *et al*[49], more than 60% of the male partners of the patients would advise her against a transvaginal CHE, mostly for fear of decreased postoperative sexual content. Of the 100 patients of our short-term analysis[25], a follow up of 24 mo was possible for 46 TVC- and 42 LC-patients[26]. However, only 36 patients of the TVC- and 25 patients of the LC-group were sexually active, and only these 61 patients could answer respective questions. The other patients had not been sexually active in the six preoperative months, either, which is why we did not rate postoperative sexual inactivity as a result of the operation. We found no differences in sexual function regarding satisfaction, dyspareunia, menstruation, vaginal discharge and pain, especially not in the lower abdomen. Indeed, TVC-patients were less impaired by the operation, and they could resume everyday activity earlier and were more satisfied with the esthetic result and the overall result. On the other hand, there was no difference in the postoperative long-term complication rate. Most likely as a result of the latter, significantly more TVC-patients would recommend the Hybrid-NOTES technique to friends and relatives. As a result, for the first time we could document the safety of the technique and the improved satisfaction of TVC-patients after a two-year follow-up in a cohort analysis. In addition, we could show that TVC-patients did not have sexual intercourse at a later postoperative point in time than did the LC-group, as suggested to the participants in a survey by Bucher *et al*[48]. Therefore, a respective difference should not be stated in future surveys or consultations regarding TVC. Additionally, our data rebut the fear of negative influences of the transvaginal access on sexual life. The previously frequently reported apprehension regarding the hygienic aspect of transvaginal access with the consequence of an intraperitoneal bacterial contamination could already be disproved in a study by Linke *et al*[50]. This way, our study could confirm earlier studies with a shorter follow up of up to 12 mo that showed no negative influence of TVC on sexual life[44,51-53].

Because all previous results came from non-randomized studies, we initiated a prospective randomized, unblinded, single center trial[29]. Because we wanted to compare TVC *vs* the least invasive laparoscopic multi trocar technique, we chose the needlescopic 3-trocar cholecystectomy as reference group. Here, two 3.9-mm and one 11-mm trocars were used. Based on the results of our cohort study,

the primary end point was postoperative pain until the morning of the second postoperative day, which was significantly less after TVC in this randomized trial. The rate of intra- and postoperative complications was again comparable, while in opposite to the cohort analysis, postoperative hospital stay was not different. Lower analgesic consumption and comparable procedural time for TVC were confirmed, although in this study all procedures from the reference group were performed by the same single surgeon. Additionally, TVC-patients were significantly more satisfied with the early postoperative esthetic result than NC-patients and had a significantly better quality of life on postoperative day ten as measured by the Eypasch GIQLI[34]. Meanwhile, two further randomized studies exist that compared TVC with LC. The three armed study by Noguera *et al*[54], which also included a transumbilical single-incision-CHE, could neither detect any differences between the techniques regarding the primary end-point complication rate, nor with regard to the secondary end-points pain, hospital stay and sick leave. Of note, the study was clearly underpowered according to the given sample-size calculation. The latest published randomized study by Borchert *et al*[55] compared 41 TVC-patients with 51 LC-patients in a double blind study design with a follow up of seven days and found a longer procedural time for TVC and neither a significant difference in complication rate nor in postoperative pain. A closer look reveals postoperative pain after TVC on the VRS (0-10) to be up to 0.8 points lower than after LC. However, despite the larger case number, this result was not significant. Unfortunately, analgesic consumption was not stated, so the respective influence of this factor could not be detected. Additionally, twelve surgeons with an average case load of 4.9 per group took part in the study, which might blur the potential differences by interindividual effects. Additionally, there was a relatively high conversion rate of 10%, possibly also due to the large number of surgeons. Interestingly, the average procedural time for TVC in our randomized trial[29], compared to that of our cohort study[25], namely the first 50 TVC at our clinic, was more than 24 min shorter (53.6 min *vs* 77.8 min). This fact underlines the assumption of an existing learning curve for TVC.

Following the confirmation of the early postoperative advantages in the cohort analysis[25] by the randomized trial[29], the documentation of more secondary end-points three and six months postoperatively could show the high acceptance and the absence of long-term problems also in a randomized study design[30]. Sexual function, measured three months postoperatively, again with the domains "satisfaction" and "pain" in the FSFI-d[32,33], was comparable for both groups, as was abdominal pain. The aesthetic results after three and six months were considered significantly better in the TVC-group, whereas satisfaction with the overall result was not rated differently. The six-month gynecological follow-up of all TVC-patients had no case with a pathological result. Thus, no access related disadvantages were found in this randomized study design. A limitation was that the case-number was calculated for the primary outcome parameter and that small but possibly relevant differences require an extremely high case number. This is especially true for wound infections and trocar hernias. For the latter, the follow up period of our study was certainly too short. The case number for a study that could theoretically prove a statistically significant reduction of trocar hernias by TVC would be 1500 patients per study arm[56]. Because this is unfeasible, this theoretical advantage will remain unproven.

Finally, the study aims mentioned in the introduction could be answered by our investigations as follows: Hybrid-NOTES cholecystectomy in clinical practice is feasible and safe, with a low conversion rate. Access related complications are rare and comprised of urinary bladder injury in patients with previous hysterectomy and bowel injury. Following Hybrid-NOTES cholecystectomy, there is less postoperative pain compared with traditional laparoscopic cholecystectomy. Hybrid-NOTES cholecystectomy does not lead to impairment of sexual function by the transvaginal access and results in a higher patient satisfaction with esthetic results. Even in long-term follow up, no local morphological damage can be attributed to the transvaginal access.

COMMENTS

Background

Laparoscopic cholecystectomy (LC) leads to less postoperative pain, a shorter length of stay, an earlier everyday life and a better aesthetic result comparing to the conventional technique so that LC becomes the gold standard for gallbladder disease. Nevertheless, the gallbladder has to be removed from the intraperitoneal cavity. This required trauma to the abdominal wall results in wound pain and scar formation as well as a notable risk of infection and incisional hernia. The transvaginal cholecystectomy with rigid instruments (TVC), the assistance of a 5 mm-trocar in the umbilicus and removal of the gallbladder *via* the transvaginal access is an alternative that eliminates the mentioned risks of removing the specimen *via* the abdominal wall. The aim of the authors was to evaluate this new technique concomitantly during its clinical establishment in regard to its advantages and specific complications and to compare it with the traditional laparoscopic technique by means of a registry analysis, a matched cohort analysis and a randomized clinical trial. In this review, the authors discuss these results in the context and with the existing literature.

Research frontiers

During the uncontrolled implementation and distribution of LC in the early 1990s, the benefits of the new procedure were unknown and the rate of bile duct injuries increased. Hence, the implementation of TVC as a new technique needed to be scientifically controlled and critically supervised.

Innovations and breakthroughs

The authors succeeded in continuously evaluating a new surgical technique by a registry analysis, a matched cohort analysis and a randomized clinical trial. Therefore, they could show the feasibility of TVC with a low conversion rate and a low complication rate. Access specific complications exist with injuries

of the bladder and rectum as well as urinary tract infections. In comparison to LC and needlescopic 3-trocar cholecystectomy, respectively, TVC leads to less postoperative pain despite less postoperative analgesic consumption, better quality of life in short-term and higher satisfaction with the aesthetic result even in long-term. The complication rate is not significantly different and TVC has no significant influence on sexual intercourse regarding to satisfaction and pain.

Applications

According to the authors' research, TVC is a feasible and safe procedure with benefits most notably in regard to less postoperative pain.

Terminology

TVC means a laparoscopic Hybrid-NOTES procedure in which the gallbladder is removed by a transvaginal access with the assistance of a transumbilical 5 mm-trocar.

Peer-review

Congratulations to the authors on a very good paper on this relatively new topic. This paper is for the most part all written with good information. There are certainly limitations to the database data for example, but this is addressed in the manuscript.

REFERENCES

1 **Seiler CM**, Deckert A, Diener MK, Knaebel HP, Weigand MA, Victor N, Büchler MW. Midline versus transverse incision in major abdominal surgery: a randomized, double-blind equivalence trial (POVATI: ISRCTN60734227). *Ann Surg* 2009; **249**: 913-920 [PMID: 19474689 DOI: 10.1097/SLA.0b013e3181a77c92]

2 **Mahmoud NN**, Turpin RS, Yang G, Saunders WB. Impact of surgical site infections on length of stay and costs in selected colorectal procedures. *Surg Infect* (Larchmt) 2009; **10**: 539-544 [PMID: 19708769 DOI: 10.1089/sur.2009.006]

3 **Aimaq R**, Akopian G, Kaufman HS. Surgical site infection rates in laparoscopic versus open colorectal surgery. *Am Surg* 2011; **77**: 1290-1294 [PMID: 22127072]

4 **Penninckx FM**, Poelmans SV, Kerremans RP, Beckers JP. Abdominal wound dehiscence in gastroenterological surgery. *Ann Surg* 1979; **189**: 345-352 [PMID: 426566]

5 **Kenig J**, Richter P, Żurawska S, Lasek A, Zbierska K. Risk factors for wound dehiscence after laparotomy - clinical control trial. *Pol Przegl Chir* 2012; **84**: 565-573 [PMID: 23399620 DOI: 10.2478/v10035-012-0094-0]

6 **Ramneesh G**, Sheerin S, Surinder S, Bir S. A prospective study of predictors for post laparotomy abdominal wound dehiscence. *J Clin Diagn Res* 2014; **8**: 80-83 [PMID: 24596730 DOI: 10.7860/JCDR/2014/7348.3921]

7 **Fink C**, Baumann P, Wente MN, Knebel P, Bruckner T, Ulrich A, Werner J, Büchler MW, Diener MK. Incisional hernia rate 3 years after midline laparotomy. *Br J Surg* 2014; **101**: 51-54 [PMID: 24281948 DOI: 10.1002/bjs.9364]

8 **Asa Z**, Greenberg R, Ghinea R, Inbar R, Wasserberg N, Avital S. Grading of complications and risk factor evaluation in laparoscopic colorectal surgery. *Surg Endosc* 2013; **27**: 3748-3753 [PMID: 23636522 DOI: 10.1007/s00464-013-2960-1]

9 **Davies SJ**, Francis J, Dilley J, Wilson RJ, Howell SJ, Allgar V. Measuring outcomes after major abdominal surgery during hospitalization: reliability and validity of the Postoperative Morbidity Survey. *Perioper Med* (Lond) 2013; **2**: 1 [PMID: 24472150 DOI: 10.1186/2047-0525-2-1]

10 **Semm K**. Endoscopic appendectomy. *Endoscopy* 1983; **15**: 59-64 [PMID: 6221925 DOI: 10.1055/s-2007-1021466]

11 **Mühe E**. [Laparoscopic cholecystectomy]. *Z Gastroenterol Verh* 1991; **26**: 204-206 [PMID: 1714149]

12 **Moore MJ**, Bennett CL. The learning curve for laparoscopic cholecystectomy. The Southern Surgeons Club. *Am J Surg* 1995; **170**: 55-59 [PMID: 7793496]

13 **Hoffman MS**, DeCesare S, Kalter C. Abdominal hysterectomy versus transvaginal morcellation for the removal of enlarged uteri. *Am J Obstet Gynecol* 1994; **171**: 309-313; discussion 313-315 [PMID: 8059807]

14 **Teng FY**, Muzsnai D, Perez R, Mazdisnian F, Ross A, Sayre JW. A comparative study of laparoscopy and colpotomy for the removal of ovarian dermoid cysts. *Obstet Gynecol* 1996; **87**: 1009-1013 [PMID: 8649681]

15 **Childers JM**, Huang D, Surwit EA. Laparoscopic trocar-assisted colpotomy. *Obstet Gynecol* 1993; **81**: 153-155 [PMID: 8416452]

16 **Kalloo AN**, Singh VK, Jagannath SB, Niiyama H, Hill SL, Vaughn CA, Magee CA, Kantsevoy SV. Flexible transgastric peritoneoscopy: a novel approach to diagnostic and therapeutic interventions in the peritoneal cavity. *Gastrointest Endosc* 2004; **60**: 114-117 [PMID: 15229442]

17 **Zornig C**, Emmermann A, von Waldenfels HA, Mofid H. Laparoscopic cholecystectomy without visible scar: combined transvaginal and transumbilical approach. *Endoscopy* 2007; **39**: 913-915 [PMID: 17968809]

18 **Marescaux J**, Dallemagne B, Perretta S, Wattiez A, Mutter D, Coumaros D. Surgery without scars: report of transluminal cholecystectomy in a human being. *Arch Surg* 2007; **142**: 823-826; discussion 826-827 [PMID: 17875836]

19 **Bessler M**, Stevens PD, Milone L, Parikh M, Fowler D. Transvaginal laparoscopically assisted endoscopic cholecystectomy: a hybrid approach to natural orifice surgery. *Gastrointest Endosc* 2007; **66**: 1243-1245 [PMID: 17892873]

20 **Zorrón R**, Filgueiras M, Maggioni LC, Pombo L, Lopes Carvalho G, Lacerda Oliveira A. NOTES. Transvaginal cholecystectomy: report of the first case. *Surg Innov* 2007; **14**: 279-283 [PMID: 18178917 DOI: 10.1177/1553350607311090]

21 **Dolz C**, Noguera JF, Martín A, Vilella A, Cuadrado A. [Transvaginal cholecystectomy (NOTES) combined with minilaparoscopy]. *Rev Esp Enferm Dig* 2007; **99**: 698-702 [PMID: 18290693]

22 **Emmermann A**, Zornig C, Peiper M, Weh HJ, Broelsch CE. Laparoscopic splenectomy. Technique and results in a series of 27 cases. *Surg Endosc* 1995; **9**: 924-927 [PMID: 8525451]

23 **Delvaux G**, Devroey P, De Waele B, Willems G. Transvaginal removal of gallbladders with large stones after laparoscopic cholecystectomy. *Surg Laparosc Endosc* 1993; **3**: 307-309 [PMID: 8269249]

24 **Lehmann KS**, Ritz JP, Wibmer A, Gellert K, Zornig C, Burghardt J, Büsing M, Runkel N, Kohlhaw K, Albrecht R, Kirchner TG, Arlt G, Mall JW, Butters M, Bulian DR, Bretschneider J, Holmer C, Buhr HJ. The German registry for natural orifice translumenal endoscopic surgery: report of the first 551 patients. *Ann Surg* 2010; **252**: 263-270 [PMID: 20585238 DOI: 10.1097/SLA.0b013e3181e6240f]

25 **Bulian DR**, Trump L, Knuth J, Siegel R, Sauerwald A, Ströhlein MA, Heiss MM. Less pain after transvaginal/transumbilical cholecystectomy than after the classical laparoscopic technique: short-term results of a matched-cohort study. *Surg Endosc* 2013; **27**: 580-586 [PMID: 22926893 DOI: 10.1007/s00464-012-2490-2]

26 **Bulian DR**, Trump L, Knuth J, Cerasani N, Heiss MM. Long-term results of transvaginal/transumbilical versus classical laparoscopic cholecystectomy--an analysis of 88 patients. *Langenbecks Arch Surg* 2013; **398**: 571-579 [PMID: 23456357 DOI: 10.1007/s00423-013-1071-8]

27 **Hosono S**, Osaka H. Minilaparoscopic versus conventional laparoscopic cholecystectomy: a meta-analysis of randomized controlled trials. *J Laparoendosc Adv Surg Tech A* 2007; **17**: 191-199 [PMID: 17484646]

28 **Gurusamy KS**, Samraj K, Ramamoorthy R, Farouk M, Fusai G, Davidson BR. Miniport versus standard ports for laparoscopic cholecystectomy. *Cochrane Database Syst Rev* 2010; (**3**): CD006804 [PMID: 20238350 DOI: 10.1002/14651858.CD006804.pub2]

29 **Bulian DR**, Knuth J, Cerasani N, Sauerwald A, Lefering R, Heiss MM. Transvaginal/transumbilical hybrid--NOTES--versus 3-trocar needlescopic cholecystectomy: short-term results of a randomized clinical trial. *Ann Surg* 2015; **261**: 451-458 [PMID: 24108196 DOI: 10.1097/SLA.0000000000000218]

30 **Bulian DR**, Knuth J, Cerasani N, Lange J, Ströhlein MA, Sauerwald A, Heiss MM. Transvaginal hybrid NOTES cholecystectomy--results of a randomized clinical trial after 6 months. *Langenbecks Arch Surg* 2014; **399**: 717-724 [PMID: 24952726 DOI: 10.1007/s00423-014-1218-2]

31 **Hartrick CT**, Kovan JP, Shapiro S. The numeric rating scale for clinical pain measurement: a ratio measure? *Pain Pract* 2003; **3**: 310-316 [PMID: 17166126 DOI: 10.1111/j.1530-7085.2003.03034.x]

32 **Rosen R**, Brown C, Heiman J, Leiblum S, Meston C, Shabsigh R, Ferguson D, D'Agostino R. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. *J Sex Marital Ther* 2000; **26**: 191-208 [PMID: 10782451 DOI: 10.1080/009262300278597]

33 **Berner MM**, Kriston L, Zahradnik HP, Härter M, Rohde A. [Validity and Reliability of the German Female Sexual Function Index (FSFI-d)]. *Geburtsh Frauenheilk* 2004; **64**: 293-303 [DOI: 10.1055/s-2004-815815]

34 **Eypasch E**, Williams JI, Wood-Dauphinee S, Ure BM, Schmülling C, Neugebauer E, Troidl H. Gastrointestinal Quality of Life Index: development, validation and application of a new instrument. *Br J Surg* 1995; **82**: 216-222 [PMID: 7749697]

35 **Richardson MC**, Bell G, Fullarton GM. Incidence and nature of bile duct injuries following laparoscopic cholecystectomy: an audit of 5913 cases. West of Scotland Laparoscopic Cholecystectomy Audit Group. *Br J Surg* 1996; **83**: 1356-1360 [PMID: 8944450]

36 **Deziel DJ**, Millikan KW, Economou SG, Doolas A, Ko ST, Airan MC. Complications of laparoscopic cholecystectomy: a national survey of 4,292 hospitals and an analysis of 77,604 cases. *Am J Surg* 1993; **165**: 9-14 [PMID: 8418705]

37 **Keus F**, de Jong JA, Gooszen HG, van Laarhoven CJ. Laparoscopic versus open cholecystectomy for patients with symptomatic cholecystolithiasis. *Cochrane Database Syst Rev* 2006; (**4**): CD006231 [PMID: 17054285 DOI: 10.1002/14651858.CD006231]

38 **Lehmann KS**, Zornig C, Arlt G, Butters M, Bulian DR, Manger R, Burghardt J, Runkel N, Pürschel A, Köninger J, Buhr HJ. [Natural orifice transluminal endoscopic surgery in Germany: Data from the German NOTES registry]. *Chirurg* 2015; **86**: 577-586 [PMID: 24994591 DOI: 10.1007/s00104-014-2808-9]

39 **Hensel M**, Schernikau U, Schmidt A, Arlt G. [Comparison between transvaginal and laparoscopic cholecystectomy - a retrospective case-control study]. *Zentralbl Chir* 2012; **137**: 48-54 [PMID: 20446249 DOI: 10.1055/s-0030-1247332]

40 **Kilian M**, Raue W, Menenakos C, Wassersleben B, Hartmann J. Transvaginal-hybrid vs. single-port-access vs. 'conventional' laparoscopic cholecystectomy: a prospective observational study. *Langenbecks Arch Surg* 2011; **396**: 709-715 [PMID: 21384187 DOI: 10.1007/s00423-011-0769-8]

41 **Solomon D**, Shariff AH, Silasi DA, Duffy AJ, Bell RL, Roberts KE. Transvaginal cholecystectomy versus single-incision laparoscopic cholecystectomy versus four-port laparoscopic cholecystectomy: a prospective cohort study. *Surg Endosc* 2012; **26**: 2823-2827 [PMID: 22549370 DOI: 10.1007/s00464-012-2253-0]

42 **Borchert D**, Federlein M, Rückbeil O, Burghardt J, Fritze F, Gellert K. Prospective evaluation of transvaginal assisted cholecystectomy. *Surg Endosc* 2012; **26**: 3597-3604 [PMID: 22717796 DOI: 10.1007/s00464-012-2378-1]

43 **Santos BF**, Teitelbaum EN, Arafat FO, Milad MP, Soper NJ, Hungness ES. Comparison of short-term outcomes between transvaginal hybrid NOTES cholecystectomy and laparoscopic cholecystectomy. *Surg Endosc* 2012; **26**: 3058-3066 [PMID: 22549379 DOI: 10.1007/s00464-012-2313-5]

44 **Zornig C**, Siemssen L, Emmermann A, Alm M, von Waldenfels HA, Felixmüller C, Mofid H. NOTES cholecystectomy: matched-pair analysis comparing the transvaginal hybrid and conventional laparoscopic techniques in a series of 216 patients. *Surg Endosc* 2011; **25**: 1822-1826 [PMID: 21181204 DOI: 10.1007/s00464-010-1473-4]

45 **Noguera JF**, Cuadrado A, Dolz C, Olea JM, Morales R, Vicens C, Pujol JJ. [Non-randomised, comparative, prospective study of transvaginal endoscopic cholecystectomy versus transparietal laparoscopic cholecystectomy]. *Cir Esp* 2009; **85**: 287-291 [PMID: 19376502]

46 **Peterson CY**, Ramamoorthy S, Andrews B, Horgan S, Talamini M, Chock A. Women's positive perception of transvaginal NOTES surgery. *Surg Endosc* 2009; **23**: 1770-1774 [PMID: 19057953 DOI: 10.1007/s00464-008-0206-4]

47 **Strickland AD**, Norwood MG, Behnia-Willison F, Olakkengil SA, Hewett PJ. Transvaginal natural orifice translumenal endoscopic surgery (NOTES): a survey of women's views on a new technique. *Surg Endosc* 2010; **24**: 2424-2431 [PMID: 20224999 DOI: 10.1007/s00464-010-0968-3]

48 **Bucher P**, Ostermann S, Pugin F, Morel P. Female population perception of conventional laparoscopy, transumbilical LESS, and transvaginal NOTES for cholecystectomy. *Surg Endosc* 2011; **25**: 2308-2315 [PMID: 21301884 DOI: 10.1007/s00464-010-1554-4]

49 **Kobiela J**, Stefaniak T, Dobrowolski S, Makarewicz W, Lachiński AJ, Sledziński Z. Transvaginal NOTES cholecystectomy in my partner? No way! *Wideochir Inne Tech Maloinwazyjne* 2011; **6**: 236-241 [PMID: 23255986 DOI: 10.5114/wiitm.2011.26258]

50 **Linke GR**, Tarantino I, Bruderer T, Celeiro J, Warschkow R, Tarr PE, Müller-Stich BP, Zerz A. Transvaginal access for NOTES: a cohort study of microbiological colonization and contamination. *Endoscopy* 2012; **44**: 684-689 [PMID: 22528675 DOI: 10.1055/s-0032-1309390]

51 **Zornig C**, Mofid H, Siemssen L, Emmermann A, Alm M, von Waldenfels HA, Felixmüller C. Transvaginal NOTES hybrid cholecystectomy: feasibility results in 68 cases with mid-term follow-up. *Endoscopy* 2009; **41**: 391-394 [PMID: 19418391]

52 **Linke GR**, Luz S, Janczak J, Zerz A, Schmied BM, Siercks I, Warschkow R, Beutner U, Tarantino I. Evaluation of sexual function in sexually active women 1 year after transvaginal NOTES: a prospective cohort study of 106 patients. *Langenbecks Arch Surg* 2013; **398**: 139-145 [PMID: 22922839 DOI: 10.1007/s00423-012-0993-x]

53 **Pugliese R**, Forgione A, Sansonna F, Ferrari GC, Di Lernia S, Magistro C. Hybrid NOTES transvaginal cholecystectomy: operative and long-term results after 18 cases. *Langenbecks Arch Surg* 2010; **395**: 241-245 [PMID: 19588162 DOI: 10.1007/s00423-009-0528-2]

54 **Noguera JF**, Cuadrado A, Dolz C, Olea JM, García JC. Prospective randomized clinical trial comparing laparoscopic cholecystectomy and hybrid natural orifice transluminal endoscopic surgery (NOTES) (NCT00835250). *Surg Endosc* 2012; **26**: 3435-3441 [PMID: 22648123 DOI: 10.1007/s00464-012-2359-4]

55 **Borchert DH**, Federlein M, Fritze-Büttner F, Burghardt J, Liersch-Löhn B, Atas Y, Müller V, Rückbeil O, Wagenpfeil S, Gräber S, Gellert K. Postoperative pain after transvaginal cholecystectomy: single-center, double-blind, randomized controlled trial. *Surg Endosc* 2014; **28**: 1886-1894 [PMID: 24464385 DOI: 10.1007/s00464-013-3409-2]

56 **Bulian DR**, Knuth J, Ströhlein MA, Sauerwald A, Heiss MM. [Transvaginal/transumbilical hybrid NOTES appendicectomy: Comparison of techniques in uncomplicated and complicated appendicitis]. *Chirurg* 2015; **86**: 366-372 [PMID: 24969344 DOI: 10.1007/s00104-014-2774-2]

P- Reviewer: Farkas DT **S- Editor**: Ma YJ **L- Editor**: A
E- Editor: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i38.10926

World J Gastroenterol 2015 October 14; 21(38): 10926-10930
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Hilar cholangiocarcinoma with intratumoral calcification: A case report

Kazuho Inoko, Takahiro Tsuchikawa, Takehiro Noji, Yo Kurashima, Yuma Ebihara, Eiji Tamoto, Toru Nakamura, Soichi Murakami, Keisuke Okamura, Toshiaki Shichinohe, Satoshi Hirano

Kazuho Inoko, Takahiro Tsuchikawa, Takehiro Noji, Yo Kurashima, Yuma Ebihara, Eiji Tamoto, Toru Nakamura, Soichi Murakami, Keisuke Okamura, Toshiaki Shichinohe, Satoshi Hirano, Department of Gastroenterological Surgery II, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido 060-8648, Japan

Author contributions: Inoko K and Tsuchikawa T wrote the manuscript; Shichinohe T and Hirano S revised the manuscript; Okamura K, Nakamura T and Tamoto E performed the surgery; Murakami S, Ebihara Y, Kurashima Y and Noji T acquired the data.

Informed consent statement: The patient gave written informed consent before treatment.

Conflict-of-interest statement: The authors have no conflicts of interest relevant to this article.

Correspondence to: Kazuho Inoko, MD, Department of Gastroenterological Surgery II, Hokkaido University Graduate School of Medicine, North-15, West-7, Kita-ku, Sapporo, Hokkaido 060-8648, Japan. k.inoko@icloud.com
Telephone: +81-11-7067714
Fax: +81-11-7067158

Received: March 26, 2015
Peer-review started: March 27, 2015
First decision: April 24, 2015
Revised: May 27, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 14, 2015

Abstract

This report describes a rare case of hilar cholangiocarcinoma with intratumoral calcification that mimicked hepatolithiasis. A 73-year-old man presented to a local hospital with a calcified lesion in the hepatic hilum. At first, hepatolithiasis was diagnosed, and he underwent endoscopic stone extraction *via* the transpapillary route. This treatment strategy failed due to biliary stricture. He was referred to our hospital, and further examination suggested the existence of cholangiocarcinoma. He underwent left hepatectomy with caudate lobectomy and extrahepatic bile duct resection. Pathological examination revealed hilar cholangiocarcinoma with intratumoral calcification, while no stones were found. To the best of our knowledge, only one case of calcified hilar cholangiocarcinoma has been previously reported in the literature. Here, we report a rare case of calcified hilar cholangiocarcinoma and reveal its clinicopathologic features.

Key words: Cholangiocarcinoma; Klatskin tumor; Calcification; Lithiasis; Differentiation

Core tip: Our report describes an extremely rare case of hilar cholangiocarcinoma with intratumoral calcification. Imaging findings of this case were confusingly similar to those of hepatolithiasis, and it was extremely difficult to make an accurate diagnosis using available radiological findings. We performed pathological examination and observed hilar cholangiocarcinoma with intratumoral calcification. We herein report a rare case of calcified hilar cholangiocarcinoma and reveal its clinicopathologic features.

E, Nakamura T, Murakami S, Okamura K, Shichinohe T, Hirano S. Hilar cholangiocarcinoma with intratumoral calcification: A case report. *World J Gastroenterol* 2015; 21(38): 10926-10930 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10926.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10926

INTRODUCTION

Calcification in the hepatic hilum is commonly caused by hepatolithiasis, which is defined as the presence of bile stones in the bile ducts proximal to the confluence of the right and left hepatic ducts[1]. The incidence of hepatolithiasis is as high as 18%-45% of patients with gallstone disease in East Asia[1], and the disease has become more prevalent in Western countries due to an increase in migrants from endemic regions[2,3]. By contrast, hilar cholangiocarcinoma with intratumoral calcification is an extremely rare cause of calcification in the hepatic hilus, with only one previously reported case in the English literature[4].

Although imaging findings are quite similar, the differential diagnosis between hepatolithiasis and calcified Klatskin tumor is crucial because of their different prognoses. We present a rare case of calcified hilar cholangiocarcinoma mimicking hepatolithiasis.

CASE REPORT

A 73-year-old man was admitted to a local hospital for investigation of a calcified lesion in the hepatic hilum associated with a dilated left intrahepatic bile duct. He initially received a diagnosis of hepatolithiasis. Endoscopic stone extraction *via* the trans-papillary route was attempted but was unsuccessful due to stricture of the left hepatic duct. He was referred to our hospital for further examination.

On admission, a blood test revealed mild elevation of carbohydrate antigen 19-9 (46.3 U/mL), though no elevation of carcinoembryonic antigen. All other laboratory findings, including serum concentrations of total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and γ-glutamyl transpeptidase, were within normal ranges. Hepatic ultrasonography showed a 33.5 mm × 26.5 mm intraductal mass at the confluence of the right and left hepatic duct. In addition, a highly echogenic mass with posterior acoustic shadowing was observed, suggesting a calcified lesion (Figure 1). Enhanced multi-detector-row computed tomography (CT) revealed marked dilatation of the left intrahepatic bile ducts upstream from the calcification and enhancement of the bile duct wall distal to the calcification (Figure 2). Endoscopic retrograde cholangiography (ERC) showed significant biliary stricture and disruption at the left hepatic duct, but no defect area (Figure 3). Furthermore, biopsy of the left hepatic duct was suggestive of adenocarcinoma.

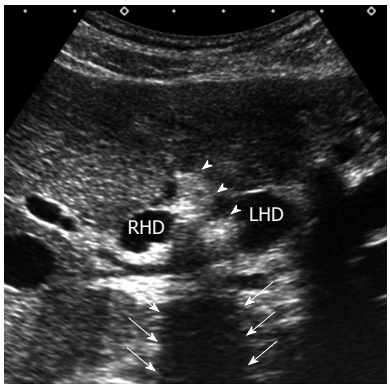


Figure 1 Finding of ultrasonography. Transverse ultrasonography shows a highly echogenic mass (arrow head) with posterior acoustic shadowing (arrow) at the confluence of the right and left hepatic duct. RHD: Right hepatic duct; LHD: Left hepatic duct.

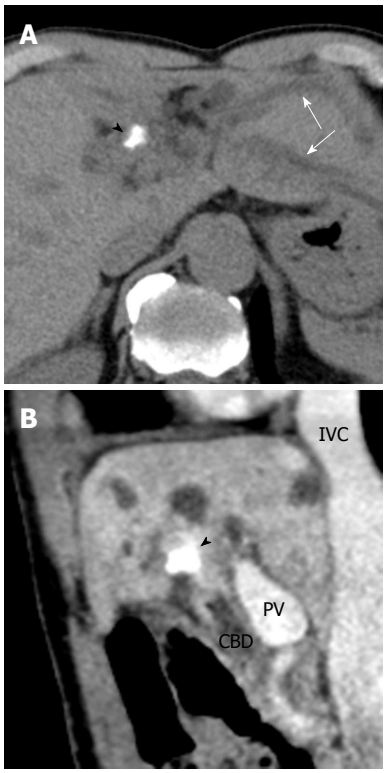


Figure 2 Findings of computed tomography. A: Plain transverse computed tomography (CT) reveals a high-density area at the liver hilus (arrow head) with dilated left intrahepatic bile ducts (arrows); B: Sagittal plane of enhanced CT shows the perihilar high-density area (arrow head). PV: Portal vein; IVC: Inferior vena cava; CBD: Common bile duct.

Based on these findings, we diagnosed hilar cholangiocarcinoma complicated by the hepatolithiasis and decided to perform left hepatectomy with caudate lobectomy and extrahepatic bile duct resection. The patient underwent the scheduled operation. The operative time was 625 min, and the total blood loss was 1165 mL. No blood transfusion was required.

On histopathological examination, the tumor was found to have spread from the hepatic hilum to the left hepatic duct. The tumor was mucus-secreting

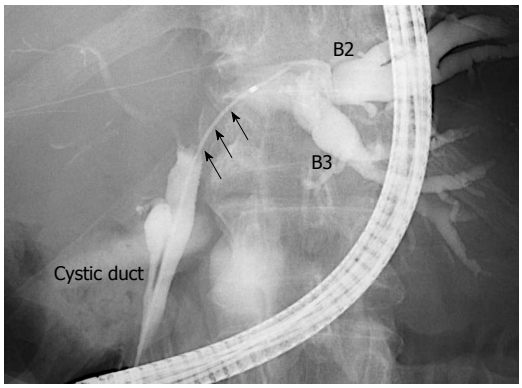


Figure 3 Finding of endoscopic retrograde cholangiography. Cholangiography *via* the trans-papillary route shows marked biliary stricture and disruption at the left hepatic duct (arrows), but no defect area.

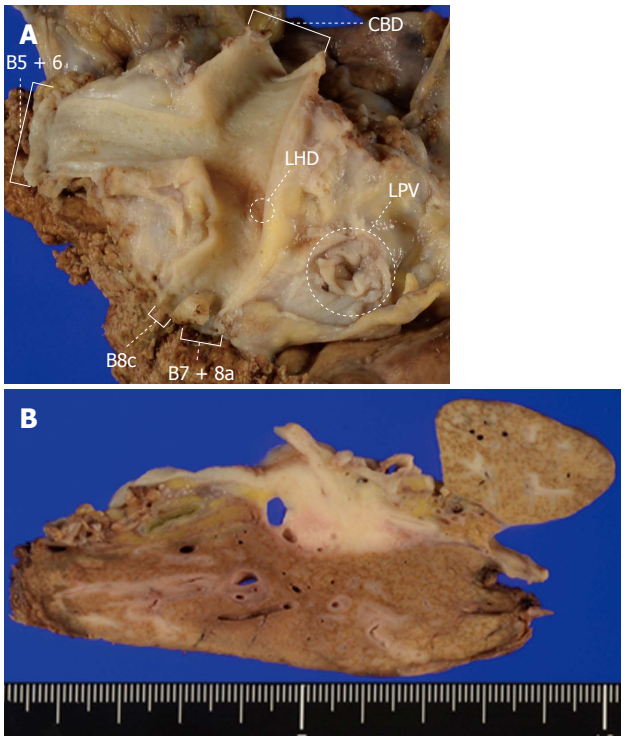


Figure 4 Macroscopic findings. A: Opened bile duct of the resected specimen. The left hepatic duct is highly obstructed; B: Gross appearance of the cut surface of the resected liver shows a gray-white tumor measuring 28 mm × 21 mm in the hepatic hilum to the left hepatic duct, while no stones are found in the hepatic hilum. CBD: Common bile duct; LHD: Left hepatic duct; LPV: Left portal vein.

and very hard, measuring 28 mm × 21 mm. It was a nodular-infiltrating type cholangiocarcinoma mainly existing in the perihilar bile duct with an abundance of fine calcification. No stones were found by the gross examination in the hilar lesion (Figure 4).

A regional lymph node in the hepatoduodenal ligament was invaded by tumor cells. Microscopically, this tumor was a gastric foveolar type adenocarcinoma and was rich in mucus. On the other hand, the majority of the calcified material was located within or replaced the tumor glands (Figure 5).

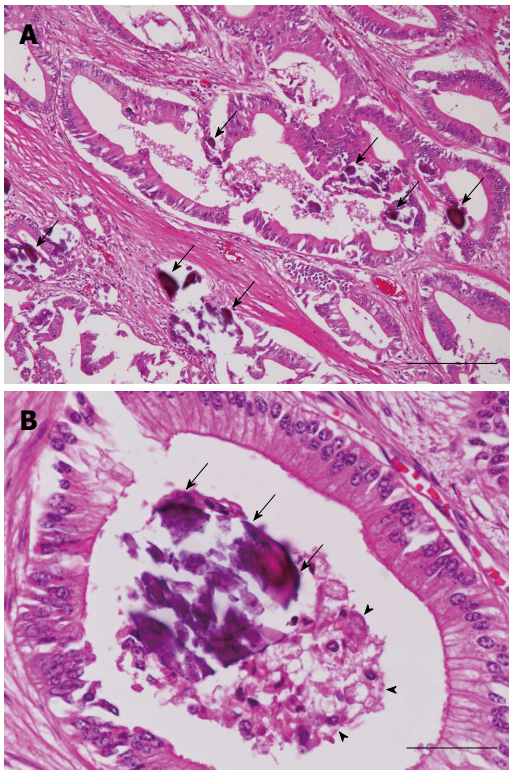


Figure 5 Histopathological findings (hematoxylin and eosin staining). A: Microscopic examination reveals a mucus-secreting, gastric foveolar type adenocarcinoma with numerous fine calcifications (arrows) (bar = 200 μm); B: The calcified material (arrows) is located in the mucus (arrow head) in the tumor glands (bar = 50 μm).

The postoperative course was uneventful, and the patient was discharged 21 d after surgery. At 7 mo after the surgery, the patient was well, without evidence of a recurrence of the disease.

DISCUSSION

This case report represents an unusual calcified hilar cholangiocarcinoma, which mimicked hepatolithiasis in clinical findings. To the best of our knowledge, there is only one article describing calcified Klatskin tumor in the literature[4].

The typical cause of calcified lesion in the porta hepatis is a calculus; in patients with primary calcium bilirubinate hepatolithiasis, stones are frequently located in the large bile ducts, such as the main hepatic ducts[5]. Histologically, in patients with hepatolithiasis, fibrotic changes in the bile duct walls and periductal hepatic parenchyma are often seen and lead to stricture formation[6,7]. Furthermore, hepatolithiasis is closely associated with concomitant cholangiocarcinoma with an incidence of approximately 5%[8], and this malignant lesion is also associated with biliary stricture[3]. Thus, among patients with hepatolithiasis, a calcified lesion is often associated with a biliary stenosis lesion caused by periductal fibrosis or cholangiocarcinoma[3].

In the present case, initial imaging findings showed

a perihilar calcified lesion associated with the dilated proximal bile duct and the stricture lesion distal to the calcification. These findings were confusingly similar to those of hepatolithiasis associated with biliary stricture, and it was extremely difficult to make an accurate diagnosis using available radiological findings. In the previously reported case, the radiologic findings of the calcified Klatskin tumor were also very similar to those of hepatolithiasis, which resulted in biliary obstruction and proximal ductal dilation[4]. Because of these similarities in imaging findings, even more than the rarity of the disease, it can be difficult to make an accurate preoperative diagnosis of calcified hilar cholangiocarcinoma. ERC in this case, however, did not show a defective area, which is typically found in hepatolithiasis. In retrospect, this point might have suggested that the calcification in this case originated from diseases other than that of hepatolithiasis.

The pathogenesis of intratumoral calcification is mainly dystrophic calcification, which refers to the macroscopic deposition of calcium salts in injured tissues with normal serum levels of calcium[9]. In the dystrophic process, deranged cells form seed crystals that become encrusted with the mineral deposits, and the acquisition of outer layers gradually creates its lamellated configurations[10]. This type of calcification is most commonly observed in papillary thyroid carcinoma, meningioma, and papillary serous cyst adenocarcinoma of the ovary at the frequency of 40%-50%, 45%, and 33% of cases respectively[10]. Additionally, mucus-producing adenocarcinoma contributes to the deposition of calcium by *via* the actions of mucin glycoproteins as an ion-exchange resin[11]. Mucinous adenocarcinoma, which is a typical and frequently occurring histological type of colorectal cancer, can have fine or punctate calcification due to its mucinous component[12,13]. In intrahepatic cholangiocarcinoma, it is usually thought that, in addition to necrotic areas of the tumor, calcification occurs as a secondary reaction to mucin secretions[13,14]. In the present case, the intratumoral calcification was observed in the absence of a dysfunction in calcium metabolism, so it was thought that the calcification had formed in the dystrophic process. In addition, at the time of microscopic examination in this case, the tumor was noted to be a gastric foveolar type adenocarcinoma and was rich in mucus. Calcium deposition was seen in foveolar cells itself and the mucus in the glands. Therefore, the tumoral calcification in this case appears to reflect an accumulation of calcium secondary to dystrophy of tumor tissue and a mucus component produced by the tumor.

In conclusion, we presented an extremely rare case of hilar cholangiocarcinoma with intratumoral calcification. Radiological findings of this case were similar to those of hepatolithiasis. The pathogenic mechanism of this calcification was thought to be a dystrophic process that was associated with mucus.

COMMENTS

Case characteristics
A 73-year-old man presented with an asymptomatic calcified lesion in the hepatic hilum.

Clinical diagnosis
Upon physical examination, the patient had no clinical abnormality.

Differential diagnosis
Hepatolithiasis.

Laboratory diagnosis
A blood test revealed mild elevation of carbohydrate antigen (CA) 19-9 (46.3 U/mL), while metabolic panel and liver function test were within normal limits.

Imaging diagnosis
Ultrasonography/computed tomography revealed a highly echogenic mass or high-density area at the hepatic hilum with dilated left intrahepatic bile ducts.

Pathological diagnosis
Pathological examination revealed a nodular-infiltrating type cholangiocarcinoma mainly in the perihilar bile duct with abundance of fine calcification.

Treatment
The patient underwent left hepatectomy with caudate lobectomy and extrahepatic bile duct resection.

Related reports
Hilar cholangiocarcinoma with intratumoral calcification is an extremely rare cause of calcification in the hepatic hilum, with only one previous case report in the English literature.

Term explanation
Dystrophic calcification refers to the macroscopic deposition of calcium salts in injured tissues.

Experiences and lessons
This case report describes a rare case of calcified hilar cholangiocarcinoma and reveals its clinicopathologic features. Imaging findings of this case were confusingly similar to those of hepatolithiasis, and it was extremely difficult to make an accurate diagnosis based on available radiological findings.

Peer-review
This article highlights the clinical characteristics of calcified hilar cholangiocarcinoma and discusses the pathogenic mechanism of this calcification.

REFERENCES

1 **Pausawasdi A**, Watanapa P. Hepatolithiasis: epidemiology and classification. *Hepatogastroenterology* 1997; **44**: 314-316 [PMID: 9164496]

2 **Al-Sukhni W**, Gallinger S, Pratzer A, Wei A, Ho CS, Kortan P, Taylor BR, Grant DR, McGilvray I, Cattral MS, Langer B, Greig PD. Recurrent pyogenic cholangitis with hepatolithiasis--the role of surgical therapy in North America. *J Gastrointest Surg* 2008; **12**: 496-503 [PMID: 17999121 DOI: 10.1007/s11605-007-0398-2]

3 **Park HS**, Lee JM, Kim SH, Jeong JY, Kim YJ, Lee KH, Choi SH, Han JK, Choi BI. CT Differentiation of cholangiocarcinoma from periductal fibrosis in patients with hepatolithiasis. *AJR Am J Roentgenol* 2006; **187**: 445-453 [PMID: 16861550 DOI: 10.2214/ajr.05.0247]

4 **Park HS**, Han JK, Lee HS, Lee KH, Kim SH, Kim KW, Kim YJ,

Kim HC, Choi BI. Calcified Klatskin tumor mimicking intrahepatic stone: case report. *Abdom Imaging* 2005; **30**: 90-92 [PMID: 15647877 DOI: 10.1007/s00261-004-0232-1]

5 **Kondo S**, Nimura Y, Hayakawa N, Kamiya J, Nagino M, Miyachi M, Kanai M. A clinicopathologic study of primary cholesterol hepatolithiasis. *Hepatogastroenterology* 1995; **42**: 478-486 [PMID: 8751201]

6 **Nakanuma Y**, Terada T, Tanaka Y, Ohta G. Are hepatolithiasis and cholangiocarcinoma aetiologically related? A morphological study of 12 cases of hepatolithiasis associated with cholangiocarcinoma. *Virchows Arch A Pathol Anat Histopathol* 1985; **406**: 45-58 [PMID: 2986349]

7 **Chan FL**, Man SW, Leong LL, Fan ST. Evaluation of recurrent pyogenic cholangitis with CT: analysis of 50 patients. *Radiology* 1989; **170**: 165-169 [PMID: 2909092 DOI: 10.1148/radiology.170.1.2909092]

8 **Chen MF**, Jan YY, Wang CS, Hwang TL, Jeng LB, Chen SC, Chen TJ. A reappraisal of cholangiocarcinoma in patient with hepatolithiasis. *Cancer* 1993; **71**: 2461-2465 [PMID: 8384069]

9 **Strayer DS**, Rubin E. Cell adaptation, cell injury and cell death. In: Rubin R, Strayer DS, editors. Rubin's pathology: clinicopathologic foundations of medicine. 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2011: 13

10 **Das DK**. Psammoma body: a product of dystrophic calcification or of a biologically active process that aims at limiting the growth and spread of tumor? *Diagn Cytopathol* 2009; **37**: 534-541 [PMID: 19373908 DOI: 10.1002/dc.21081]

11 **Batlan LE**. Calcification within the stomach wall in gastric malignancy; case report and review of literature. *Am J Roentgenol Radium Ther Nucl Med* 1954; **72**: 788-794 [PMID: 13207491]

12 **Ko EY**, Ha HK, Kim AY, Yoon KH, Yoo CS, Kim HC, Kim JC. CT differentiation of mucinous and nonmucinous colorectal carcinoma. *AJR Am J Roentgenol* 2007; **188**: 785-791 [PMID: 17312069 DOI: 10.2214/ajr.06.0476]

13 **Stoupis C**, Taylor HM, Paley MR, Buetow PC, Marre S, Baer HU, Vock P, Ros PR. The Rocky liver: radiologic-pathologic correlation of calcified hepatic masses. *Radiographics* 1998; **18**: 675-685; quiz 726 [PMID: 9599391 DOI: 10.1148/radiographics.18.3.9599391]

14 **Ros PR**, Buck JL, Goodman ZD, Ros AM, Olmsted WW. Intrahepatic cholangiocarcinoma: radiologic-pathologic correlation. *Radiology* 1988; **167**: 689-693 [PMID: 2834769 DOI: 10.1148/radiology.167.3.2834769]

P- Reviewer: Lee HC **S- Editor**: Yu J **L- Editor**: A
E- Editor: Ma S

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