

World Journal of *Hepatology*

World J Hepatol 2015 April 8; 7(4): 638-724





Editorial Board

2014-2017

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

EDITORS-IN-CHIEF

Clara Balsano, Rome
Wan-Long Chuang, Kaohsiung

GUEST EDITORIAL BOARD MEMBERS

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

MEMBERS OF THE EDITORIAL BOARD



Algeria

Samir Rouabhia, Batna



Argentina

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

Jorge Quarleri, Buenos Aires
Adriana M Torres, Rosario



Armenia

Narina Sargsyants, Yerevan



Australia

Mark D Gorrell, Sydney



Austria

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



Bangladesh

Shahinul Alam, Dhaka
Mamun Al Mahtab, Dhaka



Belgium

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



Botswana

Francesca Cainelli, Gaborone

Sandro Vento, Gaborone



Brazil

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



Bulgaria

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



Canada

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



Chile

Luis A Videla, Santiago



China

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

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



Czech Republic

Kamil Vysloulzil, Olomouc



Denmark

Henning Gronbaek, Aarhus
 Christian Mortensen, Hvidovre



Egypt

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



France

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



Germany

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

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



Greece

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



Hungary

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



India

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



Indonesia

Cosmas RA Lesmana, Jakarta
 Neneng Ratnasari, Yogyakarta



Iran

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



Israel

Stephen DH Malnick, Rehovot



Italy

Francesco Angelico, Rome

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



Japan

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

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



Jordan

Kamal E Bani-Hani, *Zarqa*



Malaysia

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



Mexico

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



Moldova

Angela Peltec, *Chishinev*



Netherlands

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



Nigeria

CA Asabamaka Onyekwere, *Lagos*



Pakistan

Bikha Ram Devrajani, *Jamshoro*



Philippines

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



Poland

Jacek Zielinski, *Gdansk*



Portugal

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



Qatar

Reem Al Olaby, *Doha*



Romania

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



Russia

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



Saudi Arabia

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



Singapore

Ser Yee Lee, *Singapore*



South Korea

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



Spain

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

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



Sri Lanka

Niranga M Devanarayana, *Ragama*



Sudan

Hatim MY Mudawi, *Khartoum*



Sweden

Evangelos Kalaitzakis, *Lund*



Switzerland

Christoph A Maurer, *Liestal*



Thailand

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



Turkey

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

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



Ukraine

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



United Kingdom

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



United States

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

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



REVIEW

- 638 Non-invasive methods for the diagnosis of nonalcoholic fatty liver disease
Papagianni M, Sofogianni A, Tziomalos K
- 649 Strategies to improve outcome of patients with hepatocellular carcinoma receiving a liver transplantation
Guerrero-Misas M, Rodríguez-Perálvarez M, De la Mata M
- 662 Arrhythmia risk in liver cirrhosis
Mozos I
- 673 Recent advances in multidisciplinary management of hepatocellular carcinoma
Gomaa AI, Waked I

MINIREVIEWS

- 688 Cirrhosis and portal hypertension: The importance of risk stratification, the role of hepatic venous pressure gradient measurement
La Mura V, Nicolini A, Tosetti G, Primignani M
- 696 Host cellular microRNA involvement in the control of hepatitis B virus gene expression and replication
Mizuguchi Y, Takizawa T, Uchida E

ORIGINAL ARTICLE

Retrospective Study

- 703 Pre-treatment prediction of response to peginterferon plus ribavirin in chronic hepatitis C genotype 3
Marciano S, Borzi SM, Dirchwolf M, Ridruejo E, Mendizabal M, Bessone F, Sirotnsky ME, Giunta DH, Trinks J, Olivera PA, Galdame OA, Silva MO, Fainboim HA, Gadano AC

SYSTEMATIC REVIEWS

- 710 Endotipsitis: A case report with a literature review on an emerging prosthetic related infection
Navaratnam AMD, Grant M, Banach DB

CASE REPORT

- 717 Skin cancer in immunosuppressed transplant patients: Vigilance matters
Unlu O, Roach EC, Okoh A, Olayan M, Yilmaz B, Uzunaslari D, Shatnawei A
- 721 First jejunal artery, an alternative graft for right hepatic artery reconstruction
Aryal B, Komokata T, Kadono J, Motodaka H, Ueno T, Furoi A, Imoto Y

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Angela Peltec, PhD, Assistant Professor, Department of Internal Medicine, Clinic of Gastroenterology and Hepatology, University of Medicine and Pharmacy "Nicolae Testemitanu", Chishinev 2019, Moldova

AIM AND SCOPE

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

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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

INDEXING/ABSTRACTING

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

FLYLEAF

I-IV Editorial Board

EDITORS FOR THIS ISSUE

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

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

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

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

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

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

EDITORIAL OFFICE
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director
World Journal of Hepatology
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@wjnet.com
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>
<http://www.wjnet.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@wjnet.com
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>
<http://www.wjnet.com>

PUBLICATION DATE
April 8, 2015

COPYRIGHT

© 2015 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT

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

INSTRUCTIONS TO AUTHORS

Full instructions are available online at http://www.wjnet.com/1948-5182/g_info_20100316080002.htm

ONLINE SUBMISSION

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

Non-invasive methods for the diagnosis of nonalcoholic fatty liver disease

Marianthi Papagianni, Areti Sofogianni, Konstantinos Tziomalos

Marianthi Papagianni, Areti Sofogianni, Konstantinos Tziomalos, First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, 54636 Thessaloniki, Greece

Author contributions: Papagianni M and Sofogianni A drafted the paper; Tziomalos K revised the draft critically for important intellectual content.

Conflict-of-interest: We have no conflict of interest to declare.

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

Correspondence to: Konstantinos Tziomalos, MD, PhD, First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, Kiriakidi 1, 54636 Thessaloniki, Greece. ktziomalos@yahoo.com
 Telephone: +30-231-994621

Fax: +30-231-994773

Received: November 3, 2014

Peer-review started: November 3, 2014

First decision: December 4, 2014

Revised: December 13, 2014

Accepted: January 15, 2015

Article in press: January 19, 2015

Published online: April 8, 2015

Abstract

Nonalcoholic fatty liver disease (NAFLD) is the commonest chronic liver disease and includes simple steatosis and nonalcoholic steatohepatitis (NASH). Since NASH progresses to cirrhosis more frequently and increases liver-related and cardiovascular disease risk substantially more than simple steatosis, there is a great need to differentiate the two entities. Liver biopsy is the gold standard for the diagnosis of NAFLD but its disadvantages, including the risk of complications

and sampling bias, stress the need for developing alternative diagnostic methods. Accordingly, several non-invasive markers have been evaluated for the diagnosis of simple steatosis and NASH, including both serological indices and imaging methods. The present review summarizes the current knowledge on the role of these markers in the diagnosis of NAFLD. Current data suggest that ultrasound and the fibrosis-4 score are probably the most appealing methods for detecting steatosis and for distinguishing NASH from simple steatosis, respectively, because of their low cost and relatively high accuracy. However, currently available methods, both serologic and imaging, cannot obviate the need for liver biopsy for diagnosing NASH due to their substantial false positive and false negative rates. Therefore, the current role of these methods is probably limited in patients who are unwilling or have contraindications for undergoing biopsy.

Key words: Nonalcoholic steatohepatitis; Steatosis; Fibrosis; Imaging; Nonalcoholic fatty liver disease

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Current data suggest that ultrasound and the fibrosis-4 score are probably the most appealing methods for detecting steatosis and for distinguishing nonalcoholic steatohepatitis from simple steatosis, respectively, because of their low cost and relatively high accuracy. However, currently available methods, both serologic and imaging, cannot obviate the need for liver biopsy for diagnosing nonalcoholic steatohepatitis due to their substantial false positive and false negative rates. Therefore, the current role of these methods is probably limited in patients who are unwilling or have contraindications for undergoing biopsy.

Papagianni M, Sofogianni A, Tziomalos K. Non-invasive methods for the diagnosis of nonalcoholic fatty liver disease.

World J Hepatol 2015; 7(4): 638-648 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/638.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i4.638>

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of hepatic steatosis in the absence of other causes of hepatic fat accumulation^[1-3]. NAFLD includes simple steatosis, steatosis accompanied by varying degrees of inflammation and fibrosis [non-alcoholic steatohepatitis, (NASH)] and cirrhosis^[3]. NAFLD is the commonest chronic liver disease; the prevalence of simple steatosis and NASH in the general population is approximately 20%-30% and 5%-12%, respectively^[4-7]. However, in patients with obesity and type 2 diabetes mellitus (T2DM), NAFLD is substantially more common, affecting up to 70% of patients^[8,9].

Simple steatosis is associated with a relatively low risk for progression to cirrhosis^[10-12]. Moreover, it is unclear whether patients with simple steatosis have increased mortality compared with the general population^[13-15]. On the other hand, approximately 7% of patients with NASH will progress to cirrhosis within 3 years^[10-12]. In addition, several prospective studies showed that NASH is independently associated with increased mortality, from both liver disease-related and cardiovascular causes^[15,16]. Therefore, there is a clear need for differentiating patients with simple steatosis from those with NASH.

Liver biopsy remains the golden standard for the diagnosis of NAFLD and for distinguishing simple steatosis from NASH. However, biopsy is an invasive method carrying a small but not negligible risk of complications^[17,18]. Sampling bias has also been reported in patients with NAFLD and might affect both diagnosis and staging of the disease^[19]. Given these limitations of liver biopsy, several non-invasive markers have been evaluated for the diagnosis of simple steatosis and NASH, including both serological indices and imaging methods. The present review summarizes the current knowledge on the role of these markers in the diagnosis of NAFLD.

SEROLOGIC MARKERS

Serologic markers for detecting hepatic steatosis

Cytokeratin-18 (CK18) is the major intermediate filament protein in the liver and plasma levels of caspase-generated CK18 fragments reflects hepatocellular apoptosis, which is implicated in the pathogenesis of NAFLD^[20-22]. In an early study ($n = 157$ patients from Hong-Kong with biopsy-proven NAFLD), CK18 levels had an area under the receiving-operating characteristics curve (AUROC) 0.90 for detecting steatosis^[20]. However, a very recent large study ($n = 318$) performed in the United States reported a considerably lower AUROC

(0.77)^[21]. Similar results were observed in a smaller cohort from Germany^[22]. Different CK18 fragments reflecting total hepatocellular death do not appear to be more accurate^[20,22] (Table 1).

Fibroblast growth factor 21 (FGF21) is involved in the regulation of glucose and lipid metabolism^[23-26]. Patients with steatosis have elevated FGF21 levels, which also correlate with the degree of steatosis^[23-26]. Moreover, in a recent prospective study, elevated FGF21 levels independently predicted the development of steatosis^[23]. However, in a comparative study, measurement of FGF21 levels was less accurate in diagnosing steatosis than CK18 fragments^[25].

In addition to these isolated markers, several algorithms incorporating multiple clinical and biochemical parameters have been evaluated for the diagnosis of simple steatosis. Perhaps the most promising is the fatty liver index (FLI), which incorporates readily available parameters [body mass index (BMI), waist circumference and serum levels of triglycerides and γ -glutamyl-transpeptidase (GGT)] to detect hepatic steatosis. In a study in the general population, this algorithm had an AUROC of 0.84 for detecting steatosis^[27]. The Lipid Accumulation Product (LAP) is an even simpler algorithm that takes into account gender, waist circumference and fasting triglyceride levels. However, in the same population where the FLI was developed, LAP had a smaller AUROC for identifying steatosis (0.79)^[28]. In addition, the diagnostic accuracy of the FLI was reported to be similar to that of a model including BMI and FGF21 levels^[23].

The Hepatic Steatosis Index is another panel of simple biomarkers [gender, history of T2DM, BMI, alanine transaminase (ALT) and aspartate transaminase (AST)] and had an AUROC of 0.81 for diagnosing NAFLD [defined as presence of fatty liver in ultrasound (US) in the absence of other causes of chronic liver disease] in the derivation study ($n = 5362$ Korean patients)^[29]. However, this algorithm had poor agreement with magnetic resonance spectroscopy (MRS) in the assessment of steatosis^[30]. Finally, the SteatoTest includes levels of α_2 -macroglobulin, apolipoprotein A-I, haptoglobin, total bilirubin, GGT, fasting glucose, triglycerides, cholesterol and ALT adjusted for age, gender and BMI^[31]. In addition to the cost of measuring the parameters included in the SteatoTest, this index has limited sensitivity and specificity for detecting steatosis (69% and 74%, respectively)^[31]. Moreover, the SteatoTest showed poor agreement with MRS in the assessment of steatosis^[30].

In summary, among the serologic markers that have been evaluated for the detection of steatosis, measurement of CK18 levels and the FLI appear to be the most accurate. Since the FLI is inexpensive and readily available in clinical practice, it appears to be more appealing than measuring CK18 levels. However, available data for this algorithm are rather limited and it should be validated in large studies in different populations.

Table 1 Accuracy of the most-well studied serologic markers for detecting steatosis and for differentiating simple steatosis from nonalcoholic steatohepatitis

Marker	AUROC	<i>n</i>	Ref.
Serologic markers for detecting steatosis			
CK18	0.90	157	[20]
	0.77	318	[21]
FLI	0.84	496	[27]
LAP	0.79	588	[28]
Hepatic steatosis index	0.81	5362	[29]
SteatoTest	0.79	69	[31]
Serologic markers for differentiating simple steatosis from nonalcoholic steatohepatitis			
APRI	0.60	190	[34]
CK18	0.82	838	[39]
NAFLD fibrosis score	0.82	733	[43]
Comparative studies of serologic markers for differentiating simple steatosis from nonalcoholic steatohepatitis			
FIB-4	0.86	145	[46]
NAFLD fibrosis score	0.81		
BARD score	0.77		
APRI	0.67		
FIB-4	0.96	165	[47]
NAFLD fibrosis score	0.94		
BARD score	0.84		
FIB-4	0.80	541	[48]
NAFLD fibrosis score	0.77		
BARD score	0.70		
APRI	0.73		
FIB-4	0.87	576	[49]
NAFLD fibrosis score	0.86		
APRI	0.79		
BARD score	0.76		

AUROC: Area under the receiving-operating characteristics curve; AST: Aspartate transaminase; CK18: Cytokeratin-18; FLI: Fatty liver index; LAP: Lipid accumulation product; APRI: AST/platelet ratio index; NAFLD: Non-alcoholic fatty liver disease; FIB-4: Fibrosis-4; BARD: BMI, AST/ALT Ratio, Diabetes; BMI: Body mass index; ALT: Alanine transaminase.

Serologic markers for differentiating simple steatosis from NASH

Isolated markers have limited accuracy for the diagnosis of NASH. Thus, normal ALT levels do not exclude the presence of advanced fibrosis or cirrhosis and ALT levels do not correlate with the severity of fibrosis^[32,33]. Other simple markers, such as the AST/platelet ratio index (APRI), defined as (AST/upper limit of normal AST levels)*100/platelet count, also have very low accuracy (AUROC < 0.60)^[34,35]. In contrast with its satisfactory performance in detecting steatosis, CK18 fragments also have moderate accuracy (AUROC = 0.70-0.83) in the diagnosis of NASH^[20-22,36-38]. Moreover, measurement of CK18 has limited accuracy in distinguishing fibrosis stages^[21,22,37]. Different CK18 fragments reflecting total hepatocellular death do not appear to be more accurate^[20,22]. In a recent meta-analysis of 10 studies in patients with NASH (*n* = 838), uncleaved and caspase-cleaved CK18 fragments had an AUROC of 0.82 and 0.84 for diagnosing NASH, respectively^[39]. A recent study suggested that measuring serum levels of Fas, a regulator of apoptotic death, might improve the ability of CK18 to diagnose NASH but additional studies

are needed to validate these findings^[40]. Another marker used to detect steatosis, FGF21, is elevated in patients with NASH compared with controls^[25]. However, in a comparative study, measurement of FGF21 levels was less accurate in diagnosing NASH than CK18 fragments^[25]. Nevertheless, combining the measurement of FGF21 and CK18 improved the accuracy of CK18 alone^[25].

Given the suboptimal diagnostic performance of isolated markers for distinguishing NASH from simple steatosis, several algorithms that combine different parameters have been developed. Some of these panels include readily available variables. The BMI, AST/ALT ratio, diabetes (BARD) score takes into account BMI, AST/ALT ratio and the presence of T2DM and had a high negative predictive value for excluding advanced fibrosis (stages 3-4) in a population of obese patients from the United States^[41]. This score was validated in a Polish population where it showed similarly high negative predictive value (97%)^[42]. The NAFLD fibrosis score incorporates age, BMI, hyperglycemia (fasting glucose levels ≥ 110 mg/dL or previously diagnosed T2DM), platelet count, albumin and AST/ALT ratio and had an AUROC of 0.82 for detecting advanced fibrosis in the study in United States where it was developed (*n* = 733)^[43]. In a validation study in 162 Chinese patients with NAFLD, this score had 91% negative predicted value and obviated the need for 79% of liver biopsies^[44]. The Nippon score includes gender, age and history of T2DM or hypertension and had an AUROC of 0.78 for detecting severe fibrosis (stages 3-4) in the derivation study (*n* = 182 Japanese patients with biopsy-proven NAFLD)^[45].

Fibrosis-4 (FIB-4) appears to be the most promising simple scoring system for distinguishing NASH from steatosis and incorporates age, AST, ALT and platelet count. Indeed, in a comparative study from the United Kingdom (*n* = 145), FIB-4 had greater AUROC for detecting advanced fibrosis compared with the AST/ALT ratio, NAFLD fibrosis score, BARD score and APRI (0.86, 0.83, 0.81, 0.77 and 0.67, respectively)^[46]. In another study in 165 Caucasian patients with NAFLD, the FIB-4 score had similar accuracy with the NAFLD fibrosis score and greater than the BARD score (AUROC 0.96, 0.94 and 0.84, respectively)^[47]. In a larger study performed in the United States (*n* = 541), FIB-4 again was more predictive of advanced fibrosis than the latter scores, even though reported AUROCs were smaller (0.70-0.80)^[48]. Moreover, accuracies for detecting significant fibrosis (stage 2-4) were even lower (AUROC 0.68-0.75)^[48]. In a more recent large comparative study in 576 Japanese patients with NAFLD, FIB-4 again had better accuracy than the NAFLD fibrosis score, APRI, age/platelet index, AST/ALT ratio, BARD score and Nippon score for the diagnosis of advanced fibrosis (0.87, 0.86, 0.82, 0.81, 0.79, 0.76 and 0.71, respectively)^[49]. In the above-mentioned studies, the sensitivity and

specificity of a cut-off value of 1.30-1.45 of the FIB-4 score for detecting advanced fibrosis was 74%-90% and 64%-88%, respectively, whereas a cut-off value of 3.25 had sensitivity of 26%-40% and specificity of 95%-100%^[46-49]. However, in a retrospective study in 320 Caucasian patients with NAFLD, the NAFLD fibrosis score appeared to be a better indicator of the risk for development of liver-related complications or death than the FIB-4 and BARD scores and the APRI^[50].

Other simple algorithms for the detection of fibrosis have been studied less extensively. FibroMeter NAFLD includes age, weight, platelet count and ferritin, glucose, AST and ALT levels and had better accuracy than the NAFLD fibrosis score and the APRI for detecting significant fibrosis (AUROC 0.91, 0.86 and 0.84, respectively) in a French study ($n = 235$)^[51]. The Koeln-Essen index includes age, AST, AST/ALT ratio and total bilirubin levels and had similar AUROC with the FIB-4 score and the NAFLD fibrosis score (0.97, 0.93 and 0.96, respectively) but greater than the AST/ALT ratio and the BARD score (0.81 and 0.67, respectively) in a German study ($n = 267$)^[52].

In addition to these simple algorithms, other scoring systems incorporate more sensitive but less readily available and therefore more expensive variables. However, the latter scores do not appear to be more accurate in detecting NASH than the simple algorithms. The NAFLD liver fat score includes the history of T2DM or metabolic syndrome and serum ALT, AST and insulin levels and had an AUROC of 0.87 for detecting NAFLD (either simple steatosis or NASH) in the derivation study ($n = 470$ Caucasian patients)^[53]. This score showed similar accuracy in an independent cohort of Caucasian patients with NAFLD^[36]. The NASH score includes AST and fasting insulin levels as well as the patatin-like phospholipase domain containing-3 genotype and had an AUROC of 0.76 in a cohort of 380 obese Caucasian patients^[54]. FibroTest-FibroSURE is composed of $\alpha 2$ -macroglobulin, apolipoprotein A-I, haptoglobin, GGT and total bilirubin levels adjusted for sex and age^[55]. In the derivation study in 267 patients with NAFLD, the FibroTest had an AUROC of 0.81 for detecting significant fibrosis but only 0.59 for differentiating NASH from simple steatosis^[55]. Moreover, a more recent study ($n = 190$ Caucasian patients) reported a considerably smaller AUROC for detecting significant fibrosis (0.59)^[34]. The NashTest includes age, gender, height, weight and serum levels of triglycerides, total cholesterol, ALT, AST, total bilirubin, GGT, $\alpha 2$ -macroglobulin, haptoglobin and apolipoprotein AI and had an AUROC of 0.79 for detecting NASH in the derivation study ($n = 257$ French patients)^[56]. Another composite index developed by Palekar *et al*^[57] includes age, gender, BMI, AST, AST/ALT ratio and hyaluronic acid levels. In the small derivation study performed in the United States ($n = 80$), this index had an AUROC of 0.76 for distinguishing NASH from simple steatosis^[57].

Very few studies compared algorithms based on simple parameters and scoring systems that

incorporate more elaborate variables. The Antwerp NAFLD significant fibrosis score includes waist, AST and fasting C-peptide levels^[58]. In the Caucasian population where it was developed ($n = 313$), it had greater AUROC than the NAFLD fibrosis score, the FIB-4 score, the BARD score and the APRI^[58]. Interestingly, measurement of CK18 levels did not improve the accuracy of this algorithm^[58]. The enhanced liver fibrosis panel (ELF) consists of tissue inhibitor of matrix metalloproteinase 1, hyaluronic acid and aminoterminal peptide of pro-collagen III^[59]. In a pivotal study in 192 patients with NAFLD, ELF had an AUROC of 0.82 for identifying significant fibrosis^[59]. In a subgroup of patients ($n = 91$), the ELF and the NAFLD fibrosis score had comparable AUROCs (0.90 and 0.86, respectively) whereas the combination of the 2 scores marginally increased the AUROC to 0.93^[59]. Another algorithm, the NAFIC score, including serum ferritin, insulin and type IV collagen 7S levels, had an AUROC of 0.85 and 0.78 for distinguishing NASH from simple steatosis in the derivation and validation studies, respectively, in Japanese patients with biopsy-proven NAFLD ($n = 177$ and 442, respectively)^[60]. The AUROC for detecting significant and advanced fibrosis was 0.83 and 0.86, respectively^[60]. In the same study, the NAFIC score had greater AUROC for distinguishing NASH from simple steatosis than the BARD score, the Nippon score, the NAFLD fibrosis score and the score developed by Palekar *et al*^[57] (0.80, 0.63, 0.67, 0.68 and 0.73, respectively).

In summary, a large number of algorithms have been developed for differentiating between simple steatosis and NASH. Among the existing algorithms, the FIB-4 score appears to be the most accurate. Moreover, this algorithm has been validated in several studies and consists of readily available and inexpensive variables. More elaborate and costly algorithms appear to be less accurate than the FIB-4 score but comparative studies are limited.

IMAGING METHODS

Imaging methods for detecting hepatic steatosis

US: US is a widely available and inexpensive method for evaluating the presence of steatosis that does not expose the patient to radiation and allows repeated examinations. However, US has several drawbacks: it is operator-dependent and cannot provide information regarding fibrosis^[61-63]. In US, a diffuse increase in hepatic echogenicity (bright liver) suggests the presence of steatosis^[61]. Additional sonographic features, such as hepatorenal contrast (*i.e.*, the difference in echogenicity between liver and right kidney cortex) or blurring of hepatic vein have similar sensitivity with hepatic echogenicity whereas other characteristics such as portal vein blurring or posterior attenuation have lower sensitivity^[61]. However, the combined evaluation of hepatic echogenicity and portal vein blurring improved the sensitivity of US^[61]. In a recent study in 79 patients

(21 with NAFLD) who underwent both US and liver biopsy, the sensitivity and specificity of the US for detecting macrovesicular steatosis $\geq 5\%$ of total hepatocyte area were 82% and 100%, respectively, but the sensitivity and specificity for detecting microvesicular steatosis were only 59% and 74%, respectively^[61]. In patients with steatosis $\geq 20\%$ of total hepatocyte area, sensitivity increased to 96% for macrovesicular steatosis but only to 67% for microvesicular steatosis; specificity decreased to 98% and 66%, respectively^[61]. In contrast, a larger study in 94 patients with NAFLD reported an AUROC of 0.98 of US for detecting steatosis; the sensitivity and specificity was 92% and 100%, respectively^[64]. In a meta-analysis of 49 studies ($n = 4720$), US had an AUROC of 0.93 for detecting steatosis; the sensitivity and specificity was 85% and 94%, respectively^[65]. Moreover, in 5 small comparative studies ($n = 215$), US was as accurate as computed tomography (CT), magnetic resonance imaging (MRI) and MRS for detecting steatosis and had a sensitivity and specificity of 94% and 80%, respectively^[65].

CT: CT provides an objective evaluation of the presence of steatosis but is more expensive than US and exposes the patient to radiation. Similar to US, CT cannot distinguish NASH from simple steatosis^[63]. In a comparative study, CT was less accurate than dual gradient-echo MRI and MRS for identifying steatosis $\geq 5\%$ whereas the latter 2 methods had similar accuracy (AUROC 0.65, 0.88 and 0.85, respectively)^[66]. Notably, at higher degrees of steatosis ($\geq 30\%$), the accuracy of the 3 methods was similar (AUROC 0.92, 0.99 and 0.91, respectively)^[66]. In the same study, CT was also less accurate than US^[66]. However, other studies reported similar accuracy of MRI, CT and US in assessing steatosis^[63,65].

MRI: In patients with NAFLD, MRI has shown excellent accuracy for detecting steatosis^[67-70], which is similar with the accuracy of MRS^[66,71-73] and superior or similar compared with US and CT^[63,65,74]. However, in patients with advanced fibrosis or cirrhosis, MRI appears to be less reliable for grading steatosis^[67,73]. Compared with CT, MRI has the advantage that it does not expose the patient to radiation and can therefore be used for follow-up. On the other hand, MRI is more expensive than CT, it cannot be performed in patients with claustrophobia and the measurements are affected by hepatic iron deposition, which is frequently present in patients with NAFLD^[75,76]. MRI also does not provide information regarding the presence of fibrosis. Indeed, in a small study in 10 patients with NAFLD, chemical-shift MRI was very accurate in identifying steatosis but could not differentiate between NASH and isolated steatosis^[77]. A larger study in 25 patients with NAFLD also showed that MRI is not useful in distinguishing NASH from simple steatosis^[63].

¹H-magnetic resonance spectroscopy: ¹H-magnetic

resonance spectroscopy is an accurate method for evaluating hepatic steatosis^[66,72,73,78,79]. Furthermore, MRS is operator-independent and fast^[66,78,79]. However, MRS has some important disadvantages, including limited availability and high cost^[66,78,79]. The results might also be affected by respiratory movements, since MRS is a free-breathing method^[66]. Some studies also suggested that advanced fibrosis is also associated with less accurate evaluation of steatosis using MRS^[73]. Claustrophobia and the presence of implanted devices are additional limitations in the use of MRS^[66,78,79].

In summary, the different imaging methods for detecting steatosis appear to have comparable accuracy. Since US is the least expensive, readily available, does not expose the patient to radiation and can be used for repeat evaluations, it appears to represent the most useful imaging method for detecting steatosis.

Imaging methods for differentiating simple steatosis from NASH

Transient elastography: In transient elastography (TE), an M-probe that includes an ultrasonic transducer is used. The transducer is placed above the right lobe of the liver through an intercostal space and produces a vibration that generates a wave, which is transmitted through the skin into the liver. The velocity of the wave correlates directly with liver stiffness. In turn, liver stiffness correlates inversely with the degree of fibrosis^[80-82]. In a large study ($n = 246$ patients with NAFLD), TE had an AUROC of 0.84 and 0.93 for detecting significant and severe fibrosis, respectively^[83]. In the same study, TE was more accurate in identifying fibrosis than APRI, FIB-4 score, NAFLD fibrosis score and BARD score^[83]. Other smaller studies in Caucasian and Japanese patients with NAFLD also reported similarly high accuracies of TE in detecting significant and severe fibrosis^[79,82,84,85] (Table 2).

Liver stiffness evaluation with TE is considered reliable when the interquartile range/median ratio of measurements is ≤ 0.30 ^[86]. Unreliable measurements are more frequent in older subjects and in overweight or obese patients^[83,87,88]. In a large study that analyzed 13369 examinations of liver stiffness using TE (13.7% with NAFLD), 15.8% of the examinations yielded unreliable measurements^[89]. In overweight and obese patients, 24% and 35% of measurements, respectively, were considered unreliable^[89]. Obesity not only hampers the measurement of liver stiffness but also increases liver stiffness independently of the presence of fibrosis^[90]. The presence of steatosis also appears to affect liver stiffness evaluation, particularly in non-cirrhotic patients^[80,81,88]. To overcome these limitations, another probe has been developed, the XL-probe, which provides more reliable measurements in obese patients^[84,87,91,92]. The XL-probe generates a lower frequency (1.75 MHz vs 3.5 MHz with the M-probe) and higher amplitude (3 mm and 2 mm, respectively) vibration resulting in greater measurement depth (3.5-7.5 cm vs 2.5-6.5 cm, respectively) and yields

Table 2 Accuracy of imaging methods for differentiating simple steatosis from nonalcoholic steatohepatitis

Imaging method	AUROC	n	Ref.
Imaging methods for differentiating simple steatosis from nonalcoholic steatohepatitis			
TE	0.84	246	[83]
	0.87	75	[84]
ARFI	0.86	77	[101]
	0.90	172	[100]
RTE	0.85	181	[106]
MRE	0.93	58	[110]
	0.95	142	[111]
Comparative studies of imaging methods for differentiating simple steatosis from nonalcoholic steatohepatitis			
TE	0.99	54	[104]
ARFI	0.97		
TE	0.73	13	[108]
ARFI	0.71		
RTE	0.51		

TE: Transient elastography; ARFI: Acoustic radiation force impulse; RTE: Real-time shear wave elastography; MRE: Magnetic resonance elastography; AUROC: Area under the receiving-operating characteristics curve.

reliable results in approximately 57%-63% of patients with unreliable M-probe measurements^[84,87,92,93]. Therefore, the combined use of both probes enables assessment of liver stiffness in > 90% of patients^[87]. Even though liver stiffness values are lower when the XL probe is used, both probes yield similar results regarding the presence of fibrosis^[84,87,92,94]. However, even when the XL probe is used, both the reliability of measurements and the accuracy of detecting fibrosis are decreasing with the increase of BMI^[87,91,93,95].

Transient elastography can also be used in the evaluation of steatosis by calculating the controlled attenuation parameter (CAP) using an algorithm included in the system. CAP has an AUROC of 0.79-0.93 and 0.76-0.94 in identifying steatosis \geq stage 1 and \geq stage 2, respectively^[79,94,96-98]. In comparative studies, it had similar accuracy with MRS^[79] and better accuracy than US^[97]. CAP also had better or similar accuracy with the FLI and better accuracy than the hepatic steatosis index and the SteatoTest^[97,98]. However, the accuracy of CAP appears to be lower in patients with more advanced fibrosis^[98].

Acoustic radiation force impulse imaging: Acoustic radiation force impulse (ARFI) imaging is an US-based elastography method integrated in conventional US machines where a region of interest in the liver is mechanically excited with an acoustic pulse inducing localized tissue displacement, which results in shear wave propagation^[99]. The velocity of wave propagation correlates with liver stiffness and fibrosis^[100]. In a meta-analysis of 4 early studies in patients with NAFLD ($n = 77$), ARFI imaging had an AUROC of 0.86 for diagnosing both significant and advanced fibrosis^[101]. In a more recent large study in 172 patients with biopsy-diagnosed NAFLD, the AUROC of the method

for detecting advanced fibrosis was 0.90^[100]. Compared with TE, ARFI has similar accuracy but lower rates of measurement failures^[101-104].

Real-time shear wave elastography: Real-time shear wave elastography (RTE) is based on the same principle with TE but provides real-time measurements of liver stiffness^[105]. In a recent study in 181 patients with NAFLD, RTE had an AUROC of 0.85 and 0.88 for detecting advanced and severe fibrosis, respectively^[106]. In the same study, RTE had similar accuracy with the FIB-4 score for detecting all stages of fibrosis and better accuracy than the NAFLD fibrosis score, BARD score and the score developed by Palekar *et al.*^[57]. Smaller studies reported similar accuracy rates^[107]. However, in a small comparative study that included 13 patients with NAFLD, RTE had lower accuracy in detecting advanced fibrosis than TE and ARFI, whereas the latter two methods had comparable accuracy (AUROC 0.51, 0.73 and 0.71, respectively)^[108]. Failure rates are similar with RTE and TE^[105].

Magnetic resonance elastography: Magnetic resonance elastography (MRE) evaluates fibrosis by estimating liver elasticity through the application of mechanical excitation and motion-sensitive magnetic resonance sequences^[109]. In a study in 72 patients with biopsy-proven hepatic fibrosis (8 with NASH), MRE had an AUROC of 0.91, 0.92 and 0.97 for detecting fibrosis stage ≥ 1 , ≥ 2 and ≥ 3 , respectively^[109]. In a more recent study in 58 patients with NAFLD, MRE had an AUROC of 0.93 for discriminating NASH from isolated steatosis^[110]. In a larger study in 142 patients with NAFLD, MRE had superior accuracy for detecting advanced fibrosis than the FIB-4 score, NAFLD fibrosis score, APRI and BARD score (AUROC 0.95, 0.83, 0.79, 0.74 and 0.71, respectively)^[111]. Compared with RTE, MRE has similar accuracy in excluding the presence of fibrosis and lower rates of unreliable measurements^[112].

In summary, among the different imaging methods for distinguishing simple steatosis from NASH, TE has been studied more extensively and appears to be more or equally accurate compared with the other techniques. However, very few studies compared these imaging methods with serological markers and it is unclear whether imaging is more accurate than the less expensive and more widely available serological algorithms.

CONCLUSION

A large number of serologic markers and imaging methods have been evaluated for the diagnosis of simple steatosis and NASH. However, most serologic markers have not been validated in independent cohorts whereas very few studies compared the different imaging methods. Current data suggest that US and the FIB-4 score are probably the most appealing methods

for detecting steatosis and for distinguishing NASH from simple steatosis, respectively, because of their low cost and relatively high accuracy. However, currently available methods, both serologic and imaging, cannot obviate the need for liver biopsy for diagnosing NASH due to their substantial false positive and false negative rates. The current role of these methods is probably limited in patients who are unwilling or have contraindications for undergoing biopsy.

REFERENCES

- Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]
- Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2011; **33**: 525-540 [PMID: 21198708 DOI: 10.1111/j.1365-2036.2010.04556.x]
- Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]
- Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]
- Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, Srishord M. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol* 2011; **9**: 524-530.e1; quiz e60 [PMID: 21440669 DOI: 10.1016/j.cgh.2011.03.020]
- Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]
- Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]
- Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009; **29**: 113-119 [PMID: 18384521 DOI: 10.1111/j.1478-3231.2008.01718.x]
- Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, Day C, Arcaro G. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007; **30**: 1212-1218 [PMID: 17277038 DOI: 10.2337/dc06-2247]
- Wong VW, Wong GL, Choi PC, Chan AW, Li MK, Chan HY, Chim AM, Yu J, Sung JJ, Chan HL. Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. *Gut* 2010; **59**: 969-974 [PMID: 20581244 DOI: 10.1136/gut.2009.205088]
- Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sørensen TI, Becker U, Bendtsen F. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut* 2004; **53**: 750-755 [PMID: 15082596 DOI: 10.1136/gut.2003.019984]
- Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]
- Haring R, Wallaschofski H, Nauck M, Dörr M, Baumeister SE, Völzke H. Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated serum gamma-glutamyl transpeptidase levels. *Hepatology* 2009; **50**: 1403-1411 [PMID: 19670414 DOI: 10.1002/hep.23135]
- Targher G, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G, Arcaro G. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. *Diabetes Care* 2007; **30**: 2119-2121 [PMID: 17519430 DOI: 10.2337/dc07-0349]
- Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]
- Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941 DOI: 10.1053/j.gastro.2005.04.014]
- Myers RP, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver Int* 2008; **28**: 705-712 [PMID: 18433397 DOI: 10.1111/j.1478-3231.2008.01691.x]
- Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 2000; **32**: 477-481 [PMID: 10960438 DOI: 10.1053/jhep.2000.16602]
- Ratzliff V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T, LIDO Study Group. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; **128**: 1898-1906 [PMID: 15940625 DOI: 10.1053/j.gastro.2005.03.084]
- Shen J, Chan HL, Wong GL, Chan AW, Choi PC, Chan HY, Chim AM, Yeung DK, Yu J, Chu WC, Wong VW. Assessment of non-alcoholic fatty liver disease using serum total cell death and apoptosis markers. *Aliment Pharmacol Ther* 2012; **36**: 1057-1066 [PMID: 23066946 DOI: 10.1111/apt.12091]
- Cusi K, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, Ortiz-Lopez C, Hecht J, Feldstein AE, Webb A, Loudon C, Goros M, Tio F. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **60**: 167-174 [PMID: 23973932 DOI: 10.1016/j.jhep.2013.07.042]
- Joka D, Wahl K, Moeller S, Schlue J, Vaske B, Bahr MJ, Manns MP, Schulze-Osthoff K, Bantel H. Prospective biopsy-controlled evaluation of cell death biomarkers for prediction of liver fibrosis and nonalcoholic steatohepatitis. *Hepatology* 2012; **55**: 455-464 [PMID: 21993925 DOI: 10.1002/hep.24734]
- Li H, Dong K, Fang Q, Hou X, Zhou M, Bao Y, Xiang K, Xu A, Jia W. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. *J Hepatol* 2013; **58**: 557-563 [PMID: 23142063 DOI: 10.1016/j.jhep.2012.10.029]
- Li H, Fang Q, Gao F, Fan J, Zhou J, Wang X, Zhang H, Pan X, Bao Y, Xiang K, Xu A, Jia W. Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. *J Hepatol* 2010; **53**: 934-940 [PMID: 20675007 DOI: 10.1016/j.jhep.2010.05.018]
- Shen J, Chan HL, Wong GL, Choi PC, Chan AW, Chan HY, Chim AM, Yeung DK, Chan FK, Woo J, Yu J, Chu WC, Wong VW. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. *J Hepatol* 2012; **56**: 1363-1370 [PMID: 22314419 DOI: 10.1016/j.jhep.2011.12.025]
- Dushay J, Chui PC, Gopalakrishnan GS, Varela-Rey M, Crawley M, Fisher FM, Badman MK, Martinez-Chantar ML, Maratos-Flier E. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. *Gastroenterology* 2010; **139**: 456-463 [PMID: 20451522 DOI: 10.1053/j.gastro.2010.04.054]

- 27 **Bedogni G**, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293 DOI: 10.1186/1471-230X-6-33]
- 28 **Bedogni G**, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. *BMC Gastroenterol* 2010; **10**: 98 [PMID: 20738844 DOI: 10.1186/1471-230X-10-98]
- 29 **Lee JH**, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW, Lee HS. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010; **42**: 503-508 [PMID: 19766548 DOI: 10.1016/j.dld.2009.08.002]
- 30 **Guiu B**, Crevisy-Girod E, Binquet C, Duvillard L, Masson D, Lepage C, Hamza S, Krausé D, Verges B, Minello A, Cercueil JP, Hillon P, Petit JM. Prediction for steatosis in type-2 diabetes: clinico-biological markers versus ¹H-MR spectroscopy. *Eur Radiol* 2012; **22**: 855-863 [PMID: 22101800 DOI: 10.1007/s00330-011-2326-9]
- 31 **Poynard T**, Ratziu V, Naveau S, Thabut D, Charlotte F, Messous D, Capron D, Abella A, Massard J, Ngo Y, Munteanu M, Mercadier A, Manns M, Albrecht J. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005; **4**: 10 [PMID: 16375767 DOI: 10.1186/1476-5926-4-10]
- 32 **Fracanzani AL**, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; **48**: 792-798 [PMID: 18752331 DOI: 10.1002/hep.22429]
- 33 **Mofrad P**, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003; **37**: 1286-1292 [PMID: 12774006 DOI: 10.1053/jhep.2003.50229]
- 34 **Sebastiani G**, Castera L, Halfon P, Pol S, Mangia A, Di Marco V, Pirisi M, Voiculescu M, Bourliere M, Alberti A. The impact of liver disease aetiology and the stages of hepatic fibrosis on the performance of non-invasive fibrosis biomarkers: an international study of 2411 cases. *Aliment Pharmacol Ther* 2011; **34**: 1202-1216 [PMID: 21981787 DOI: 10.1111/j.1365-2036.2011.04861.x]
- 35 **Loaeza-del-Castillo A**, Paz-Pineda F, Oviedo-Cárdenas E, Sánchez-Avila F, Vargas-Vorácková F. AST to platelet ratio index (APRI) for the noninvasive evaluation of liver fibrosis. *Ann Hepatol* 2008; **7**: 350-357 [PMID: 19034235]
- 36 **Musso G**, Gambino R, Durazzo M, Cassader M. Noninvasive assessment of liver disease severity with liver fat score and CK-18 in NAFLD: Prognostic value of liver fat equation goes beyond hepatic fat estimation. *Hepatology* 2010; **51**: 715-717 [PMID: 19821531 DOI: 10.1002/hep.23255]
- 37 **Feldstein AE**, Wiecekowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology* 2009; **50**: 1072-1078 [PMID: 19585618 DOI: 10.1002/hep.23050]
- 38 **Malik R**, Chang M, Bhaskar K, Nasser I, Curry M, Schuppan D, Byrnes V, Afdhal N. The clinical utility of biomarkers and the nonalcoholic steatohepatitis CRN liver biopsy scoring system in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2009; **24**: 564-568 [PMID: 19378390 DOI: 10.1111/j.1440-1746.2008.05731.x]
- 39 **Chen J**, Zhu Y, Zheng Q, Jiang J. Serum cytokeratin-18 in the diagnosis of non-alcoholic steatohepatitis: A meta-analysis. *Hepatol Res* 2014; **44**: 854-862 [PMID: 23834322 DOI: 10.1111/hepr.12197]
- 40 **Tamimi TI**, Elgouhari HM, Alkhouri N, Yerian LM, Berk MP, Lopez R, Schauer PR, Zein NN, Feldstein AE. An apoptosis panel for nonalcoholic steatohepatitis diagnosis. *J Hepatol* 2011; **54**: 1224-1229 [PMID: 21145805 DOI: 10.1016/j.jhep.2010.08.023]
- 41 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]
- 42 **Raszeja-Wyszomirska J**, Szymanik B, Ławniczak M, Kajor M, Chwist A, Milkiewicz P, Hartleb M. Validation of the BARD scoring system in Polish patients with nonalcoholic fatty liver disease (NAFLD). *BMC Gastroenterol* 2010; **10**: 67 [PMID: 20584330 DOI: 10.1186/1471-230X-10-67]
- 43 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Thorneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]
- 44 **Wong VW**, Wong GL, Chim AM, Tse AM, Tsang SW, Hui AY, Choi PC, Chan AW, So WY, Chan FK, Sung JJ, Chan HL. Validation of the NAFLD fibrosis score in a Chinese population with low prevalence of advanced fibrosis. *Am J Gastroenterol* 2008; **103**: 1682-1688 [PMID: 18616651 DOI: 10.1111/j.1572-0241.2008.01933.x]
- 45 **Miyaaki H**, Ichikawa T, Nakao K, Yatsushashi H, Furukawa R, Ohba K, Omagari K, Kusumoto Y, Yanagi K, Inoue O, Kinoshita N, Ishibashi H, Yano M, Eguchi K. Clinicopathological study of nonalcoholic fatty liver disease in Japan: the risk factors for fibrosis. *Liver Int* 2008; **28**: 519-524 [PMID: 17976158 DOI: 10.1111/j.1478-3231.2007.01614.x]
- 46 **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]
- 47 **Demir M**, Lang S, Nierhoff D, Drebbler U, Hardt A, Wedemeyer I, Schulte S, Quasdorff M, Goeser T, Töx U, Steffen HM. Stepwise combination of simple noninvasive fibrosis scoring systems increases diagnostic accuracy in nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2013; **47**: 719-726 [PMID: 23442837 DOI: 10.1097/MCG.0b013e3182819a89]
- 48 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ; Nash Clinical Research Network. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]
- 49 **Sumida Y**, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Fujita K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012; **12**: 2 [PMID: 22221544 DOI: 10.1186/1471-230X-12-2]
- 50 **Angulo P**, Bugianesi E, Björnsson ES, Charatcharoenwithaya P, Mills PR, Barrera F, Haflidadottir S, Day CP, George J. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013; **145**: 782-789.e4 [PMID: 23860502 DOI: 10.1053/j.gastro.2013.06.057]
- 51 **Calès P**, Lainé F, Boursier J, Deugnier Y, Moal V, Oberti F, Hunault G, Rousselet MC, Hubert I, Laafi J, Ducluzeaux PH, Lunel F. Comparison of blood tests for liver fibrosis specific or not to NAFLD. *J Hepatol* 2009; **50**: 165-173 [PMID: 18977552 DOI: 10.1016/j.jhep.2008.07.035]
- 52 **Demir M**, Lang S, Schlattjan M, Drebbler U, Wedemeyer I, Nierhoff D, Kaul I, Sowa J, Canbay A, Töx U, Steffen HM. NIKEI: a new inexpensive and non-invasive scoring system to exclude advanced fibrosis in patients with NAFLD. *PLoS One* 2013; **8**: e58360 [PMID: 23555578 DOI: 10.1371/journal.pone.0058360]
- 53 **Kotronen A**, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstråle M, Groop L, Orho-Melander M, Yki-Järvinen H. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology* 2009; **137**: 865-872 [PMID: 19524579 DOI: 10.1053/j.gastro.2009.06.005]
- 54 **Hyysalo J**, Männistö VT, Zhou Y, Arola J, Kärjä V, Leivonen M,

- Juuti A, Jaser N, Lallukka S, Käkälä P, Venesmaa S, Simonen M, Saltevo J, Moilanen L, Korpi-Hyövalti E, Keinänen-Kiukaanniemi S, Oksa H, Orho-Melander M, Valenti L, Fargion S, Pihlajamäki J, Peltonen M, Yki-Järvinen H. A population-based study on the prevalence of NASH using scores validated against liver histology. *J Hepatol* 2014; **60**: 839-846 [PMID: 24333862 DOI: 10.1016/j.jhep.2013.12.009]
- 55 **Poynard T**, Lebray P, Ingiliz P, Varaut A, Varsat B, Ngo Y, Norha P, Munteanu M, Drane F, Messous D, Bismut FI, Carrau JP, Massard J, Ratzu V, Giordanella JP. Prevalence of liver fibrosis and risk factors in a general population using non-invasive biomarkers (FibroTest). *BMC Gastroenterol* 2010; **10**: 40 [PMID: 20412588 DOI: 10.1186/1471-230X-10-40]
- 56 **Poynard T**, Ratzu V, Charlotte F, Messous D, Munteanu M, Imbert-Bismut F, Massard J, Bonyhay L, Tahiri M, Thabut D, Cadranet JF, Le Bail B, de Ledinghen V. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholic steato hepatitis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 34 [PMID: 17096854 DOI: 10.1186/1471-230X-6-34]
- 57 **Palekar NA**, Naus R, Larson SP, Ward J, Harrison SA. Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2006; **26**: 151-156 [PMID: 16448452 DOI: 10.1111/j.1478-3231.2005.01209.x]
- 58 **Franck SM**, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, Michielsens P, Van Gaal L. Noninvasive assessment of nonalcoholic fatty liver disease in obese or overweight patients. *Clin Gastroenterol Hepatol* 2012; **10**: 1162-1168; quiz e87 [PMID: 22796457 DOI: 10.1016/j.cgh.2012.06.019]
- 59 **Guha IN**, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Aithal GP, Day CP, Rosenberg WM. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008; **47**: 455-460 [PMID: 18038452 DOI: 10.1002/hep.21984]
- 60 **Sumida Y**, Yoneda M, Hyogo H, Yamaguchi K, Ono M, Fujii H, Eguchi Y, Suzuki Y, Imai S, Kanemasa K, Fujita K, Chayama K, Yasui K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Okanoue T. A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 257-268 [PMID: 20842510 DOI: 10.1007/s00535-010-0305-6]
- 61 **Dasarathy S**, Dasarathy J, Khyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J Hepatol* 2009; **51**: 1061-1067 [PMID: 19846234 DOI: 10.1016/j.jhep.2009.09.001]
- 62 **Mathiesen UL**, Franzén LE, Aselius H, Resjö M, Jacobsson L, Foberg U, Frydén A, Bodemar G. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. *Dig Liver Dis* 2002; **34**: 516-522 [PMID: 12236486 DOI: 10.1016/S1590-8658(02)80111-6]
- 63 **Saadeh S**, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 745-750 [PMID: 12198701 DOI: 10.1053/gast.2002.35354]
- 64 **Hamaguchi M**, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, Kato T, Takeda N, Okuda J, Ida K, Kawahito Y, Yoshikawa T, Okanoue T. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol* 2007; **102**: 2708-2715 [PMID: 17894848 DOI: 10.1111/j.1572-0241.2007.01526.x]
- 65 **Hernaez R**, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011; **54**: 1082-1090 [PMID: 21618575 DOI: 10.1002/hep.24452]
- 66 **Lee SS**, Park SH, Kim HJ, Kim SY, Kim MY, Kim DY, Suh DJ, Kim KM, Bae MH, Lee JY, Lee SG, Yu ES. Non-invasive assessment of hepatic steatosis: prospective comparison of the accuracy of imaging examinations. *J Hepatol* 2010; **52**: 579-585 [PMID: 20185194 DOI: 10.1016/j.jhep.2010.01.008]
- 67 **Permutt Z**, Le TA, Peterson MR, Seki E, Brenner DA, Sirlin C, Loomba R. Correlation between liver histology and novel magnetic resonance imaging in adult patients with non-alcoholic fatty liver disease - MRI accurately quantifies hepatic steatosis in NAFLD. *Aliment Pharmacol Ther* 2012; **36**: 22-29 [PMID: 22554256 DOI: 10.1111/j.1365-2036.2012.05121.x]
- 68 **Tang A**, Tan J, Sun M, Hamilton G, Bydder M, Wolfson T, Gamst AC, Middleton M, Brunt EM, Loomba R, Lavine JE, Schwimmer JB, Sirlin CB. Nonalcoholic fatty liver disease: MR imaging of liver proton density fat fraction to assess hepatic steatosis. *Radiology* 2013; **267**: 422-431 [PMID: 23382291 DOI: 10.1148/radiol.12120896]
- 69 **Hatta T**, Fujinaga Y, Kadoya M, Ueda H, Murayama H, Kurozumi M, Ueda K, Komatsu M, Nagaya T, Joshita S, Kodama R, Tanaka E, Uehara T, Sano K, Tanaka N. Accurate and simple method for quantification of hepatic fat content using magnetic resonance imaging: a prospective study in biopsy-proven nonalcoholic fatty liver disease. *J Gastroenterol* 2010; **45**: 1263-1271 [PMID: 20625773 DOI: 10.1007/s00535-010-0277-6]
- 70 **Mennesson N**, Dumortier J, Hervieu V, Milot L, Guillaud O, Scoazec JY, Pilleul F. Liver steatosis quantification using magnetic resonance imaging: a prospective comparative study with liver biopsy. *J Comput Assist Tomogr* 2009; **33**: 672-677 [PMID: 19820490 DOI: 10.1097/RCT.0b013e318199d883]
- 71 **Yokoo T**, Bydder M, Hamilton G, Middleton MS, Gamst AC, Wolfson T, Hassanein T, Patton HM, Lavine JE, Schwimmer JB, Sirlin CB. Nonalcoholic fatty liver disease: diagnostic and fat-grading accuracy of low-flip-angle multiecho gradient-recalled-echo MR imaging at 1.5 T. *Radiology* 2009; **251**: 67-76 [PMID: 19221054 DOI: 10.1148/radiol.2511080666]
- 72 **Noworolski SM**, Lam MM, Merriman RB, Ferrell L, Qayyum A. Liver steatosis: concordance of MR imaging and MR spectroscopic data with histologic grade. *Radiology* 2012; **264**: 88-96 [PMID: 22723561 DOI: 10.1148/radiol.12110673]
- 73 **McPherson S**, Jonsson JR, Cowin GJ, O'Rourke P, Clouston AD, Volp A, Horsfall L, Jothamani D, Fawcett J, Galloway GJ, Benson M, Powell EE. Magnetic resonance imaging and spectroscopy accurately estimate the severity of steatosis provided the stage of fibrosis is considered. *J Hepatol* 2009; **51**: 389-397 [PMID: 19505740 DOI: 10.1016/j.jhep.2009.04.012]
- 74 **Fishbein M**, Castro F, Cheruku S, Jain S, Webb B, Gleason T, Stevens WR. Hepatic MRI for fat quantitation: its relationship to fat morphology, diagnosis, and ultrasound. *J Clin Gastroenterol* 2005; **39**: 619-625 [PMID: 16000931 DOI: 10.1097/00004836-200508000-00012]
- 75 **Bugianesi E**, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**: 179-187 [PMID: 14752836 DOI: 10.1002/hep.20023]
- 76 **Chitturi S**, Weltman M, Farrell GC, McDonald D, Kench J, Liddle C, Samarasinghe D, Lin R, Abeygunasekera S, George J. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. *Hepatology* 2002; **36**: 142-149 [PMID: 12085358 DOI: 10.1053/jhep.2002.33892]
- 77 **Kalra N**, Duseja A, Das A, Dhiman RK, Virmani V, Chawla Y, Singh P, Khandelwal N. Chemical shift magnetic resonance imaging is helpful in detecting hepatic steatosis but not fibrosis in patients with nonalcoholic fatty liver disease (NAFLD). *Ann Hepatol* 2009; **8**: 21-25 [PMID: 19221529]
- 78 **Roldan-Valadez E**, Favila R, Martínez-López M, Uribe M, Ríos C, Méndez-Sánchez N. In vivo 3T spectroscopic quantification of liver fat content in nonalcoholic fatty liver disease: Correlation with biochemical method and morphometry. *J Hepatol* 2010; **53**: 732-737 [PMID: 20594607 DOI: 10.1016/j.jhep.2010.04.018]
- 79 **Karlas T**, Petroff D, Garnov N, Böhm S, Tenckhoff H, Wittekind C, Wiese M, Schiefke I, Linder N, Schaudinn A, Busse H, Kahn T,

- Mössner J, Berg T, Tröltzsch M, Keim V, Wiegand J. Non-invasive assessment of hepatic steatosis in patients with NAFLD using controlled attenuation parameter and 1H-MR spectroscopy. *PLoS One* 2014; **9**: e91987 [PMID: 24637477 DOI: 10.1371/journal.pone.0091987]
- 80 **Ziol M**, Kettaneh A, Ganne-Carrié N, Barget N, Tennghe-Barna I, Beaugrand M. Relationships between fibrosis amounts assessed by morphometry and liver stiffness measurements in chronic hepatitis or steatohepatitis. *Eur J Gastroenterol Hepatol* 2009; **21**: 1261-1268 [PMID: 19478678 DOI: 10.1097/MEG.0b013e32832a20f5]
- 81 **Gaia S**, Carenzi S, Barilli AL, Bugianesi E, Smedile A, Brunello F, Marzano A, Rizzetto M. Reliability of transient elastography for the detection of fibrosis in non-alcoholic fatty liver disease and chronic viral hepatitis. *J Hepatol* 2011; **54**: 64-71 [PMID: 20932598 DOI: 10.1016/j.jhep.2010.06.022]
- 82 **Lupsor M**, Badea R, Stefanescu H, Grigorescu M, Serban A, Radu C, Crişan D, Sparchez Z, Iancu S, Maniu A. Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis. *J Gastrointest Liver Dis* 2010; **19**: 53-60 [PMID: 20361076]
- 83 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462 [PMID: 20101745 DOI: 10.1002/hep.23312]
- 84 **Myers RP**, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, Beaton M, Levstik M, Crotty P, Elkashab M. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. *Hepatology* 2012; **55**: 199-208 [PMID: 21898479 DOI: 10.1002/hep.24624]
- 85 **Yoneda M**, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, Yonemitsu K, Higurashi T, Takahashi H, Kobayashi N, Kirikoshi H, Abe Y, Inamori M, Kubota K, Saito S, Tamano M, Hiraishi H, Maeyama S, Yamaguchi N, Togo S, Nakajima A. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Liver Dis* 2008; **40**: 371-378 [PMID: 18083083 DOI: 10.1016/j.dld.2007.10.019]
- 86 **Boursier J**, Zarski JP, de Lédinghen V, Rousselet MC, Sturm N, Le Bail B, Fouchard-Hubert I, Gallois Y, Oberti F, Bertrais S, Calès P; Multicentric Group from ANRS/HC/EP23 FIBROSTAR Studies. Determination of reliability criteria for liver stiffness evaluation by transient elastography. *Hepatology* 2013; **57**: 1182-1191 [PMID: 22899556 DOI: 10.1002/hep.25993]
- 87 **de Lédinghen V**, Wong VW, Vergniol J, Wong GL, Foucher J, Chu SH, Le Bail B, Choi PC, Chermak F, Yiu KK, Merrouche W, Chan HL. Diagnosis of liver fibrosis and cirrhosis using liver stiffness measurement: comparison between M and XL probe of FibroScan®. *J Hepatol* 2012; **56**: 833-839 [PMID: 22173167 DOI: 10.1016/j.jhep.2011.10.017]
- 88 **Fraquelli M**, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, Colombo M. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968-973 [PMID: 17255218 DOI: 10.1136/gut.2006.111302]
- 89 **Castéra L**, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, Couzigou P, de Lédinghen V. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology* 2010; **51**: 828-835 [PMID: 20063276 DOI: 10.1002/hep.23425]
- 90 **Baba M**, Furuya K, Bandou H, Kasai K, Sadaoka K. Discrimination of individuals in a general population at high-risk for alcoholic and non-alcoholic fatty liver disease based on liver stiffness: a cross section study. *BMC Gastroenterol* 2011; **11**: 70 [PMID: 21669003 DOI: 10.1186/1471-230X-11-70]
- 91 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan AW, Chermak F, Choi PC, Merrouche W, Chu SH, Pesque S, Chan HL, de Lédinghen V. Liver stiffness measurement using XL probe in patients with nonalcoholic fatty liver disease. *Am J Gastroenterol* 2012; **107**: 1862-1871 [PMID: 23032979 DOI: 10.1038/ajg.2012.331]
- 92 **Friedrich-Rust M**, Hadji-Hosseini H, Kriener S, Herrmann E, Sircar I, Kau A, Zeuzem S, Bojunga J. Transient elastography with a new probe for obese patients for non-invasive staging of non-alcoholic steatohepatitis. *Eur Radiol* 2010; **20**: 2390-2396 [PMID: 20526777 DOI: 10.1007/s00330-010-1820-9]
- 93 **Şirli R**, Sporea I, Deleanu A, Culcea L, Szilaski M, Popescu A, Dănilă M. Comparison between the M and XL probes for liver fibrosis assessment by transient elastography. *Med Ultrason* 2014; **16**: 119-122 [PMID: 24791843 DOI: 10.11152/mu.201.3.2066.162.rslis2]
- 94 **Friedrich-Rust M**, Romen D, Vermehren J, Kriener S, Sadet D, Herrmann E, Zeuzem S, Bojunga J. Acoustic radiation force impulse-imaging and transient elastography for non-invasive assessment of liver fibrosis and steatosis in NAFLD. *Eur J Radiol* 2012; **81**: e325-e331 [PMID: 22119555 DOI: 10.1016/j.ejrad.2011.10.029]
- 95 **Myers RP**, Pomier-Layrargues G, Kirsch R, Pollett A, Beaton M, Levstik M, Duarte-Rojo A, Wong D, Crotty P, Elkashab M. Discordance in fibrosis staging between liver biopsy and transient elastography using the FibroScan XL probe. *J Hepatol* 2012; **56**: 564-570 [PMID: 22027584 DOI: 10.1016/j.jhep.2011.10.007]
- 96 **Shen F**, Zheng RD, Mi YQ, Wang XY, Pan Q, Chen GY, Cao HX, Chen ML, Xu L, Chen JN, Cao Y, Zhang RN, Xu LM, Fan JG. Controlled attenuation parameter for non-invasive assessment of hepatic steatosis in Chinese patients. *World J Gastroenterol* 2014; **20**: 4702-4711 [PMID: 24782622 DOI: 10.3748/wjg.v20.i16.4702]
- 97 **de Lédinghen V**, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. *Liver Int* 2012; **32**: 911-918 [PMID: 22672642 DOI: 10.1111/j.1478-3231.2012.02820.x]
- 98 **Myers RP**, Pollett A, Kirsch R, Pomier-Layrargues G, Beaton M, Levstik M, Duarte-Rojo A, Wong D, Crotty P, Elkashab M. Controlled Attenuation Parameter (CAP): a noninvasive method for the detection of hepatic steatosis based on transient elastography. *Liver Int* 2012; **32**: 902-910 [PMID: 22435761 DOI: 10.1111/j.1478-3231.2012.02781.x]
- 99 **Palmeri ML**, Wang MH, Dahl JJ, Frinkley KD, Nightingale KR. Quantifying hepatic shear modulus in vivo using acoustic radiation force. *Ultrasound Med Biol* 2008; **34**: 546-558 [PMID: 18222031 DOI: 10.1016/j.ultrasmedbio.2007.10.009]
- 100 **Palmeri ML**, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, Diehl AM, Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. *J Hepatol* 2011; **55**: 666-672 [PMID: 21256907 DOI: 10.1016/j.jhep.2010.12.019]
- 101 **Friedrich-Rust M**, Nierhoff J, Lupsor M, Sporea I, Fierbinteanu-Braticevici C, Strobel D, Takahashi H, Yoneda M, Suda T, Zeuzem S, Herrmann E. Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis. *J Viral Hepat* 2012; **19**: e212-e219 [PMID: 22239521 DOI: 10.1111/j.1365-2893.2011.01537.x]
- 102 **Ebinuma H**, Saito H, Komuta M, Ojio K, Wakabayashi K, Usui S, Chu PS, Umeda R, Ishibashi Y, Takayama T, Kikuchi M, Nakamoto N, Yamagishi Y, Kanai T, Ohkuma K, Sakamoto M, Hibi T. Evaluation of liver fibrosis by transient elastography using acoustic radiation force impulse: comparison with Fibroscan®. *J Gastroenterol* 2011; **46**: 1238-1248 [PMID: 21779759 DOI: 10.1007/s00535-011-0437-3]
- 103 **Boursier J**, Isselin G, Fouchard-Hubert I, Oberti F, Dib N, Lebilot J, Bertrais S, Gallois Y, Calès P, Aubé C. Acoustic radiation force impulse: a new ultrasonographic technology for the widespread noninvasive diagnosis of liver fibrosis. *Eur J Gastroenterol Hepatol* 2010; **22**: 1074-1084 [PMID: 20440210 DOI: 10.1097/MEG.0b013e328339e0a1]
- 104 **Yoneda M**, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, Saito S, Nakajima A. Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography. *Radiology* 2010;

- 256: 640-647 [PMID: 20529989 DOI: 10.1148/radiol.10091662]
- 105 **Poynard T**, Munteanu M, Luckina E, Perazzo H, Ngo Y, Royer L, Fedchuk L, Sattonnet F, Pais R, Lebray P, Rudler M, Thabut D, Ratzu V. Liver fibrosis evaluation using real-time shear wave elastography: applicability and diagnostic performance using methods without a gold standard. *J Hepatol* 2013; **58**: 928-935 [PMID: 23321316 DOI: 10.1016/j.jhep.2012.12.021]
- 106 **Ochi H**, Hirooka M, Koizumi Y, Miyake T, Tokumoto Y, Soga Y, Tada F, Abe M, Hiasa Y, Onji M. Real-time tissue elastography for evaluation of hepatic fibrosis and portal hypertension in nonalcoholic fatty liver diseases. *Hepatology* 2012; **56**: 1271-1278 [PMID: 22488593 DOI: 10.1002/hep.25756]
- 107 **Orlacchio A**, Bolacchi F, Antonicoli M, Coco I, Costanzo E, Tosti D, Francioso S, Angelico M, Simonetti G. Liver elasticity in NASH patients evaluated with real-time elastography (RTE). *Ultrasound Med Biol* 2012; **38**: 537-544 [PMID: 22341049 DOI: 10.1016/j.ultrasmedbio.2011.12.023]
- 108 **Chung JH**, Ahn HS, Kim SG, Lee YN, Kim YS, Jeong SW, Jang JY, Lee SH, Kim HS, Kim BS. The usefulness of transient elastography, acoustic-radiation-force impulse elastography, and real-time elastography for the evaluation of liver fibrosis. *Clin Mol Hepatol* 2013; **19**: 156-164 [PMID: 23837140 DOI: 10.3350/cmh.2013.19.2.156]
- 109 **Asbach P**, Klatt D, Schlosser B, Biermer M, Muche M, Rieger A, Loddenkemper C, Somasundaram R, Berg T, Hamm B, Braun J, Sack I. Viscoelasticity-based staging of hepatic fibrosis with multifrequency MR elastography. *Radiology* 2010; **257**: 80-86 [PMID: 20679447 DOI: 10.1148/radiol.10092489]
- 110 **Chen J**, Talwalkar JA, Yin M, Glaser KJ, Sanderson SO, Ehman RL. Early detection of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease by using MR elastography. *Radiology* 2011; **259**: 749-756 [PMID: 21460032 DOI: 10.1148/radiol.11101942]
- 111 **Kim D**, Kim WR, Talwalkar JA, Kim HJ, Ehman RL. Advanced fibrosis in nonalcoholic fatty liver disease: noninvasive assessment with MR elastography. *Radiology* 2013; **268**: 411-419 [PMID: 23564711 DOI: 10.1148/radiol.13121193]
- 112 **Yoon JH**, Lee JM, Woo HS, Yu MH, Joo I, Lee ES, Sohn JY, Lee KB, Han JK, Choi BI. Staging of hepatic fibrosis: comparison of magnetic resonance elastography and shear wave elastography in the same individuals. *Korean J Radiol* 2013; **14**: 202-212 [PMID: 23483022 DOI: 10.3348/kjr.2013.14.2.202]

P- Reviewer: Pan JJ, Pan TL, Quarleri J **S- Editor:** Tian YL
L- Editor: A **E- Editor:** Liu SQ



Strategies to improve outcome of patients with hepatocellular carcinoma receiving a liver transplantation

Marta Guerrero-Misas, Manuel Rodríguez-Perálvarez, Manuel De la Mata

Marta Guerrero-Misas, Manuel Rodríguez-Perálvarez, Manuel De la Mata, Department of Hepatology and Liver Transplantation, Reina Sofia University Hospital, Maimónides Institute of Biomedical Research of Córdoba, CIBERehd, 14004 Córdoba, Spain

Author contributions: All authors contributed equally to the manuscript; Guerrero-Misas M and Rodríguez-Perálvarez M performed research and wrote the paper; De la Mata M designed research and contributed new reagents.

Conflict-of-interest: The authors of the present manuscript do not have any conflict of interest to disclose.

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

Correspondence to: Manuel De la Mata, MD, PhD, Head, Department of Hepatology and Liver Transplantation, Reina Sofia University Hospital, Maimónides Institute of Biomedical Research of Córdoba, CIBERehd, Avda/ Menéndez Pidal s/n, 14004 Córdoba, Spain. mdelamatagarcia@gmail.com

Telephone: +34-957-010450

Fax: +34-957-736014

Received: August 25, 2014

Peer-review started: August 25, 2014

First decision: November 27, 2014

Revised: December 15, 2014

Accepted: January 15, 2015

Article in press: January 19, 2015

Published online: April 8, 2015

However, tumour recurrence rates are as high as 20%, and once the recurrence is established the therapeutic options are scarce and with little impact on prognosis. Strategies to minimize tumour recurrence and thus to improve outcome may be classified into 3 groups: (1) An adequate selection of candidates for liver transplantation by using the Milan criteria; (2) An optimized management within waiting list including prioritization of patients at high risk of tumour progression, and the implementation of bridging therapies, particularly when the expected length within the waiting list is longer than 6 mo; and (3) Tailored immunosuppression comprising reduced exposure to calcineurin inhibitors, particularly early after liver transplantation, and the addition of mammalian target of rapamycin inhibitors. In the present manuscript the available scientific evidence supporting these strategies is comprehensively reviewed, and future directions are provided for novel research approaches, which may contribute to the final target: to cure more patients with hepatocellular carcinoma and with an improved long term outcome.

Key words: Hepatocellular carcinoma; Recurrence; Bridging therapy; Milan criteria; Immunosuppression; Liver transplantation

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Liver transplantation is the only therapeutic option which allows to treat both, the hepatocellular carcinoma and the underlying liver disease. However, tumour recurrence rates are 15%-20% with a very poor prognosis. Strategies to minimize tumour recurrence and thus to improve outcome are focused in a careful selection of candidates for liver transplantation, an optimized management within waiting list and a tailored immunosuppression. The available scientific evidence supporting these strategies is reviewed, and future directions are provided for novel research approaches, which may contribute to the final target: to cure more

Abstract

Liver transplantation is the only therapeutic option which allows to treat both, the hepatocellular carcinoma and the underlying liver disease. Indeed, liver transplantation is considered the standard of care for a subset of patients with cirrhosis and hepatocellular carcinoma.

patients with hepatocellular carcinoma with an improved long term outcome.

Guerrero-Misas M, Rodríguez-Perálvarez M, De la Mata M. Strategies to improve outcome of patients with hepatocellular carcinoma receiving a liver transplantation. *World J Hepatol* 2015; 7(4): 649-661 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/649.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i4.649>

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer, which accounts for 70%-80% of the hepatic malignancies^[1]. It generally appears on a cirrhotic liver, and thus the common causes of cirrhosis are also involved in the oncogenesis of HCC, being particularly relevant hepatitis B virus (HBV) and HCV chronic viral infections^[2-4]. It has been estimated that HBV is responsible for 50%-80% of HCC cases, whereas 10%-20% of cases are related to HCV^[1].

HCC represents the fifth most common malignancy (554000 cases worldwide) and the second leading cause of cancer-related mortality, (746000 deaths annually)^[5]. HCC rates are 2-4 fold increased in male population. The incidence is higher in developing countries, although its incidence in developed countries is increasing, mainly due to HCV infection^[6]. Among developed countries the highest incidence rates are found in North America (9.3 cases/100000) and southern Europe (9.5 cases/100000)^[5]. However this picture may change in the next decades given the exponential raising of infant obesity in United States and Europe which may trigger the number of HCCs attributable to non-alcoholic fatty liver disease^[7-9].

Liver Transplantation (LT) is the only therapeutic option able to treat both the HCC and the underlying liver disease, and it is currently considered the standard of care for patients with small unresectable HCC. The proportion of patients diagnosed at early stage, who potentially would benefit from LT, is expected to increase due to the screening programs with ultrasound implemented for patients with chronic liver disease^[10]. Compared to liver resection and locoregional ablative therapies, LT offers an improved long term survival (70% at 5 years). However the tumour recurrence rates after LT are 15%-20%, and the therapeutic options are very limited in this situation^[11].

Current strategies to minimize HCC recurrence after LT are grouped in: (1) Adequate selection of candidates for LT; (2) Waiting list management and bridging locoregional therapies; and (3) Tailored immunosuppression. In the present manuscript the scientific evidence supporting these strategies is comprehensively reviewed, and future directions are drawn in order to improve long term outcome of LT patients with HCC.

SELECTION OF HCC PATIENTS FOR LT

The initial experiences of LT to treat HCC were disappointing since the tumour recurrence was frequent, and survival was shorter when compared with other LT aetiologies (5-year survival rates 18%-40%)^[12]. Many institutions included the HCC as a formal contraindication for LT until the early 90's, when some studies showed that patients with small HCC undergoing LT had reduced tumour recurrence rates when compared to liver resection. Clinicians soon became aware of the improved outcomes when LT was restricted to patients with a limited tumour burden. Indeed in a cohort of 221 consecutive LT patients [38 patients with small HCC (< 5 cm) and 136 with cirrhosis without HCC], the survival rates were similar irrespective of whether HCC was present or not (63% vs 68% at 5 years respectively; $P = 0.84$). In 1996 Mazzaferro *et al.*^[13] prospectively analyzed a cohort of 48 patients with small unresectable HCC undergoing LT. The authors considered LT when there was a single nodule ≤ 5 cm or up to 3 nodules, ≤ 3 cm each, in the absence of macrovascular invasion or extrahepatic metastases. The 4-year survival rate was 75%, with a recurrence-free survival rate of 83%. These premises, currently known as Milan criteria, found a wide acceptance and most LT institutions implemented them as the standard of care to select HCC patients for LT^[10]. However according to the Milan criteria, only 6% of patients with HCC would be eligible for LT^[14], and therefore these criteria may be considered too restrictive. A significant proportion of patients above Milan criteria could still benefit from LT without increasing HCC recurrence rates^[15].

There have been many attempts to expand Milan criteria, being the most frequently used summarized in Table 1. The expansion of the Milan criteria may be performed by increasing the diameter and/or the number of nodules allowed, and in some cases with additional criteria such as histological tumour differentiation, serum PIVKA-II or α -fetoprotein.

One of the most popular criteria is the so called "up-to-seven", according to which patients without macrovascular invasion could be candidates for LT as long as the sum of the number of nodules and the diameter of the largest nodule is ≤ 7 . These criteria were derived from a retrospective multicenter study with 1556 patients with HCC, from which 1070 had a HCC beyond Milan criteria. Patients outside Milan criteria but within the up-to-seven criteria had an overall 5 year-survival rate of 71.2%, similar to those found in patients within Milan criteria (73.3%). HCC recurrence rates were also similar provided that microvascular invasion was absent^[16]. However this study had important limitations. The tumour was evaluated in the explanted liver rather than by using radiological techniques, and a certain grade of disagreement between both approaches is expected in clinical practice. Most importantly, the favorable outcome showed by patients within the up-to-seven criteria was only present after excluding patients

Table 1 Summary of the most relevant criteria used to select candidates with hepatocellular carcinoma for liver transplantation

Ref.	Name of criteria	No. of nodules	Diameter	Tumour different	PIVKA-II	Prospective validation	n	Outcomes within Milan criteria	Outcomes within proposed criteria	P
Mazzaferro <i>et al</i> ^[3]	Milan	1 or ≤ 3	≤ 5 cm or ≤ 3 cm each	-	-	+	48	4-yr survival rate 75% 4-yr recurrence free survival rate 83%	NA	
Mazzaferro <i>et al</i> ^[6]	Up-to-seven	TTN + DLN ≤ 7								
Yao <i>et al</i> ^[8]	UCSF criteria	1 or ≤ 3	≤ 6.5 cm or ≤ 4.5 cm the largest one but with a TTD ≤ 8 cm	-	-	+ ^[19-14]	1556	5-yr survival rate 73.3% 5-yr survival rate 72.4%	5-yr survival rate 71.2% 5-yr survival rate 75.2%	> 0.05 0.87
Herrero <i>et al</i> ^[23]	Universidad de Navarra criteria	1 or 2-3	≤ 6 cm or ≤ 5 cm	-	-	-	47	Not reported	5-yr survival rate 79% 3-yr recurrence free survival rate 70%	-
Lee <i>et al</i> ^[23]	Asan criteria	≤ 6	≤ 5 cm	-	-	-	221	5-yr survival rate 76% 3-yr recurrence rate 13.6%	5-yr survival rate 81.6% 3-yr recurrence rate 9.1%	0.334 0.554
DuBay <i>et al</i> ^[27]	Toronto criteria	No	No	+	-	-	294	5-yr overall survival rate 72% 5-yr disease free survival rate 70%	5-yr overall survival rate 70% 5-yr disease free survival 66%	0.63 0.25
Ito <i>et al</i> ^[24]	Kyoto criteria	≤ 10	≤ 5 cm	-	+	-	125	Not reported	5-yr survival 86.7% 5-yr recurrence 4.9%	-

TTN: Total tumor nodules; DLN: Diameter of the largest nodule; TTD: Total tumor diameter; NA: Not applicable.

with microvascular invasion, which cannot be assessed pre-LT^[17]. Thus the up-to-seven criteria cannot be considered a safe approach to expand Milan criteria.

The UCSF criteria were described in 2001 by Yao *et al*^[18], who prospectively analyzed the outcome of 70 patients with HCC undergoing LT in a single institution. Patients with a solitary tumor ≤ 6.5 cm, or up to 3 nodules, being the largest lesion ≤ 4.5 cm and the total tumor diameter ≤ 8 cm, had 1-year survival rates of 90% (vs 50% for patients exceeding these criteria; *P* = 0.0005). Although the tumour assessment was performed in the explanted liver, the UCSF criteria have been subsequently validated in independent retrospective studies, some of them based on pre-LT imaging techniques^[19-22].

The criteria from the University of Navarra include a single tumor ≤ 6 cm or 2-3 nodules up to 5 cm, without macrovascular invasion or extra-hepatic disease^[23]. The original study included 160 LT patients from a single institution (47 patients had HCC, and 12 were above Milan criteria). There were no differences between patients within Milan criteria and above Milan criteria but within the Navarra criteria. The limitations of this proposal are the limited number of patients included, the retrospective design and the lack of external validation.

In the Kyoto criteria up to 10 nodules, less than 5 cm each, were permitted if serum PIVKA-II was < 400 mU/mL^[24]. These factors were obtained by a multivariate approach in a retrospective study with 125 LT with HCC (70 patients met the Milan criteria, and 55 patients did not). Patients above Milan criteria but within the Kyoto criteria had similar 5-year tumour recurrence rates when compared with patients within the Milan criteria (7.3% vs 9.7%; *P* = 0.87). When serum PIVKA-II, which is a tumour marker related to an aggressive histological behavior of HCC, was included in the selection criteria the patients within Kyoto criteria showed reduced 5-year HCC recurrence rates (4.9%) when compared to patients above Kyoto criteria (60.5%) (*P* = 0.0001). Similarly, 5-year survival rates significantly differed between these groups (86.7% vs 34.4%, respectively; *P* = 0.0001). Similar results were described in independent cohorts^[25,26].

In the Toronto criteria there were no limits in terms of diameter of the main nodule or the number of nodules, provided that extrahepatic metastasis, macrovascular invasion and poor histological differentiation were ruled out, and there was a preserved performance status^[27]. There were similar 5-year survival rates and 5-year disease-free survival rates between the patients within the Milan criteria and patients beyond Milan criteria but within Toronto criteria. Interestingly, a histological parameter was implemented in the Toronto criteria, as it is histological differentiation according to the Edmonson scale^[28]. Far from being a limitation, the addition of histological differentiation may add relevant information^[29,30] and it can be assessed, although with some limitations, in a regular liver biopsy performed before the LT. However most of the patients with HCC are diagnosed by imaging techniques and the liver biopsy would not be performed otherwise. The risk of needle track seeding

after the liver biopsy^[31] casts doubts on recommending this procedure to all patients with HCC before LT. Unfortunately the Toronto criteria have not been validated in independent cohorts.

In the Asan criteria ≤ 6 tumors with a maximum tumor diameter of ≤ 5 cm, and without macrovascular invasion or extra-hepatic involvement were considered^[32]. The original study analyzed the outcome of 221 patients with HCC undergoing LT in a single institution. The 5-year survival rates within Milan criteria were 76%, similar to those found in patients above Milan criteria and within Asan criteria (76.3%) ($P = 0.334$).

Hitherto these attempts to expand Milan criteria had a little impact in clinical practice because of inherent methodological limitations. Further studies are needed to identify the best approach to expand Milan criteria safely. Although there is a general agreement to exclude HCC patients with macrovascular invasion or extrahepatic spreading, the intrahepatic tumour burden allowed is a matter of debate, as they are the additional parameters to be included. The ideal criteria to select candidates with HCC for LT should be based on solid data, and future studies addressing this issue should fulfill the following premises: (1) Enough sample size and statistical power; (2) Criteria based on objective parameters with prognostic capability, easily measured before LT; (3) Cut-off points derived from robust statistical methods; (4) Similar overall and disease free survival rates as Milan criteria; and (5) External validation in a prospective multicentric cohort.

On the other hand the expansion of the Milan criteria should be tempered. A liberal policy would not only impair outcomes, but would also limit the access to LT of patients with other liver diseases, particularly in areas with increased incidence rates of HCC. Thus any attempt to expand Milan criteria need to provide similar long term outcome when compared to other aetiologies for LT, and specific studies will be needed in each area considering the HCC prevalence, the number of donors available, and the impact of this strategy on the waiting list.

The idea of expanding Milan criteria by using only the size of the tumour and the number of nodules is too simplistic. Each series may show a different threshold for the maximum tumour diameter or for the number of nodules permitted, but the results may not be reproducible in other countries, or even in a different institution within the same country. The reason may be that the biological tumour behavior is widely variable between patients with similar HCC burden. There are many surrogate markers related to an aggressive tumour behavior in HCC, which can be categorized into histological and serum markers.

Microvascular invasion (mVI) occurs when the tumour phenotype is sufficiently evolved to degrade extracellular matrix which surround vascular structures, and invades the vascular lumen. HCC cells are then free to metastasize either in a different location of the liver

(multinodular disease), or in other organs (extrahepatic spreading). Thus mVI is a critical hallmark in HCC progression, and the strongest prognostic factor as demonstrated in a metaanalysis of observational studies ($RR = 3.41$ for tumour recurrence and $RR = 2.41$ for mortality at 3 years)^[17]. However the diagnosis of mVI has proven to be difficult even for experienced pathologists with the whole HCC specimen^[33]. The mVI assessment in a regular liver biopsy has not been validated, but implementing this information to the selection of HCC patients for LT would allow to expand safely the Milan criteria^[10,34]. Hitherto there have been many attempts to identify surrogate biomarkers of mVI, including serum markers [*i.e.*, alpha-fetoprotein (AFP), PIVKA-II, neutrophil to lymphocyte ratio]^[35-38], histological markers [*i.e.*, poor differentiation and extranodular growth]^[39-41], and imaging techniques [*i.e.*, presence of capsule in an magnetic resonance imaging, smooth margin in computed tomography scan or positive positron emission tomography]^[42-44], but none of them are reliable enough to impact on the decision-making process. Further studies are needed either to identify new not invasive biomarkers of mVI, or to validate its diagnosis in a regular liver biopsy. There are other histological features related to poor prognosis in HCC patients such as capsular invasion, lymphatic permeation, presence of satellite nodules and tumour differentiation, being the later the only one able to be detected in a liver biopsy before LT. Many studies have shown that patients with poorly differentiated tumours have increased risk of recurrence and reduced survival rates^[39-41].

Among serum markers, AFP is the most widely used. Monitoring AFP levels was used in the past as an screening to detect early HCC in patients with chronic liver disease, but in the most recent guidelines the only recognized screening technique was liver ultrasound^[10]. AFP was abandoned because of its suboptimal sensitivity. In patients with HCC candidates to LT there is controversy about what is the best threshold to exclude a patient from the waiting list^[45,46]. In addition AFP serum levels may be modified within waiting list by the use of locoregional therapies such as transarterial chemoembolization (TACE)^[47]. Serum PIVKA-II, also known as Des-gamma-carboxyprothrombin, is preferred to AFP in some LT institutions, particularly in eastern countries, because of an increased accuracy reported in some studies^[48]. However AFP appears more sensitive than PIVKA-II for early HCC^[49]. Increased serum PIVKA-II concentrations are found in patients with more advanced HCC, and in patients with mVI^[50-52]. The combination of PIVKA-II and AFP provided increased accuracy than any of them alone^[53]. Other biomarkers related to systemic inflammation such as neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and inflammation-based index have been associated with poor survival in HCC^[54,55], but their role in predicting HCC recurrence after LT is controversial^[29,56,57], probably because these parameters are highly influenced by

many environmental factors different from tumour progression.

The combination of demographic features, the underlying liver disease, tumour burden, histological characteristics and serum biomarkers by using novel multivariate approaches allowing to manage an increased amount of information such as machine learning classifiers or artificial neural networks, which have already proven their utility in other LT scenarios^[58], might be the key for a safe expansion of the Milan criteria.

WAITING LIST MANAGEMENT AND BRIDGING THERAPY

The shortage of donors is universal and causes an imbalance between candidates for LT and number of organs available. The patients included in the waiting list should face a risk of drop-out, either because of death or due to a significant worsening of the underlying liver disease. In patients with HCC the drop-out is usually related to tumour progression. The waiting list management should be programmed carefully, and adapted to each clinical scenario in order to guarantee an equal access to LT for patients with different aetiologies of liver disease.

From 2002 the Model for end stage liver disease (MELD) was widely accepted as the standard of care to predict short term survival within waiting list, and has been adopted to prioritize the sickest patients for LT. However the MELD score does not reflect the risk of HCC progression. The proposal to palliate this problem consisted in adding extra-MELD points on an empirical basis according to the time within the waiting list. First experiences resulted in an increased benefit to patients with HCC, with more patients transplanted, decreased waiting list mortality and drop-out rates^[59,60]. Indeed this was a very positive picture for HCC patients but it was linked to an imbalance in the access to LT between HCC patients and patients with other liver diseases^[59-63].

A moderate delay within the waiting list would allow for a better selection of HCC candidates for LT according to some authors. The patients with the most aggressive tumours would experience an early tumour progression and they would not be transplanted. Indeed a recent analysis of a nationwide American database showed that a longer waiting time for LT resulted in improved survival rates after LT for HCC patients, while the disparities in the access to LT among different aetiologies were reduced^[64]. The optimal balance between the length within waiting list and the outcome after LT for HCC patients has not been established yet. The current allocation policy for patients with HCC is to add extra-MELD points only when there is a significant risk of drop-out (*i.e.*, T2 HCC stage). This may palliate the problem but it is far from solving it. A recent study used Monte-Carlo simulations and multiple logistic regression to calculate a corrected MELD score for HCC patients. The so called HCC-MELD formula included also

AFP and provided the same priority for HCC patients as the equivalent of conventional MELD score did for other LT aetiologies^[65]. The main limitations were the lack of consideration of tumour volume at listing and tumour progression within the waiting list. In addition changes in AFP after bridging therapies may decrease the HCC-MELD score in patients with positive response to therapy, and these patients have shown particularly reduced tumour recurrence rates.

Neoadjuvant locoregional therapies are recommended when the expected time to LT is longer than 6 mo in order to prevent drop-out and increase long term survival, while minimizing the risk of tumour recurrence after LT^[23,66-72]. However many LT institutions treat most of the patients within the waiting list, since the actual time to LT may be unpredictable, and this strategy has demonstrated a favourable cost-effect balance^[73]. The radiological response to bridging therapy may also help to assess the HCC biological behavior^[74-76], and to prioritize HCC patients for LT^[77-81]. Patients with tumour growth beyond Milan criteria after locoregional therapies should not undergo LT.

There are different modalities of locoregional therapies to be used as bridging for LT. The most frequently applied are liver resection (LR), TACE and radiofrequency ablation (RFA). None of these therapies have shown to be superior to the others and the selection should be tailored according to the BCLC schema^[72]. LR can be used as a first line-bridging therapy procedure to LT in experienced units, with a morbidity of 39%, and an early mortality rate of 3%^[82]. The main advantage of LR is that the whole HCC specimen will be available for histological examination. The information coming from the histology is very valuable as noted above, and may serve to identify predictors of poor outcome. In the presence of these factors the tumour recurrence is almost universal and many LT teams include high-risk patients within waiting list for LT immediately after LR. Other authors would consider LT only in patients with tumour recurrence after LR (salvage LT), but this strategy may result in worse survival rates and increased recurrence rates, unless a careful selection of cases is carried out^[83-87].

TACE is the most frequently used locoregional bridging therapy for LT. It has been hypothesized an increased risk of arterial and biliary complications after LT in patients with a previous TACE due to an endothelial damage, but this was not confirmed in a recent study with 456 HCC transplanted patients^[88]. The use of TACE with drug eluting beads has improved the performance of the technique with complete tumour necrosis rates as high as 76.2%, and with a better safety profile^[67,89]. In spite of this, RFA is preferred for single tumours less than 5 cm^[80]. The available studies comparing RFA vs TACE suggested that complete response is more frequent with RFA, while drop-out rates are diminished^[80,90]. In addition, RFA is a safer procedure with reduced rates of adverse events (4.6%)^[91,92]. However the new protocols of TACE with drug eluting

beads have not been tested against RFA in a randomized fashion. The heterogeneity in reporting outcome and in the inclusion criteria among the available studies make impossible to perform pooled data analysis, and no recommendation can be made of which is the optimal bridging protocol in HCC patients candidates for LT. Other locoregional therapies have been evaluated with promising results (*i.e.*, percutaneous ethanol injection, percutaneous laser ablation, microwave ablation, and radioembolization)^[72,91,93], but further studies are needed to confirm their utility, and to describe which patients may benefit the most of these novel approaches.

Sorafenib is an oral multi-tyrosine kinase inhibitor with antiangiogenic properties which has shown to prolong survival in patients with advanced HCC^[94]. The role of sorafenib in the LT setting has been nicely reviewed by Castelli *et al.*^[95]. Theoretically, sorafenib would be used as an adjuvant therapy to locoregional ablation to reduce tumour recurrence after LT, and this approach is thought to be cost-effective for T2 HCC patients^[96]. However the antiangiogenic effects of sorafenib could be deleterious in the perioperative period, and important safety concerns were arisen in the available series including biliary complications and hepatic artery thrombosis^[97]. The combination of radioembolization and sorafenib as bridging for LT was poorly tolerated in a pilot prospective study with 23 patients, and the risk of biliary complications after LT was enhanced^[98,99]. The combination of sorafenib with locoregional therapies as bridging for LT should not be recommended.

STRATEGIES TO IMPROVE OUTCOME AFTER LIVER TRANSPLANTATION

Despite a careful selection of candidates for LT by the Milan criteria, and an optimization of bridging locoregional therapies within the waiting list, HCC recurrence rates are 15%-20%^[100]. In addition pre-LT imaging techniques may lead to misdiagnosis either by not detecting HCC nodules (incidental HCC), or by inducing a wrong staging, which usually means patients transplanted above Milan criteria and increased tumour recurrence rates^[16]. Even in some patients fulfilling Milan criteria the histological evaluation shows features of poor prognosis such as mVI, poor differentiation or capsular invasion. In these situations the implementation of post-LT strategies to minimize HCC recurrence may be the only option to improve outcome^[101].

The whole concept of tumour recurrence requires that a remnant of circulating HCC cells should be left behind after the LT, and remained unnoticed by the immune system. The use of immunosuppressive drugs after LT is needed to prevent the consequences of acute cellular rejection, including chronic rejection and graft loss^[102]. In normal conditions the immune system is able

to detect tumour cells and to destroy them^[103]. However the use of high doses of immunosuppressants may abolish the immune surveillance in the early post-LT period^[104-108], as happens in other immunosuppressive conditions such as HIV chronic infection^[109].

In LT patients with HCC the relationship between immunosuppression and tumour recurrence is poorly understood, but it is attracting more attention in the recent years. However the variability in the immunosuppression protocols among different institutions make it difficult to design studies addressing this issue with reduced risk of bias^[106]. The current evidence is mainly based in observational studies, most of them retrospective and with a limited sample size, and thus their results should be taken with caution. Among immunosuppressive drugs used in LT patients, only calcineurin inhibitors and mammalian target of rapamycin (mTOR) inhibitors have shown to influence HCC recurrence, increasing and decreasing the risk respectively. Azathioprine is preferred in some centres for patients transplanted with HCV claiming for amelioration of viral recurrence and prevention of graft loss^[110]. Long term use of azathioprine increases the risk of non-melanoma skin cancer^[111] and lymphoma, the later when high doses are used especially in elderly patients^[112], but there is no proved role on HCC. On the other hand mycophenolate seems to be protective against malignancy in the transplant population^[113,114] but again there is no evidence supporting any significant effect on HCC. With regard to induction agents, the anti-interleukin-2 receptor basiliximab does not increase the risk of cancer, but anti-thymocyte globulins have been associated with an increased risk of lymphoma^[115].

The use of calcineurin inhibitors, which are the mainstay of immunosuppression protocols after LT, is able to activate proto-oncogenes and pathways of cancer in a dose-dependent fashion such as transforming growth factor beta, thus promoting tumour proliferation, resistance to apoptosis and metastasis^[116,117]. In a retrospective study with 70 LT patients receiving cyclosporine, the drug exposure calculated with the trapezoidal rule was increased in patients with HCC recurrence (trough concentrations 278.3 ng/mL vs 169.9 ng/mL; $P < 0.001$)^[118]. However there were only 7 patients with HCC recurrence in this cohort, and it was not possible to control for confounding factors. In another study from the same group, 139 LT patients with HCC were analyzed, being 60 patients under tacrolimus and 79 patients under cyclosporine^[119]. The rates of HCC recurrence were increased in patients with higher exposure to calcineurin inhibitors defined as tacrolimus > 10 ng/mL or cyclosporine > 220 ng/mL (RR = 4.01; $P = 0.014$). However the wide interval of recruitment with patients transplanted before and after the implementation of the Milan criteria, the heterogeneous length of drug exposure considered for each patient, and the lack of control for concomitant immunosuppression weakened the

conclusions. Another study with 219 LT patients from two European institutions evaluated the exposure to calcineurin inhibitors within the first month after LT with respect to HCC recurrence^[120]. After controlling for possible confounding factors such as tumour features and concomitant immunosuppression, the increased exposure to calcineurin inhibitors within the first month after LT (tacrolimus > 10 ng/mL or cyclosporine > 300 ng/mL) was an independent predictor of HCC recurrence (RR = 2.82; $P = 0.005$), either if the patient was within or above Milan criteria. The exposure to calcineurin inhibitors after the first month post-LT was similar in patients with and without tumour recurrence, highlighting the early post-LT period as one in which the minimization of calcineurin inhibitors should be encouraged.

The mTOR is a serine/threonine kinase involved in cellular growth, proliferation, metabolism and angiogenesis. The mTOR pathway is up-regulated in approximately half of patients with HCC^[121]. The mTOR inhibitors sirolimus and everolimus have shown anti-cancer properties in animal models including HCC^[121-123]. The mTOR inhibitors are able to prevent acute cellular rejection after LT, and allow for reducing the exposure to calcineurin inhibitors, and thus acting as renal sparing agents^[124]. Regarding prevention of HCC recurrence there are five retrospective studies with sirolimus^[125-129], whose results have been recently summarized in two meta-analyses^[130,131] with the same conclusion: sirolimus may protect against HCC recurrence after LT, and patients treated with sirolimus showed improved overall survival rates. However the level of evidence is poor. These studies are heterogeneous, retrospective, and with an increased risk of reporting bias. There are no randomized controlled trials evaluating the role of mTOR inhibitors in preventing HCC recurrence. The SILVER study is a multicentre randomized controlled trial which preliminary results are expected to be available in 2016, and may shed some light in the actual role of sirolimus in LT patients with HCC^[132]. The major concern with sirolimus relies in its safety profile. A large phase II randomized trial ($n = 222$) evaluated *de novo* sirolimus and reduced tacrolimus after liver transplantation compared with a control arm composed by conventional tacrolimus^[133]. The study had to be prematurely stopped due to an imbalance of adverse outcomes between groups. Patients under sirolimus experienced increased rates of graft failure (26.4% vs 12.5%) and mortality (20% vs 8% at 24 mo; $P = 0.010$), and a trend towards more hepatic artery thrombosis (8.3% vs 2.7%; $P = 0.065$). In addition the analysis of the Scientific Registry of Transplant Recipients (SRTR) database ($n = 26414$) showed an increased risk of all-cause mortality in patients with hepatitis C treated with sirolimus (HR = 1.29; 95%CI: 1.08-1.55)^[134]. Everolimus has a selective effect on mTOR complex 1, and it has been proposed to be more potent than sirolimus^[135], and with an improved metabolic profile^[136]. Unfortunately

the evidence linking everolimus and HCC recurrence after LT is lacking. In the current scenario the systematic use of mTOR inhibitors after LT to prevent HCC recurrence may not be justified, but selected patients either with features of poor prognosis in the explanted liver (*i.e.*, above Milan criteria, poor tumour differentiation and/or microvascular invasion), or with up-regulated mTOR pathway may benefit of combining early tacrolimus minimization and *de novo* everolimus. Future randomized controlled trials should evaluate the convenience, efficacy and safety of this approach.

Sorafenib have shown to delay HCC recurrence and metastasis after LT in a rat model^[137]. In a prospective not randomized pilot study, 7 patients with HCC above Milan criteria were treated with sorafenib after LT, and compared with 12 matched historical controls in whom sorafenib had not been used^[138]. Sorafenib was well tolerated with no severe adverse effects and there was a trend to less HCC recurrence in the group treated with sorafenib (29% vs 75%; $P = 0.07$). The combination of sorafenib and mTOR inhibitors should be avoided because of increased risk of severe adverse events^[95]. At any rate these are very early experiences and no further recommendations should be derived until larger randomized controlled trials are performed.

CONCLUSION

The efforts to improve outcome of patients with HCC undergoing LT should be driven to prevent tumour recurrence by combining the following approaches: (1) Adequate selection of candidates for LT by using Milan criteria. A moderate expansion of the Milan criteria may be possible without a significant increase in HCC recurrence rates, but this expansion should be based in objective criteria strongly associated with the biological tumour behavior; (2) Optimization in waiting-list management. Bridging locoregional therapies should be used whenever possible to prevent drop-out and to minimize HCC recurrence after LT, particularly when the expected time to LT is longer than 6 mo. The best protocol to be used remains as a matter of debate; and (3) Tailored immunosuppression protocols: Currently, early minimization of calcineurin inhibitors combined with an mTOR inhibitor may be the most rationale schema, but specific randomized controlled trials are needed for a general recommendation.

Taken as a whole the scientific evidence regarding strategies to prevent HCC recurrence after LT needs to be strengthened. Research projects addressing this issue face important caveats such as the increased sample size needed, prolonged length of recruitment and follow up of patients, and increased costs. Further studies are needed to identify non-invasive biomarkers of HCC with prognostic capability, to establish the optimal management within waiting list, and to develop new immunosuppressive drugs with antiproliferative properties, able to prevent tumour recurrence in high-

risk patients.

ACKNOWLEDGMENTS

We would like to thank Encarna Díaz Sillero and Maribel Gómez Nuñez for their continuous effort in improving medical care, and to make possible the clinical research within our department.

REFERENCES

- Rosenthal GE, Mettler G, Pare S, Riegger M, Ward M, Landefeld CS. A new diagnostic index for predicting cervical infection with either Chlamydia trachomatis or Neisseria gonorrhoeae. *J Gen Intern Med* 2010; **5**: 319-326 [PMID: 2115576 DOI: 10.1634/theoncologist.2010-S4-05]
- Raimondi S, Bruno S, Mondelli MU, Maisonneuve P. Hepatitis C virus genotype 1b as a risk factor for hepatocellular carcinoma development: a meta-analysis. *J Hepatol* 2009; **50**: 1142-1154 [PMID: 19395111 DOI: 10.1016/j.jhep.2009.01.019]
- Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol* 2008; **48**: 335-352 [PMID: 18096267 DOI: 10.1016/j.jhep.2007.11.011]
- El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34 [PMID: 15508094 DOI: 10.1053/j.gastro.2004.09.013]
- World Health Organizations. Globocan 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. [Cited 2014 July 24]. Available from: URL: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx
- Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0S0140-6736(11)61347-0]
- Haslam DW, James WP. Obesity. *Lancet* 2005; **366**: 1197-1209 [PMID: 16198769 DOI: 10.1016/S0140-6736(05)67483-1]
- Sun B, Karin M. Obesity, inflammation, and liver cancer. *J Hepatol* 2012; **56**: 704-713 [PMID: 22120206 DOI: 10.1016/j.jhep.2011.09.020]
- Rocchini AP. Childhood obesity and a diabetes epidemic. *N Engl J Med* 2002; **346**: 854-855 [PMID: 11893799 DOI: 10.1056/NEJM200203143461112]
- European Association For Research Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438]
- Galuppo R, McCall A, Gedaly R. The role of bridging therapy in hepatocellular carcinoma. *Int J Hepatol* 2013; **2013**: 419302 [PMID: 24455285 DOI: 10.1155/2013/419302]
- Mazzaferro V, Chun YS, Poon RT, Schwartz ME, Yao FY, Marsh JW, Bhoori S, Lee SG. Liver transplantation for hepatocellular carcinoma. *Ann Surg Oncol* 2008; **15**: 1001-1007 [PMID: 18236119 DOI: 10.1245/s10434-007-9559-5]
- Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
- Ulahannan SV, Duffy AG, McNeel TS, Kish JK, Dickie LA, Rahma OE, McGlynn KA, Greten TF, Altekruse SF. Earlier presentation and application of curative treatments in hepatocellular carcinoma. *Hepatology* 2014; **60**: 1637-1644 [PMID: 24996116 DOI: 10.1002/hep.27288]
- Silva MF, Sherman M. Criteria for liver transplantation for HCC: what should the limits be? *J Hepatol* 2011; **55**: 1137-1147 [PMID: 21718672 DOI: 10.1016/j.jhep.2011.05.012]
- Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
- Rodríguez-Perálvarez M, Luong TV, Andreana L, Meyer T, Dhillon AP, Burroughs AK. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. *Ann Surg Oncol* 2013; **20**: 325-339 [PMID: 23149850 DOI: 10.1245/s10434-012-2513-1]
- Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]
- Yao FY, Bass NM, Nikolai B, Davern TJ, Kerlan R, Wu V, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: analysis of survival according to the intention-to-treat principle and dropout from the waiting list. *Liver Transpl* 2002; **8**: 873-883 [PMID: 12360427 DOI: 10.1053/jlts.2002.34923]
- Yao FY, Xiao L, Bass NM, Kerlan R, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007; **7**: 2587-2596 [PMID: 17868066 DOI: 10.1111/j.1600-6143.2007.01965.x]
- Duffy JP, Vardanian A, Benjamin E, Watson M, Farmer DG, Ghobrial RM, Lipshutz G, Yersiz H, Lu DS, Lassman C, Tong MJ, Hiatt JR, Busuttil RW. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg* 2007; **246**: 502-509; discussion 509-511 [PMID: 17717454]
- Chen JW, Kow L, Verran DJ, McCall JL, Munn S, Balderson GA, Fawcett JW, Gow PJ, Jones RM, Jeffrey GP, House AK, Strasser SI. Poorer survival in patients whose explanted hepatocellular carcinoma (HCC) exceeds Milan or UCSF Criteria. An analysis of liver transplantation in HCC in Australia and New Zealand. *HBP (Oxford)* 2009; **11**: 81-89 [PMID: 19590628 DOI: 10.1111/j.1477-2574.2009.00022.x]
- Herrero JL, Sangro B, Quiroga J, Pardo F, Herraiz M, Cienfuegos JA, Prieto J. Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2001; **7**: 631-636 [PMID: 11460231 DOI: 10.1053/jlts.2001.25458]
- Ito T, Takada Y, Ueda M, Haga H, Maetani Y, Oike F, Ogawa K, Sakamoto S, Ogura Y, Egawa H, Tanaka K, Uemoto S. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl* 2007; **13**: 1637-1644 [PMID: 18044766 DOI: 10.1002/lt.21281]
- Kaido T, Ogawa K, Mori A, Fujimoto Y, Ito T, Tomiyama K, Takada Y, Uemoto S. Usefulness of the Kyoto criteria as expanded selection criteria for liver transplantation for hepatocellular carcinoma. *Surgery* 2013; **154**: 1053-1060 [PMID: 24074704 DOI: 10.1016/j.surg.2013.04.056]
- Fujiki M, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, Uemoto S. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant* 2009; **9**: 2362-2371 [PMID: 19656125 DOI: 10.1111/j.1600-6143.2009.02783.x]
- DuBay D, Sandroussi C, Sandhu L, Cleary S, Guba M, Cattral MS, McGilvray I, Ghanekar A, Selzner M, Greig PD, Grant DR. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann Surg* 2011; **253**: 166-172 [PMID: 21294289 DOI: 10.1097/SLA.0b013e31820508f1]
- Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer* 1954; **7**: 462-503 [PMID: 13160935]
- Bertuzzo VR, Cescon M, Ravaioli M, Grazi GL, Ercolani G, Del Gaudio M, Cucchetti A, D'Errico-Grigioni A, Golfieri R, Pinna

- AD. Analysis of factors affecting recurrence of hepatocellular carcinoma after liver transplantation with a special focus on inflammation markers. *Transplantation* 2011; **91**: 1279-1285 [PMID: 21617590 DOI: 10.1097/TP.0b013e3182187cf0]
- 30 **Bhangui P**, Vibert E, Majno P, Salloum C, Andreani P, Zocrato J, Ichai P, Saliba F, Adam R, Castaing D, Azoulay D. Intention-to-treat analysis of liver transplantation for hepatocellular carcinoma: living versus deceased donor transplantation. *Hepatology* 2011; **53**: 1570-1579 [PMID: 21520172 DOI: 10.1002/hep.24231]
 - 31 **Silva MA**, Hegab B, Hyde C, Guo B, Buckels JA, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut* 2008; **57**: 1592-1596 [PMID: 18669577 DOI: 10.1136/gut.2008.149062]
 - 32 **Lee SG**, Hwang S, Moon DB, Ahn CS, Kim KH, Sung KB, Ko GY, Park KM, Ha TY, Song GW. Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. *Liver Transpl* 2008; **14**: 935-945 [PMID: 18581465 DOI: 10.1002/lt.21445]
 - 33 **Fan L**, Mac MT, Frishberg DP, Fan X, Dhall D, Balzer BL, Geller SA, Wang HL. Interobserver and intraobserver variability in evaluating vascular invasion in hepatocellular carcinoma. *J Gastroenterol Hepatol* 2010; **25**: 1556-1561 [PMID: 20796155 DOI: 10.1111/j.1440-1746.2010.06304.x]
 - 34 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762]
 - 35 **McHugh PP**, Gilbert J, Vera S, Koch A, Ranjan D, Gedaly R. Alpha-fetoprotein and tumour size are associated with microvascular invasion in explanted livers of patients undergoing transplantation with hepatocellular carcinoma. *HPB (Oxford)* 2010; **12**: 56-61 [PMID: 20495646 DOI: 10.1111/j.1477-2574.2009.00128.x]
 - 36 **Eguchi S**, Takatsuki M, Hidaka M, Soyama A, Tomonaga T, Muraoka I, Kanematsu T. Predictor for histological microvascular invasion of hepatocellular carcinoma: a lesson from 229 consecutive cases of curative liver resection. *World J Surg* 2010; **34**: 1034-1038 [PMID: 20127241 DOI: 10.1007/s00268-010-0424-5]
 - 37 **Pawlik TM**, Delman KA, Vauthey JN, Nagorney DM, Ng IO, Ikai I, Yamaoka Y, Belghiti J, Lauwers GY, Poon RT, Abdalla EK. Tumor size predicts vascular invasion and histologic grade: Implications for selection of surgical treatment for hepatocellular carcinoma. *Liver Transpl* 2005; **11**: 1086-1092 [PMID: 16123959 DOI: 10.1002/lt.20472]
 - 38 **Kaibori M**, Ishizaki M, Matsui K, Kwon AH. Predictors of microvascular invasion before hepatectomy for hepatocellular carcinoma. *J Surg Oncol* 2010; **102**: 462-468 [PMID: 20872949 DOI: 10.1002/jso.21631]
 - 39 **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]
 - 40 **Kim BK**, Han KH, Park YN, Park MS, Kim KS, Choi JS, Moon BS, Chon CY, Moon YM, Ahn SH. Prediction of microvascular invasion before curative resection of hepatocellular carcinoma. *J Surg Oncol* 2008; **97**: 246-252 [PMID: 18095300 DOI: 10.1002/jso.20953]
 - 41 **Esnaola NF**, Lauwers GY, Mirza NQ, Nagorney DM, Doherty D, Ikai I, Yamaoka Y, Regimbeau JM, Belghiti J, Curley SA, Ellis LM, Vauthey JN. Predictors of microvascular invasion in patients with hepatocellular carcinoma who are candidates for orthotopic liver transplantation. *J Gastrointest Surg* 2002; **6**: 224-232; discussion 232 [PMID: 11992808]
 - 42 **Witjes CD**, Willemssen FE, Verheij J, van der Veer SJ, Hansen BE, Verhoef C, de Man RA, Ijzermans JN. Histological differentiation grade and microvascular invasion of hepatocellular carcinoma predicted by dynamic contrast-enhanced MRI. *J Magn Reson Imaging* 2012; **36**: 641-647 [PMID: 22532493 DOI: 10.1002/jmri.23681]
 - 43 **Kornberg A**, Küpper B, Thrum K, Katenkamp K, Steenbeck J, Sappeler A, Habrecht O, Gottschild D. Increased 18F-FDG uptake of hepatocellular carcinoma on positron emission tomography independently predicts tumor recurrence in liver transplant patients. *Transplant Proc* 2009; **41**: 2561-2563 [PMID: 19715974]
 - 44 **Chou CT**, Chen RC, Lee CW, Ko CJ, Wu HK, Chen YL. Prediction of microvascular invasion of hepatocellular carcinoma by pre-operative CT imaging. *Br J Radiol* 2012; **85**: 778-783 [PMID: 21828149]
 - 45 **Hameed B**, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level < 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014; **20**: 945-951 [PMID: 24797281 DOI: 10.1002/lt.23904]
 - 46 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debette-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D. Liver transplantation for hepatocellular carcinoma: a model including α -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-994.e3; quiz e14-15 [PMID: 22750200]
 - 47 **Vibert E**, Azoulay D, Hoti E, Iacopinelli S, Samuel D, Salloum C, Lemoine A, Bismuth H, Castaing D, Adam R. Progression of alphafetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010; **10**: 129-137 [PMID: 20070666]
 - 48 **Hamamura K**, Shiratori Y, Shiina S, Imamura M, Obi S, Sato S, Yoshida H, Omata M. Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. *Cancer* 2000; **88**: 1557-1564 [PMID: 10738213]
 - 49 **Marrero JA**, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, Reddy KR, Harnois D, Llovet JM, Normolle D, Dalhgren J, Chia D, Lok AS, Wagner PD, Srivastava S, Schwartz M. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009; **137**: 110-118 [PMID: 19362088]
 - 50 **Shirabe K**, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, Aishima S, Maehara Y. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. *J Surg Oncol* 2007; **95**: 235-240 [PMID: 17323337 DOI: 10.1002/jso.20655]
 - 51 **Shirabe K**, Toshima T, Kimura K, Yamashita Y, Ikeda T, Ikegami T, Yoshizumi T, Abe K, Aishima S, Maehara Y. New scoring system for prediction of microvascular invasion in patients with hepatocellular carcinoma. *Liver Int* 2014; **34**: 937-941 [PMID: 24393295 DOI: 10.1111/liv.12459]
 - 52 **Koike Y**, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, Yoshida H, Shiina S, Omata M. Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. *Cancer* 2001; **91**: 561-569 [PMID: 11169939 DOI: 10.1002/1097-0142(20010201)91:3<561::AID-CNCR1035>3.0.CO;2-N]
 - 53 **Inagaki Y**, Tang W, Makuuchi M, Hasegawa K, Sugawara Y, Kokudo N. Clinical and molecular insights into the hepatocellular carcinoma tumour marker des- γ -carboxyprothrombin. *Liver Int* 2011; **31**: 22-35 [PMID: 20874725 DOI: 10.1111/j.1478-3231.2010.02348.x]
 - 54 **Pinato DJ**, Stebbing J, Ishizuka M, Khan SA, Wasan HS, North BV, Kubota K, Sharma R. A novel and validated prognostic index in hepatocellular carcinoma: the inflammation based index (IBI). *J Hepatol* 2012; **57**: 1013-1020 [PMID: 22732513 DOI: 10.1016/j.jhep.2012.06.022]
 - 55 **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: 10.1186/1471-2407-13-78]
- 56 **Parisi I**, Tsochatzis E, Wijewantha H, Rodríguez-Perálvarez M, De Luca L, Manousou P, Fatourou E, Pieri G, Papastergiou V, Davies N, Yu D, Luong T, Dhillon AP, Thorburn D, Patch D, O'Beirne J, Meyer T, Burroughs AK. Inflammation-based scores do not predict post-transplant recurrence of hepatocellular carcinoma in patients within Milan criteria. *Liver Transpl* 2014; **20**: 1327-1335 [PMID: 25088400 DOI: 10.1002/lt.23969]
 - 57 **Lai Q**, Castro Santa E, Rico Juri JM, Pinheiro RS, Lerut J. Neutrophil and platelet-to-lymphocyte ratio as new predictors of dropout and recurrence after liver transplantation for hepatocellular cancer. *Transpl Int* 2014; **27**: 32-41 [PMID: 24118272 DOI: 10.1111/tri.12191]
 - 58 **Briceño J**, Cruz-Ramírez M, Prieto M, Navasa M, Ortiz de Urbina J, Orti R, Gómez-Bravo MÁ, Otero A, Varo E, Tomé S, Clemente G, Bañares R, Bárcena R, Cuervas-Mons V, Solórzano G, Vinaixa C, Rubin A, Colmenero J, Valdovinos A, Ciria R, Hervás-Martínez C, de la Mata M. Use of artificial intelligence as an innovative donor-recipient matching model for liver transplantation: results from a multicenter Spanish study. *J Hepatol* 2014; **61**: 1020-1028 [PMID: 24905493 DOI: 10.1016/j.jhep.2014.05.039]
 - 59 **Wiesner RH**. Patient selection in an era of donor liver shortage: current US policy. *Nat Clin Pract Gastroenterol Hepatol* 2005; **2**: 24-30 [PMID: 16265097 DOI: 10.1038/ncpgasthep0070]
 - 60 **Wiesner RH**, Freeman RB, Mulligan DC. Liver transplantation for hepatocellular cancer: the impact of the MELD allocation policy. *Gastroenterology* 2004; **127**: S261-S267 [PMID: 15508092 DOI: 10.1053/j.gastro.2004.09.040]
 - 61 **Sharma P**, Balan V, Hernandez JL, Harper AM, Edwards EB, Rodriguez-Luna H, Byrne T, Vargas HE, Mulligan D, Rakela J, Wiesner RH. Liver transplantation for hepatocellular carcinoma: the MELD impact. *Liver Transpl* 2004; **10**: 36-41 [PMID: 14755775]
 - 62 **Park SJ**, Freise CE, Hirose R, Kerlan RK, Yao FY, Roberts JP, Vagefi PA. Risk factors for liver transplant waitlist dropout in patients with hepatocellular carcinoma. *Clin Transplant* 2012; **26**: E359-E364 [PMID: 22693962 DOI: 10.1111/j.1399-0012.2012.01668.x]
 - 63 **Piscaglia F**, Camaggi V, Ravaioli M, Grazi GL, Zanella M, Leoni S, Ballardini G, Cavarini G, Pinna AD, Bolondi L. A new priority policy for patients with hepatocellular carcinoma awaiting liver transplantation within the model for end-stage liver disease system. *Liver Transpl* 2007; **13**: 857-866 [PMID: 17539006 DOI: 10.1002/lt.21155]
 - 64 **Schlansky B**, Chen Y, Scott DL, Austin D, Naugler WE. Waiting time predicts survival after liver transplantation for hepatocellular carcinoma: a cohort study using the United Network for Organ Sharing registry. *Liver Transpl* 2014; **20**: 1045-1056 [PMID: 24838471 DOI: 10.1002/lt.23917]
 - 65 **Vitale A**, Volk ML, De Feo TM, Burra P, Frigo AC, Ramirez Morales R, De Carlis L, Belli L, Colledan M, Fagioli S, Rossi G, Andorno E, Baccarani U, Regalia E, Vivarelli M, Donatiggio M, Cillo U. A method for establishing allocation equity among patients with and without hepatocellular carcinoma on a common liver transplant waiting list. *J Hepatol* 2014; **60**: 290-297 [PMID: 24161408 DOI: 10.1016/j.jhep.2013.10.010]
 - 66 **Yamashiki N**, Tateishi R, Yoshida H, Shiina S, Teratani T, Sato S, Mine N, Kondo Y, Kawabe T, Omata M. Ablation therapy in containing extension of hepatocellular carcinoma: a simulative analysis of dropout from the waiting list for liver transplantation. *Liver Transpl* 2005; **11**: 508-514 [PMID: 15838878 DOI: 10.1002/lt.20392]
 - 67 **Nicolini D**, Svegliati-Baroni G, Candelari R, Mincarelli C, Mandolesi A, Bearzi I, Mucchegiani F, Vecchi A, Montalti R, Benedetti A, Risaliti A, Vivarelli M. Doxorubicin-eluting bead vs conventional transcatheter arterial chemoembolization for hepatocellular carcinoma before liver transplantation. *World J Gastroenterol* 2013; **19**: 5622-5632 [PMID: 24039354 DOI: 10.3748/wjg.v19.i34.5622]
 - 68 **De Luna W**, Sze DY, Ahmed A, Ha BY, Ayoub W, Keeffe EB, Cooper A, Esquivel C, Nguyen MH. Transarterial chemoinfusion for hepatocellular carcinoma as downstaging therapy and a bridge toward liver transplantation. *Am J Transplant* 2009; **9**: 1158-1168 [PMID: 19344435 DOI: 10.1111/j.1600-6143.2009.02576.x]
 - 69 **Graziadei IW**, Sandmueller H, Waldenberger P, Koenigsrainer A, Nachbaur K, Jaschke W, Margreiter R, Vogel W. Chemoembolization followed by liver transplantation for hepatocellular carcinoma impedes tumor progression while on the waiting list and leads to excellent outcome. *Liver Transpl* 2003; **9**: 557-563 [PMID: 12783395 DOI: 10.1053/jlts.2003.50106]
 - 70 **Hayashi PH**, Ludkowski M, Forman LM, Osgood M, Johnson S, Kugelmas M, Trotter JF, Bak T, Wachs M, Kam I, Durham J, Everson GT. Hepatic artery chemoembolization for hepatocellular carcinoma in patients listed for liver transplantation. *Am J Transplant* 2004; **4**: 782-787 [PMID: 15084175 DOI: 10.1111/j.1600-6143.2004.00413.x]
 - 71 **Dharancy S**, Boitard J, Decaens T, Sergent G, Boleslawski E, Duvoux C, Vanlemmens C, Meyer C, Gugenheim J, Durand F, Boillot O, Declercq N, Louvet A, Canva V, Romano O, Ernst O, Mathurin P, Pruvot FR. Comparison of two techniques of transarterial chemoembolization before liver transplantation for hepatocellular carcinoma: a case-control study. *Liver Transpl* 2007; **13**: 665-671 [PMID: 17427172 DOI: 10.1002/lt.21109]
 - 72 **Cescon M**, Cucchetti A, Ravaioli M, Pinna AD. Hepatocellular carcinoma locoregional therapies for patients in the waiting list. Impact on transplantability and recurrence rate. *J Hepatol* 2013; **58**: 609-618 [PMID: 23041304 DOI: 10.1016/j.jhep.2012.09.021S 0168-8278(12)00752-0]
 - 73 **Llovet JM**, Mas X, Aponte JJ, Fuster J, Navasa M, Christensen E, Rodés J, Bruix J. Cost effectiveness of adjuvant therapy for hepatocellular carcinoma during the waiting list for liver transplantation. *Gut* 2002; **50**: 123-128 [PMID: 11772979 DOI: 10.1136/gut.50.1.123]
 - 74 **Kim DJ**, Clark PJ, Heimbach J, Rosen C, Sanchez W, Watt K, Charlton MR. Recurrence of hepatocellular carcinoma: importance of mRECIST response to chemoembolization and tumor size. *Am J Transplant* 2014; **14**: 1383-1390 [PMID: 24801862 DOI: 10.1111/ajt.12684]
 - 75 **Millonig G**, Graziadei IW, Freund MC, Jaschke W, Stadlmann S, Ladurner R, Margreiter R, Vogel W. Response to preoperative chemoembolization correlates with outcome after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2007; **13**: 272-279 [PMID: 17256758 DOI: 10.1002/lt.21033]
 - 76 **Decaens T**, Roudot-Thoraval F, Bresson-Hadni S, Meyer C, Gugenheim J, Durand F, Bernard PH, Boillot O, Boudjema K, Calmus Y, Hardwigsen J, Ducerf C, Pageaux GP, Dharancy S, Chazouilleres O, Dhumeaux D, Cherqui D, Duvoux C. Impact of pretransplantation transarterial chemoembolization on survival and recurrence after liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2005; **11**: 767-775 [PMID: 15973710 DOI: 10.1002/lt.20418]
 - 77 **Baccarani U**, Adani GL, Serraino D, Lorenzin D, Gambato M, Buda A, Zanusi G, Vitale A, Piselli P, De Paoli A, Bresadola V, Risaliti A, Toniutto P, Cillo U, Bresadola F, Burra P. De novo tumors are a major cause of late mortality after orthotopic liver transplantation. *Transplant Proc* 2009; **41**: 1303-1305 [PMID: 19460546 DOI: 10.1016/j.transproceed.2009.03.079]
 - 78 **Yao FY**, Bass NM, Nikolai B, Merriman R, Davern TJ, Kerlan R, Ascher NL, Roberts JP. A follow-up analysis of the pattern and predictors of dropout from the waiting list for liver transplantation in patients with hepatocellular carcinoma: implications for the current organ allocation policy. *Liver Transpl* 2003; **9**: 684-692 [PMID: 12827553 DOI: 10.1053/jlts.2003.50147]
 - 79 **De Giorgio M**, Vezzoli S, Cohen E, Armellini E, Lucà MG, Verga G, Pinelli D, Nani R, Valsecchi MG, Antolini L, Colledan M, Fagioli S, Strazzabosco M. Prediction of progression-free survival in patients presenting with hepatocellular carcinoma within the Milan criteria. *Liver Transpl* 2010; **16**: 503-512 [PMID: 20373461 DOI: 10.1002/lt.22039]
 - 80 **Cucchetti A**, Cescon M, Bigonzi E, Piscaglia F, Golfieri R, Ercolani G, Cristina Morelli M, Ravaioli M, Daniele Pinna A. Priority of candidates with hepatocellular carcinoma awaiting liver

- transplantation can be reduced after successful bridge therapy. *Liver Transpl* 2011; **17**: 1344-1354 [PMID: 21837731 DOI: 10.1002/lt.22397]
- 81 **Mehta N**, Dodge JL, Goel A, Roberts JP, Hirose R, Yao FY. Identification of liver transplant candidates with hepatocellular carcinoma and a very low dropout risk: implications for the current organ allocation policy. *Liver Transpl* 2013; **19**: 1343-1353 [PMID: 24285611 DOI: 10.1002/lt.23753]
 - 82 **Xu XS**, Liu C, Qu K, Song YZ, Zhang P, Zhang YL. Liver transplantation versus liver resection for hepatocellular carcinoma: a meta-analysis. *Hepatobiliary Pancreat Dis Int* 2014; **13**: 234-241 [PMID: 24919605 DOI: 10.1016/S1499-3872(14)60037-0]
 - 83 **Hu Z**, Zhou J, Xu X, Li Z, Zhou L, Wu J, Zhang M, Zheng S. Salvage liver transplantation is a reasonable option for selected patients who have recurrent hepatocellular carcinoma after liver resection. *PLoS One* 2012; **7**: e36587 [PMID: 22574187 DOI: 10.1371/journal.pone.0036587]
 - 84 **Fuks D**, Dokmak S, Paradis V, Diouf M, Durand F, Belghiti J. Benefit of initial resection of hepatocellular carcinoma followed by transplantation in case of recurrence: an intention-to-treat analysis. *Hepatology* 2012; **55**: 132-140 [PMID: 21932387 DOI: 10.1002/hep.24680]
 - 85 **Lei JY**, Yan LN, Wang WT. Transplantation vs resection for hepatocellular carcinoma with compensated liver function after downstaging therapy. *World J Gastroenterol* 2013; **19**: 4400-4408 [PMID: 23885153 DOI: 10.3748/wjg.v19.i27.4400]
 - 86 **Wang HQ**, Yang J, Yan LN, Zhang XW, Yang JY. Liver resection in hepatitis B related-hepatocellular carcinoma: clinical outcomes and safety in elderly patients. *World J Gastroenterol* 2014; **20**: 6620-6625 [PMID: 24914386 DOI: 10.3748/wjg.v20.i21.6620]
 - 87 **Adam R**, Azoulay D, Castaing D, Eshkenazy R, Pascal G, Hashizume K, Samuel D, Bismuth H. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann Surg* 2003; **238**: 508-518; discussion 518-519 [PMID: 14530722]
 - 88 **Goel A**, Mehta N, Guy J, Fidelman N, Yao F, Roberts J, Terrault N. Hepatic artery and biliary complications in liver transplant recipients undergoing pretransplant transarterial chemoembolization. *Liver Transpl* 2014; **20**: 1221-1228 [PMID: 25045002 DOI: 10.1002/lt.23945]
 - 89 **Pompili M**, Francica G, Ponziani FR, Iezzi R, Avolio AW. Bridging and downstaging treatments for hepatocellular carcinoma in patients on the waiting list for liver transplantation. *World J Gastroenterol* 2013; **19**: 7515-7530 [PMID: 24282343 DOI: 10.3748/wjg.v19.i43.7515]
 - 90 **Huo TI**, Huang YH, Su CW, Lin HC, Chiang JH, Chiou YY, Huo SC, Lee PC, Lee SD. Validation of the HCC-MELD for dropout probability in patients with small hepatocellular carcinoma undergoing locoregional therapy. *Clin Transplant* 2008; **22**: 469-475 [PMID: 18318736 DOI: 10.1111/j.1399-0012.2008.00811.xCTR811]
 - 91 **Atabani SF**, Smith C, Atkinson C, Aldridge RW, Rodriguez-Perálvarez M, Rolando N, Harber M, Jones G, O'Riordan A, Burroughs AK, Thorburn D, O'Beirne J, Milne RS, Emery VC, Griffiths PD. Cytomegalovirus replication kinetics in solid organ transplant recipients managed by preemptive therapy. *Am J Transplant* 2012; **12**: 2457-2464 [PMID: 22594993 DOI: 10.1111/j.1600-6143.2012.04087.x]
 - 92 **Feng K**, Ma KS. Value of radiofrequency ablation in the treatment of hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 5987-5998 [PMID: 24876721 DOI: 10.3748/wjg.v20.i20.5987]
 - 93 **Mearini L**. High intensity focused ultrasound, liver disease and bridging therapy. *World J Gastroenterol* 2013; **19**: 7494-7499 [PMID: 24282341 DOI: 10.3748/wjg.v19.i43.7494]
 - 94 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Gretten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514]
 - 95 **Castelli G**, Burra P, Giacomini A, Vitale A, Senzolo M, Cillo U, Farinati F. Sorafenib use in the transplant setting. *Liver Transpl* 2014; **20**: 1021-1028 [PMID: 24809799 DOI: 10.1002/lt.23911]
 - 96 **Vitale A**, Volk ML, Pastorelli D, Lonardi S, Farinati F, Burra P, Angeli P, Cillo U. Use of sorafenib in patients with hepatocellular carcinoma before liver transplantation: a cost-benefit analysis while awaiting data on sorafenib safety. *Hepatology* 2010; **51**: 165-173 [PMID: 19877181 DOI: 10.1002/hep.23260]
 - 97 **Saidi RF**, Shah SA, Rawson AP, Grossman S, Piperdi B, Bozorgzadeh A. Treating hepatocellular carcinoma with sorafenib in liver transplant patients: an initial experience. *Transplant Proc* 2010; **42**: 4582-4584 [PMID: 21168742 DOI: 10.1016/j.transproceed.2010.09.147]
 - 98 **Hoffmann K**, Glimm H, Radeleff B, Richter G, Heining C, Schenkel I, Zahlten-Hinguranage A, Schirmacher P, Schmidt J, Büchler MW, Jaeger D, von Kalle C, Schemmer P. Prospective, randomized, double-blind, multi-center, Phase III clinical study on transarterial chemoembolization (TACE) combined with Sorafenib versus TACE plus placebo in patients with hepatocellular cancer before liver transplantation - HeLiivCa [ISRCTN24081794]. *BMC Cancer* 2008; **8**: 349 [PMID: 19036146 DOI: 10.1186/1471-2407-8-3491471-2407-8-349]
 - 99 **Kulik L**, Vouche M, Koppe S, Lewandowski RJ, Mulcahy MF, Ganger D, Habib A, Karp J, Al-Saden P, Lacouture M, Cotliar J, Abecassis M, Baker T, Salem R. Prospective randomized pilot study of Y90+/-sorafenib as bridge to transplantation in hepatocellular carcinoma. *J Hepatol* 2014; **61**: 309-317 [PMID: 24681342 DOI: 10.1016/j.jhep.2014.03.023]
 - 100 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
 - 101 **Chen K**, Man K, Metselaar HJ, Janssen HL, Peppelenbosch MP, Pan Q. Rationale of personalized immunosuppressive medication for hepatocellular carcinoma patients after liver transplantation. *Liver Transpl* 2014; **20**: 261-269 [PMID: 24376158 DOI: 10.1002/lt.23806]
 - 102 **Wiesner RH**, Fung JJ. Present state of immunosuppressive therapy in liver transplant recipients. *Liver Transpl* 2011; **17** Suppl 3: S1-S9 [PMID: 21850697 DOI: 10.1002/lt.22410]
 - 103 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]
 - 104 **Kim R**, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. *Immunology* 2007; **121**: 1-14 [PMID: 17386080 DOI: 10.1111/j.1365-2567.2007.02587.x]
 - 105 **Teng MW**, Swann JB, Koebel CM, Schreiber RD, Smyth MJ. Immune-mediated dormancy: an equilibrium with cancer. *J Leukoc Biol* 2008; **84**: 988-993 [PMID: 18515327 DOI: 10.1189/jlb.1107774]
 - 106 **Rodríguez-Perálvarez M**, De la Mata M, Burroughs AK. Liver transplantation: immunosuppression and oncology. *Curr Opin Organ Transplant* 2014; **19**: 253 [DOI: 10.1097/MOT.0000000000000069]
 - 107 **Schulz TF**. Cancer and viral infections in immunocompromised individuals. *Int J Cancer* 2009; **125**: 1755-1763 [PMID: 19588503 DOI: 10.1002/ijc.24741]
 - 108 **Mbulaitye SM**, Clarke CA, Morton LM, Gibson TM, Pawlish K, Weisenburger DD, Lynch CF, Goodman MT, Engels EA. Burkitt lymphoma risk in US solid organ transplant recipients. *Am J Hematol* 2013; **88**: 245-250 [PMID: 23386365 DOI: 10.1002/ajh.23385]
 - 109 **Grulich AE**, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 2007; **370**: 59-67 [PMID: 17617273 DOI: 10.1016/S0140-6736(07)61050-2]
 - 110 **Manousou P**, Cholongitas E, Samonakis D, Tsochatzis E, Corbani A, Dhillon AP, Davidson J, Rodríguez-Perálvarez M, Patch D, O'Beirne J, Thorburn D, Luong T, Rolles K, Davidson B, McCormick PA, Hayes P, Burroughs AK. Reduced fibrosis in

- recurrent HCV with tacrolimus, azathioprine and steroids versus tacrolimus: randomised trial long term outcomes. *Gut* 2014; **63**: 1005-1013 [PMID: 24131637 DOI: 10.1136/gutjnl-2013-305606]
- 111 **Karran P**, Attard N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. *Nat Rev Cancer* 2008; **8**: 24-36 [PMID: 18097462 DOI: 10.1038/nrc2292]
 - 112 **Smith MA**, Irving PM, Marinaki AM, Sanderson JD. Review article: malignancy on thiopurine treatment with special reference to inflammatory bowel disease. *Aliment Pharmacol Ther* 2010; **32**: 119-130 [PMID: 20412066 DOI: 10.1111/j.1365-2036.2010.04330.x]
 - 113 **Robson R**, Cecka JM, Opelz G, Budde M, Sacks S. Prospective registry-based observational cohort study of the long-term risk of malignancies in renal transplant patients treated with mycophenolate mofetil. *Am J Transplant* 2005; **5**: 2954-2960 [PMID: 16303010 DOI: 10.1111/j.1600-6143.2005.01125.x]
 - 114 **O'Neill JO**, Edwards LB, Taylor DO. Mycophenolate mofetil and risk of developing malignancy after orthotopic heart transplantation: analysis of the transplant registry of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 2006; **25**: 1186-1191 [PMID: 17045930 DOI: 10.1016/j.healun.2006.06.010]
 - 115 **Turner AP**, Knechtle SJ. Induction immunosuppression in liver transplantation: a review. *Transpl Int* 2013; **26**: 673-683 [PMID: 23651083 DOI: 10.1111/tri.12100]
 - 116 **Maluccio M**, Sharma V, Lagman M, Vyas S, Yang H, Li B, Suthanthiran M. Tacrolimus enhances transforming growth factor-beta1 expression and promotes tumor progression. *Transplantation* 2003; **76**: 597-602 [PMID: 12923450 DOI: 10.1097/01.TP.0.000081399.75231.3B]
 - 117 **Datta D**, Contreras AG, Basu A, Dormond O, Flynn E, Briscoe DM, Pal S. Calcineurin inhibitors activate the proto-oncogene Ras and promote protumorigenic signals in renal cancer cells. *Cancer Res* 2009; **69**: 8902-8909 [PMID: 19903851 DOI: 10.1158/0008-5472.CAN-09-1404]
 - 118 **Vivarelli M**, Cucchetti A, Piscaglia F, La Barba G, Bolondi L, Cavallari A, Pinna AD. Analysis of risk factors for tumor recurrence after liver transplantation for hepatocellular carcinoma: key role of immunosuppression. *Liver Transpl* 2005; **11**: 497-503 [PMID: 15838913 DOI: 10.1002/lt.20391]
 - 119 **Vivarelli M**, Cucchetti A, La Barba G, Ravaioli M, Del Gaudio M, Lauro A, Grazi GL, Pinna AD. Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. *Ann Surg* 2008; **248**: 857-862 [PMID: 18948815 DOI: 10.1097/SLA.0b013e3181896278]
 - 120 **Rodríguez-Perálvarez M**, Tsochatzis E, Naveas MC, Pieri G, García-Caparrós C, O'Beirne J, Poyato-González A, Ferrín-Sánchez G, Montero-Álvarez JL, Patch D, Thorburn D, Briceño J, De la Mata M, Burroughs AK. Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. *J Hepatol* 2013; **59**: 1193-1199 [PMID: 23867318 DOI: 10.1016/j.jhep.2013.07.012]
 - 121 **Villanueva A**, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, Tovar V, Roayaie S, Minguez B, Sole M, Battiston C, Van Laarhoven S, Fiel MI, Di Feo A, Hoshida Y, Yea S, Toffanin S, Ramos A, Martignetti JA, Mazzaferro V, Bruix J, Waxman S, Schwartz M, Meyerson M, Friedman SL, Llovet JM. Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* 2008; **135**: 1972-1983, 1983.e1-11 [PMID: 18929564 DOI: 10.1053/j.gastro.2008.08.008]
 - 122 **Gaumann A**, Schlitt HJ, Geissler EK. Immunosuppression and tumor development in organ transplant recipients: the emerging dualistic role of rapamycin. *Transpl Int* 2008; **21**: 207-217 [PMID: 18069922]
 - 123 **Schumacher G**, Oidtmann M, Rueggeberg A, Jacob D, Jonas S, Langrehr JM, Neuhaus R, Berra M, Neuhaus P. Sirolimus inhibits growth of human hepatoma cells alone or combined with tacrolimus, while tacrolimus promotes cell growth. *World J Gastroenterol* 2005; **11**: 1420-1425 [PMID: 15770715 DOI: 10.3748/wjg.v11.i10.1420]
 - 124 **De Simone P**, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, Saliba F, Jonas S, Sudan D, Fung J, Fischer L, Duvoux C, Chavin KD, Koneru B, Huang MA, Chapman WC, Foltys D, Witte S, Jiang H, Hexham JM, Junge G. Everolimus with reduced tacrolimus improves renal function in de novo liver transplant recipients: a randomized controlled trial. *Am J Transplant* 2012; **12**: 3008-3020 [PMID: 22882750 DOI: 10.1111/j.1600-6143.2012.04212.x]
 - 125 **Zhou J**, Wang Z, Wu ZQ, Qiu SJ, Yu Y, Huang XW, Tang ZY, Fan J. Sirolimus-based immunosuppression therapy in liver transplantation for patients with hepatocellular carcinoma exceeding the Milan criteria. *Transplant Proc* 2008; **40**: 3548-3553 [PMID: 19100435]
 - 126 **Vivarelli M**, Dazzi A, Zanella M, Cucchetti A, Cescon M, Ravaioli M, Del Gaudio M, Lauro A, Grazi GL, Pinna AD. Effect of different immunosuppressive schedules on recurrence-free survival after liver transplantation for hepatocellular carcinoma. *Transplantation* 2010; **89**: 227-231 [PMID: 20098287]
 - 127 **Campsen J**, Zimmerman MA, Trotter JF, Wachs M, Bak T, Steinberg T, Kam I. Clinically recurrent primary sclerosing cholangitis following liver transplantation: a time course. *Liver Transpl* 2008; **14**: 181-185 [PMID: 18236392 DOI: 10.1002/lt.21420]
 - 128 **Chinnakotla S**, Davis GL, Vasani S, Kim P, Tomiyama K, Sanchez E, Onaca N, Goldstein R, Levy M, Klintmalm GB. Impact of sirolimus on the recurrence of hepatocellular carcinoma after liver transplantation. *Liver Transpl* 2009; **15**: 1834-1842 [PMID: 19938137 DOI: 10.1002/lt.21953]
 - 129 **Toso C**, Merani S, Bigam DL, Shapiro AM, Kneteman NM. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. *Hepatology* 2010; **51**: 1237-1243 [PMID: 20187107 DOI: 10.1002/hep.23437]
 - 130 **Menon KV**, Hakeem AR, Heaton ND. Meta-analysis: recurrence and survival following the use of sirolimus in liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2013; **37**: 411-419 [PMID: 23278125 DOI: 10.1111/apt.12185]
 - 131 **Liang W**, Wang D, Ling X, Kao AA, Kong Y, Shang Y, Guo Z, He X. Sirolimus-based immunosuppression in liver transplantation for hepatocellular carcinoma: a meta-analysis. *Liver Transpl* 2012; **18**: 62-69 [PMID: 21964956 DOI: 10.1002/lt.22441]
 - 132 **Schnitzbauer AA**, Zuelke C, Graeb C, Rochon J, Bilbao I, Burra P, de Jong KP, Duvoux C, Kneteman NM, Adam R, Bechstein WO, Becker T, Beckebaum S, Chazouillères O, Cillo U, Colledan M, Fändrich F, Gugenheim J, Hauss JP, Heise M, Hidalgo E, Jamieson N, Königsrainer A, Lamby PE, Lerut JP, Mäksälä H, Margreiter R, Mazzaferro V, Mutzbauer I, Otto G, Pageaux GP, Pinna AD, Pirenne J, Rizell M, Rossi G, Rostaing L, Roy A, Turrión VS, Schmidt J, Troisi RI, van Hoek B, Valente U, Wolf P, Wolters H, Mirza DF, Scholz T, Steininger R, Soderdahl G, Strasser SI, Jauch KW, Neuhaus P, Schlitt HJ, Geissler EK. A prospective randomised, open-labeled, trial comparing sirolimus-containing versus mTOR-inhibitor-free immunosuppression in patients undergoing liver transplantation for hepatocellular carcinoma. *BMC Cancer* 2010; **10**: 190 [PMID: 20459775]
 - 133 **Asrani SK**, Wiesner RH, Trotter JF, Klintmalm G, Katz E, Maller E, Roberts J, Kneteman N, Teperman L, Fung JJ, Millis JM. De novo sirolimus and reduced-dose tacrolimus versus standard-dose tacrolimus after liver transplantation: the 2000-2003 phase II prospective randomized trial. *Am J Transplant* 2014; **14**: 356-366 [PMID: 24456026 DOI: 10.1111/ajt.12543]
 - 134 **Watt KD**, Dierkhising R, Heimbach JK, Charlton MR. Impact of sirolimus and tacrolimus on mortality and graft loss in liver transplant recipients with or without hepatitis C virus: an analysis of the Scientific Registry of Transplant Recipients Database. *Liver Transpl* 2012; **18**: 1029-1036 [PMID: 22641474 DOI: 10.1002/lt.23479]
 - 135 **Jin YP**, Valenzuela NM, Ziegler ME, Rozengurt E, Reed EF. Everolimus inhibits anti-HLA I antibody-mediated endothelial cell signaling, migration and proliferation more potently than sirolimus. *Am J Transplant* 2014; **14**: 806-819 [PMID: 24580843 DOI: 10.1111/ajt.12669]

- 136 **Lapante M**, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149**: 274-293 [PMID: 22500797]
- 137 **Yan J**, Tan C, Gu F, Jiang J, Xu M, Huang X, Dai Z, Wang Z, Fan J, Zhou J. Sorafenib delays recurrence and metastasis after liver transplantation in a rat model of hepatocellular carcinoma with high expression of phosphorylated extracellular signal-regulated kinase. *Liver Transpl* 2013; **19**: 507-520 [PMID: 23408515 DOI: 10.1002/lt.23619]
- 138 **Shetty K**, Dash C, Laurin J. Use of adjuvant sorafenib in liver transplant recipients with high-risk hepatocellular carcinoma. *J Transplant* 2014; **2014**: 913634 [PMID: 24818010 DOI: 10.1155/2014/913634]
- 139 **Lei JY**, Wang WT, Yan LN. Up-to-seven criteria for hepatocellular carcinoma liver transplantation: a single center analysis. *World J Gastroenterol* 2013; **19**: 6077-6083 [PMID: 24106409 DOI: 10.3748/wjg.v19.i36.6077]
- 140 **D'Amico F**, Schwartz M, Vitale A, Tabrizian P, Roayaie S, Thung S, Guido M, del Rio Martin J, Schiano T, Cillo U. Predicting recurrence after liver transplantation in patients with hepatocellular carcinoma exceeding the up-to-seven criteria. *Liver Transpl* 2009; **15**: 1278-1287 [PMID: 19790142 DOI: 10.1002/lt.21842]
- 141 **de Ataíde EC**, Garcia M, Mattosinho TJ, Almeida JR, Escanhoela CA, Boin IF. Predicting survival after liver transplantation using up-to-seven criteria in patients with hepatocellular carcinoma. *Transplant Proc* 2012; **44**: 2438-2440 [PMID: 23026614]

P- Reviewer: Maroni L, Narciso-Schiavon JL, Peltec A, Penkova-Radicheva MP, Shimada Y
S- Editor: Tian YL **L- Editor:** A **E- Editor:** Liu SQ



Arrhythmia risk in liver cirrhosis

Ioana Mozos

Ioana Mozos, Department of Functional Sciences, “Victor Babes” University of Medicine and Pharmacy, 300173 Timisoara, Romania

Author contributions: Mozos I reviewed the literature and wrote the manuscript.

Conflict-of-interest: No potential conflicts of interest relevant to this article were reported.

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

Correspondence to: Ioana Mozos, MD, PhD, Associate Professor, Department of Functional Sciences, “Victor Babes” University of Medicine and Pharmacy, T. Vladimirescu Street 14, 300173 Timisoara, Romania. ioanamozos@yahoo.de

Telephone: +40-745-610004

Fax: +40-256-490626

Received: August 23, 2014

Peer-review started: August 24, 2014

First decision: October 14, 2014

Revised: December 4, 2014

Accepted: January 15, 2015

Article in press: January 19, 2015

Published online: April 8, 2015

Abstract

Interactions between the functioning of the heart and the liver have been described, with heart diseases affecting the liver, liver diseases affecting the heart, and conditions that simultaneously affect both. The heart is one of the most adversely affected organs in patients with liver cirrhosis. For example, arrhythmias and electrocardiographic changes are observed in patients with liver cirrhosis. The risk for arrhythmia is influenced by factors such as cirrhotic cardiomyopathy, cardiac ion channel remodeling, electrolyte imbalances,

impaired autonomic function, hepatorenal syndrome, metabolic abnormalities, advanced age, inflammatory syndrome, stressful events, impaired drug metabolism and comorbidities. Close monitoring of cirrhotic patients is needed for arrhythmias, particularly when QT interval-prolonging drugs are given, or if electrolyte imbalances or hepatorenal syndrome appear. Arrhythmia risk may persist after liver transplantation due to possible QT interval prolongation, persistence of the parasympathetic impairment, post-transplant reperfusion and chronic immunosuppression, as well as consideration of the fact that the transplant itself is a stressful event for the cardiovascular system. The aims of the present article were to provide a review of the most important data regarding the epidemiology, pathophysiology, and biomarkers of arrhythmia risk in patients with liver cirrhosis, to elucidate the association with long-term outcome, and to propose future research directions.

Key words: Arrhythmia; Atrial fibrillation; Cirrhotic cardiomyopathy; Electrocardiography; Liver cirrhosis; Liver transplantation; Sudden cardiac death; $T_{peak}-T_{end}$ interval; Ventricular tachycardia; Long-QT syndrome

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Arrhythmias and electrocardiographic changes occur in several non-cardiac diseases, including liver cirrhosis. Supraventricular and ventricular arrhythmias, including atrial fibrillation and flutter, and premature atrial and ventricular contractions, have been reported in cirrhotic patients. It is questionable whether the prevalence of atrial fibrillation and flutter is high in patients with liver cirrhosis, or if liver cirrhosis protects against supraventricular arrhythmias.

Mozos I. Arrhythmia risk in liver cirrhosis. *World J Hepatol* 2015; 7(4): 662-672 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/662.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i4.662>

INTRODUCTION

Interactions between the functioning of the heart and the liver have been described, with heart diseases affecting the liver, liver diseases affecting the heart, and conditions that simultaneously affect both^[1,2]. Thus, it is important for both hepatologists and cardiologists to understand the relationship between the liver and the heart. Indeed, involvement of the cardiovascular system in end-stage liver disease is well recognized, and there are reports of cardiovascular symptoms in patients with liver cirrhosis, including chronotropic incompetence, cardiomyopathy, prolonged QT intervals, hyperdynamic circulation with an increased cardiac output and decreased peripheral vascular resistance, and impaired ventricular contractility in response to physiologic and pharmacologic stimuli^[3,4].

Liver cirrhosis is a fatal condition, and is most often caused by harmful alcohol consumption, metabolic syndrome related to being overweight or obese, or hepatitis B or C virus infection^[5,6]. Arrhythmias and electrocardiographic changes can occur with liver cirrhosis, for which cases of atrial fibrillation and flutter, premature atrial and ventricular contractions, and ventricular arrhythmias have been reported^[7]. The most important risk factors for arrhythmias in patients with cirrhosis include cirrhotic cardiomyopathy, cardiac ion channel remodeling, electrolyte imbalances, impaired autonomic function, hepatorenal syndrome, metabolic abnormalities, advanced age, inflammatory syndrome, and comorbidities. The aims of the present article were to provide a review of the most important data regarding the epidemiology, pathophysiology and biomarkers of arrhythmia risk in patients with liver cirrhosis, to elucidate the association with long-term outcome, and to propose future research directions.

CIRRHOTIC CARDIOMYOPATHY

The heart is one of the most adversely affected organs in patients with liver cirrhosis^[8]. Cirrhotic cardiomyopathy can appear in all forms of cirrhosis due to physical or pharmacologic stress, and includes increased cardiac output, decreased response to physiologic and pharmacologic stimuli, systolic and diastolic dysfunction, and electrophysiologic abnormalities in the absence of any known cardiac disease^[1,9-11]. Cirrhotic cardiomyopathy involves changes affecting the cardiomyocyte plasma membrane, attenuated stimulatory pathways, and increased activities of inhibitory systems^[3]. In order to differentiate between cardiomyopathy resulting from cirrhosis with cardiomyopathy due to the underlying cause of cirrhosis, Zaky *et al*^[8] prefer the term "cirrhosis-associated cardiomyopathy".

Diastolic dysfunction at rest is present in most cirrhotic patients, is more prevalent in those with ascites^[12], and precedes the development of systolic dysfunction^[9]. Although severe heart failure due to cirrhotic cardiomyopathy is rare, its prevalence is unknown, considering

that the disease is latent, and becomes apparent when the patient is subjected to a stressful event, including exercise, drugs, hemorrhage, infections, and surgery^[9,13]. At least one feature of cardiomyopathy is present in the majority of patients with severe or moderate liver failure, though the association between liver disease severity and cardiac dysfunction is controversial^[9,12]. Cirrhotic cardiomyopathy is reversible after liver transplantation^[14] and may contribute to the pathogenesis of hepatorenal syndrome^[9].

Structural and histologic changes in cardiac chambers and subsequent structural myocardial heterogeneity may contribute to electrical instability. Increased left ventricular wall thickness was described as a supportive criterion in patients with cirrhotic cardiomyopathy^[15], and it is known to impair myocardial oxygen demand. Myocardial hypertrophy (left ventricular hypertrophy and increased interventricular septum) and fibrosis cause diastolic dysfunction and contribute to structural heterogeneity and arrhythmia risk^[2]. Autopsy studies have described subendocardial and myocyte edema and patchy fibrosis, in addition to myocardial hypertrophy^[16]. However, further studies are needed to confirm the relationship between cardiac structural heterogeneity and arrhythmic events in cirrhotic patients.

VENTRICULAR ABNORMALITIES IN LIVER CIRRHOISIS

Multiple electrophysiologic abnormalities have been described in liver cirrhosis, including prolonged QT intervals, increased QT dispersion, chronotropic incompetence, and electromechanical uncoupling. These signs occur in the absence of known cardiovascular disease, and are related to autonomic dysfunction, severe portal hypertension, liver dysfunction, cytokines and endotoxins, and are independent of the cause of cirrhosis^[1,7,17-19].

The QT interval varies from daytime to nighttime due to the diurnal variations in autonomic tone, circulatory status and oxygen demands^[18,20]; the minimum value of the corrected QT (QTc), rather than the maximum value, shows a significant diurnal variation^[20]. The Bazett formula incompletely corrects the QT interval for heart rate, and the Fridericia method is therefore suggested to be the most reliable and valid^[7]. Chronotropic incompetence refers to lack of heart rate response to physiologic and pharmacologic demands, including exercise, head tilt, inotropes, and increased norepinephrine concentrations^[8], which limits exercise capacity. Electromechanical uncoupling leads to the dyssynchrony between electrical and mechanical systole^[8].

Long QT intervals

A prolonged QT interval, found incidentally by Kowalski *et al*^[21], is the electrophysiologic hallmark of cirrhotic cardiomyopathy. It represents the most common

Table 1 Factors associated with QT prolongation in liver cirrhosis

Factor	Example
Autonomic neuropathy	Plasma norepinephrine, diurnal variations
Liver dysfunction	Child-Pugh class, portal hypertension, pediatric end-stage liver disease score
Serum markers	Electrolytes, serum uric acid, serum bile salts, creatinine, plasma renin activity, aldosterone, atrial natriuretic factor, gonadal hormones, norepinephrine
Volume overload	Left ventricular end diastolic dimensions
Coronary heart disease	Risk factors: older age, male gender, smoking, arterial hypertension, diabetes mellitus
Left ventricular hypertrophy	-
Stressful events	Acute gastrointestinal bleeding
Drugs: excessive accumulation, impaired metabolization, distribution, or excretion	Erythromycin, fluoroquinolones, telipressin, sevoflurane

electrocardiographic finding in patients with liver cirrhosis, appearing in half of cirrhotic patients^[4,10,22,23], with a higher incidence than in patients with mild chronic active hepatitis^[24]. Prolongation of the QT interval predisposes the patients to a potentially fatal polymorphic ventricular tachycardia called torsade de pointes, which can degenerate into ventricular fibrillation and cause sudden cardiac death^[25]. Delayed repolarization of cardiomyocytes due to potassium channel abnormalities and sympathoadrenergic hyperactivity may contribute to QT interval prolongation^[17,18,26]. The main factors associated with QT interval prolongation in cirrhotic patients are reviewed in Table 1. Gender difference in the QTc interval is abolished in cirrhosis, which is not influenced by gonadal hormones nor restored after liver transplantation^[27].

QT prolongation in liver pathology was first described in alcoholic liver diseases^[28], and has since been associated with alcoholic etiology in patients with liver cirrhosis^[10,19,29]. Chronic, heavy alcohol consumption affects both the heart and the liver, increases the mass and impairs the function of the left ventricle^[1,30], and causes subclinical heart muscle injury, patchy delays in conduction and cardiac arrhythmias^[31]. Delays in intraventricular conduction and nonuniform myocardial involvement have been described in alcoholic cardiomyopathy^[30], and life-threatening ventricular arrhythmias are found in alcoholics without heart disease^[32]. Alcohol alters the resting membrane potential due to inhibition of sodium-potassium-ATPase, delays calcium binding and transport by the cardiac sarcoplasmic reticulum, and impairs calcium channels^[33]. Acute alcoholic states, including binge drinking and the "holiday heart syndrome," are also associated with an increased prevalence of cardiac arrhythmias and sudden cardiac death^[34]. The amount and duration of alcohol intake is related to life-threatening arrhythmias, though small quantities can be significant in susceptible individuals^[35]. On the other hand, a protective effect of moderate alcohol consumption against sudden cardiac death has also been demonstrated^[36,37], likely related to polyphenols, increased concentrations of high-density lipoprotein cholesterol, fibrinolysis, and polyunsaturated fatty acids, decreased platelet aggregation and coagulation factors,

with beneficial effects on endothelial function and inflammation^[38]. Arrhythmogenesis may be attributed to the hyperadrenergic state of drinking and withdrawal, electrolyte imbalances, impaired vagal heart rate control, repolarization abnormalities with prolonged QT intervals, worsening of myocardial ischemia, or sleep apnea^[31].

Prolonged QT intervals have been reported in patients with primary biliary cirrhosis and other chronic non-alcoholic liver diseases, and were shown to be associated with the severity of autonomic neuropathy and increased cardiovascular risk^[39], as well as with the pathophysiology of cirrhosis and liver dysfunction^[17,40,41]. A prolonged QT interval is common in children with chronic liver disease^[42], where it is related to the pediatric end-stage liver disease score, portal hypertension, and high mortality^[43]. QT interval prolongation is proportional to the Child-Pugh class^[10,17,16], and is related to the presence of portal hypertension, including mild portal hypertension^[18,22,44,43], liver dysfunction^[44], hepatic venous pressure gradient^[22], and markers of hyperdynamic circulation^[40]. Furthermore, plasma calcium level^[22], serum uric acid^[10], serum bile salts, electrolytes, creatinine, plasma renin activity, aldosterone, atrial natriuretic factor, and gonadal hormones are associated with prolonged QT intervals in patients with liver cirrhosis^[17,45]. QT interval is also related to cardiac serum markers, but not to vasodilator (endothelin-3, calcitonin gene-related peptide) or vasoconstrictor (endothelin-1) markers^[46]. A multivariate analysis showed that plasma norepinephrine was independently correlated with QTc duration, demonstrating that sympathoadrenergic hyperactivity is a risk factor for QT prolongation^[17,47]. Disturbances of excitation-contraction coupling have been reported in cirrhotic patients with QT interval prolongation, attributable to defective potassium channel function in ventricular cardiomyocytes^[18,40]. Moaref *et al*^[13] showed a positive correlation between QT prolongation and left ventricular end diastolic dimensions in cirrhotic patients, indicating a direct relationship between electrophysiologic changes and the severity of volume overload. Volume overload is related to the progression of liver cirrhosis and prolongation of the repolarization time by the stretching of myofibers, and

volume control is recommended in cirrhotic patients to prevent decompensation^[13].

Prolonged QTc is related to an increased mortality rate in patients with chronic liver diseases^[48]. Among these, patients with a QTc longer than 440 ms have a significantly lower survival rate than those with normal QTc^[17]. The clinical significance of QT prolongation in liver cirrhosis is unclear, considering that sudden cardiac death and torsade de pointes are rare^[9]. However, acute gastrointestinal bleeding further prolongs QTc in patients with liver cirrhosis, which predicts bleeding-induced mortality^[49]. QT prolongation and electromechanical dyssynchrony have not been observed in septic cardiodepression, the inflammatory phenotype of cardiac dysfunction that is mediated through cytokines^[15].

Drug-induced QT prolongation

Child-Pugh and model for end-stage liver disease scores correlate with drug clearance^[50]. As a result, patients with liver disease often require dosage adjustments in order to prevent adverse effects caused by excessive drug or metabolite accumulation^[51]. Accumulation results from altered activity of drug-metabolizing enzymes and drug distribution, as well as from impaired renal excretion. For example, the activity of cytochrome P450 3A, the most abundant hepatic drug-metabolizing enzyme, is reduced in liver cirrhosis^[51,52]. The activity of this enzyme varies according to the etiology and severity of liver disease^[51,53]. Patients with transjugular intrahepatic portosystemic shunts are at increased risk for abnormal QT prolongation when exposed to oral cytochrome P450 substrates with QT-prolonging effects^[54].

Drugs affecting the QT interval should be avoided in patients with liver cirrhosis, or used with caution under close ECG monitoring^[2]. For example, the use of fluoroquinolones as secondary prophylaxis for spontaneous bacterial peritonitis in cirrhotic patients can predict QT prolongation^[19]. Drug administration should be critically reviewed, with consideration of indications, interactions and adverse reactions, to prevent drug-induced torsade de pointes^[55] and QT prolongation, particularly in patients with hepatic failure^[56,57]. Werner *et al*^[55] described a case of secondary torsade de pointes tachycardia in a 50-year-old patient with alcoholic liver cirrhosis who was admitted for hematemesis and melena after administration of QT-active drugs. Chung *et al*^[58] reported a case of torsade de pointes after induction of anesthesia for liver transplantation with QT prolonging drugs: sevoflurane (to maintain anesthesia) and palonosetron (for postoperative nausea and vomiting). Faigel *et al*^[45] also reported prolonged QT intervals and torsade de pointes in three cirrhotic patients with bleeding esophageal varices who received endoscopic sclerotherapy, vasopressin and neuroleptics. Lehmann *et al*^[59] presented a patient with newly diagnosed cirrhosis and kidney failure who underwent cardiopulmonary resuscitation twice after terlipressin,

an analogue of vasopressin.

Ventricular repolarization

The T_{pe} corresponds to the transmural dispersion of repolarization, and is a predictor of ventricular arrhythmias and sudden cardiac death^[60-62]. The T_{pe}/QT ratio is also used as an index of ventricular arrhythmogenesis^[63]. A prolonged T_{pe} interval and T_{pe}/QT interval ratio have been reported in patients with chronic hepatitis B infection, indicating an increased ventricular repolarization heterogeneity^[64]. Liver cirrhosis affects ventricular repolarization *via* electrolyte imbalances, impaired autonomic function, subclinical cardiomyopathy, reduced β -adrenergic receptor function, post-receptor pathway defects, altered physical properties of myocyte plasma membranes, elevated levels of cardiotoxins, ion channel remodeling, portosystemic shunting, and systemic circulatory disturbances^[10,16,22,44,65,66].

Late ventricular potentials

Chronic alcoholics exhibit late ventricular potentials, low-amplitude and high-frequency waveforms appearing in the terminal part of the ECG QRS complex, which are predictors of re-entry ventricular tachycardia and sudden cardiac death^[35,67]. Late ventricular potentials are associated with histologically significant fatty liver caused by chronic alcohol intake, revealing preclinical myocardial lesions and identifying alcoholic patients at risk of lethal arrhythmias^[68].

Ion channel remodeling

Cardiac ion channel remodeling, particularly of potassium channels, occurs in patients with liver cirrhosis^[26]. Moreover, reduced transient outward and delayed rectifier potassium currents have been detected in ventricular myocytes from cirrhotic animals^[26], which prolong the action potential and the QT interval^[7]. Ionic channels, as well β -adrenergic receptors and G proteins, are altered by endotoxins and increased biliary acids in patients with cholestasis^[16].

AUTONOMIC FUNCTION

Patients with liver cirrhosis show impaired autonomic cardiovascular reflexes, with the parasympathetic system more commonly affected than the sympathetic system^[7]. The escape of systemic and intestinal vasodilators from degraded, diseased liver and the formation of new blood vessels in the gut explain arteriolar vasodilation of the systemic and splanchnic circulations^[8]. The reduction in circulating blood volume and hyperdynamic circulation enhances the activities of the sympathetic nervous and renin-angiotensin-aldosterone systems. The resulting increased cardiac output and reduced systemic vascular resistance may induce myocardial remodeling and left ventricular hypertrophy, causing systolic and diastolic dysfunction and cardiomyopathy^[7,8]. Sympathetic overactivity is

associated with an increase in inflammatory cytokines, such as interleukin-1b, -6 and -8, tumor necrosis factor (TNF)- α , and transforming growth factor- β ^[8], which is a profibrogenic and proapoptotic stimulant^[18]. Cardiovascular autonomic dysfunction has also been described in chronic alcoholic liver disease and chronic hepatitis B and C virus infections^[64].

CARDIAC MANIFESTATIONS WITH HEPATITIS

Palpitations, dyspnea, angina chest discomfort, electrocardiographic changes, tachycardia and bradycardia have all been described in patients with viral hepatitis^[69], myocarditis, acute pericarditis and cardiomyopathy^[70-72]. Sinus tachycardia occurs in most patients and is related to the febrile response^[72]. Myocarditis may be a serious extrahepatic complication, and hepatitis B virus antigens have been detected in small intramyocardial vessels^[71]. The cardiac abnormalities may be caused by viral infection, hyperbilirubinemia, hemorrhage in the myocardium and pericardium, or by immune mechanisms^[69,71]. Chronic hepatitis B infection triggers autoimmune disorders and several extrahepatic disorders may appear, including of the ganglia and the heart^[73]. Endothelial progenitor cells may serve as a virus carrier, enabling transinfection in injured endothelial cells to cause hepatitis B virus-associated myocarditis^[73]. Hayashi *et al*^[69] reported a case of fulminant hepatitis complicated with myocarditis, with myocardial infarction-like electrocardiographic changes. Hepatitis C virus infection has been detected often in patients with dilated and hypertrophic cardiomyopathy, and may be an important causal agent in the pathogenesis of the disease and cause arrhythmias^[72,74]. Interferon, successfully used to treat patients with chronic hepatitis C infections, may induce several cardiovascular complications, such as tachycardia, myocardial infarction and congestive heart failure^[75].

MARKERS OF CARDIAC DYSFUNCTION

Cell death is a central mechanism involved in liver damage, for which several promising noninvasive biomarkers have been associated with QT prolongation, including soluble cytokeratin 18, TNF and TNF-related apoptosis-inducing ligand receptors and their ligands, various isoforms of high mobility group box-1, small non-coding RNAs (microRNAs) and microparticles (extracellular vesicles)^[76]. These biomarkers could be utilized in future studies to assess arrhythmia risk in liver cirrhosis. Fibrosis serum markers, such as hyaluronic acid and laminin^[77], may also be indicators of electrophysiologic abnormalities in cirrhotic patients.

Natriuretic peptides are produced by the cardiac atrial and ventricular myocytes^[78], and are higher in myocardial ischemia, heart failure and left ventricular tachycardia, as well as in liver cirrhosis and renal failure^[79]. Plasma

levels of N-terminal pro-brain natriuretic peptide (BNP) are useful markers of increased cardiovascular risk, cardiac subclinical dysfunction, atrial volume, and early decompensation of cirrhosis, and are increased proportionate to the stage of chronic liver disease^[78]. Elevated levels of BNP are related to interventricular septal thickness and the impairment of diastolic function in asymptomatic patients with cirrhosis, and may be a marker of the presence of cirrhotic cardiomyopathy^[80]. Henriksen *et al*^[81] also reported that circulating pro-BNP and BNP are related to severity of liver disease (Child-Pugh score, serum albumin, coagulation factors and hepatic venous pressure gradient) and markers of cardiac dysfunction (QT interval, heart rate and plasma volume), but not to indicators of hyperdynamic circulation.

RELATED COMPLICATIONS AND CONDITIONS

Cirrhotic patients also have an increased risk and prevalence of coronary heart disease, which is also a cause of QT prolongation^[1,82,83]. Risk factors for coronary heart disease, such as older age, male gender, smoking and arterial hypertension^[82,84], are independent predictors of several electrocardiographic abnormalities in cirrhotic patients^[19]. Moreover, liver disease severity is associated with many electrocardiographic features of coronary heart disease^[19]. Considering low serum cholesterol, low blood pressure values and higher levels of circulating estrogens, cirrhosis should protect against coronary atherosclerosis^[82]. However, recent reports have demonstrated an increased prevalence of major risk factors for atherosclerosis and cardiovascular disease in liver cirrhosis, especially in nonalcoholic steatohepatitis-cirrhosis^[19,85]. Hypercholesterolemia in patients with primary biliary cirrhosis should be considered a cardiovascular risk factor, and further studies are needed to confirm if arrhythmias are related to it.

Arrhythmias are also associated with hypoxia and orthodeoxia due to hepatopulmonary syndrome. Hepatorenal syndrome may be another important contributor, influenced by systolic dysfunction and insufficient ventricular contractile reserve^[2,86]. Ventricular arrhythmia risk and sudden cardiac death are increased in patients with renal failure, and even mild reductions in kidney function can alter the electrophysiologic properties of the myocardium^[87]. Arrhythmia risk is related not only to renal function, but also to electrolyte imbalances, sympathetic activity, and levels of parathyroid hormone, hemoglobin, hematocrit and inflammatory markers^[87].

Accumulation of bile acids in the liver due to obstructed ducts results in high circulating concentrations^[88], with immunosuppressive effects^[89]. In addition to the concentration, the composition of bile acids is important for arrhythmogenesis^[90]. Taurocholic acid, a conjugated primary bile acid, has a negative inotropic effect and reduces the duration of the action potentials in the ventricular myocytes by reducing inward sodium and

calcium and increasing outward potassium currents^[88]. The increased level of non-ursodeoxycholic acids in patients with arrhythmias suggests that ursodeoxycholic acids provide cardioprotective and hepatoprotective effects^[90]. Although the exact intracellular effects of bile salts are not clear, they may act on muscarinic or cell-surface bile acid receptors involved in the regulation of macrophage functions^[89] or directly damage cardiac calcium channels due to the detergent-like properties^[91].

SUPRAVENTRICULAR ARRHYTHMIAS AND CONDUCTION DISORDERS IN LIVER CIRRHOSIS

Atrial fibrillation and flutter are arrhythmias that are more frequently diagnosed in cirrhotic patients, and are significantly associated with arteriosclerosis, hypercholesterolemia and diabetes mellitus^[92]. Atrial fibrillation after septic shock and sinus bradycardia with cardiac arrest were reported after living-donor liver transplantation in a 58-year-old man diagnosed with hepatocellular carcinoma and liver cirrhosis, which required resuscitation and temporary pacing^[93]. Josefsson *et al*^[19] reported several supraventricular arrhythmias in cirrhotic patients, such as atrial and junctional premature beats, atrial flutter or fibrillation, sinus tachycardia or bradycardia. Pre-transplant evaluation of cirrhotic patients also revealed atrioventricular-conduction defects, such as complete or incomplete right or left bundle branch block and intraventricular blocks.

Inflammation may promote cardiac and arrhythmogenic complications in non-alcoholic fatty liver disease^[94]. Patients with liver fibrosis have elevated plasma levels of inflammatory markers, and several studies have indicated that inflammation plays a significant role in the generation, maintenance, and perpetuation of atrial fibrillation^[95]. However, Zamirian *et al*^[96] suggested that liver cirrhosis has a protective effect against atrial fibrillation, despite significant metabolic abnormalities, inflammatory syndrome and enlarged left atria. The low prevalence of atrial fibrillation observed in their study may be the result of the accumulation of anti-arrhythmic or anti-inflammatory substances that are normally metabolized by an intact functioning liver; this would explain the development of atrial fibrillation after liver transplantation^[96]. However, no data concerning the influence of inflammation in the relationship of arrhythmias and liver cirrhosis have been reported, which should be the aim of future studies.

The low prevalence atrial fibrillation in cirrhotic patients reported by Zamirian *et al*^[96] may also have been related to the low prevalence of systemic hypertension in their patients or the administration of medications (spironolactone and beta-blockers) that reduce atrial excitability. Spironolactone reduces myocardial fibrosis of dilated atria and P-wave duration, producing an antifibrotic effect in the ventricles and

reducing QT interval duration^[97,98]. Beta-blockers are given as prophylaxis for variceal bleeding, such as for large esophageal varices, resulting in vasoconstriction in the splanchnic compartment, which increases preload and improves diastolic function^[78]. Beta-blocker therapy may also prevent bleeding from portal hypertensive gastropathy and the development of spontaneous bacterial peritonitis. However, recent studies have warned about their use in decompensated cirrhosis, as they are associated with poor survival^[99,100]. Beta-blockers decrease chronotropy and depress atrioventricular conduction, resulting in bradycardia or high-grade heart block^[100].

Myocardial fibrosis is dysrhythmogenic^[98], and atrial interstitial fibrosis is associated with changes in the electrical properties of the atria, including depressed excitability, increased refractoriness and conduction slowing or block^[96,101]. Angiotensin-converting enzyme (ACE) inhibitors protect against myocardial fibrosis and prevent cardiac remodeling^[102] and atrial fibrillation^[103]. Drugs that interfere with the renin-angiotensin system, such as angiotensin II -receptor blockers, also prevent atrial remodeling^[103]. However, ACE inhibitors and other afterload-reducing drugs should be used with caution considering the risk for aggravating the vasodilatory state^[2].

Statins are known for their pleiotropic and anti-hypertrophic effects, suppressing arrhythmogenesis and improving endothelial function^[104]. Desensitization of cardiac myocytes to catecholamines due to down-regulation of beta-adrenergic receptors in the myocardium of cirrhotic patients could also be a protective mechanism against occurrence of tachyarrhythmia and atrial fibrillation^[96].

The main mechanisms explaining the influence of cirrhosis on the higher prevalence or the protection against atrial fibrillation are reviewed in Table 2.

THERAPY

No specific therapy can be recommended for cirrhotic patients with heart conditions, but it should be supportive and directed against heart failure and pulmonary stasis^[1,15]. Surgical stress, including transjugular intra-hepatic portosystemic shunt insertion, surgical porto-systemic shunting and liver transplantation can facilitate heart failure^[15,103]. However, severe heart failure can be prevented by vasodilated peripheral circulation, which unloads the heart, and a compensatory decrease of some negatively inotropic regulatory mechanisms^[15]. Aldosterone antagonists may reduce left ventricular dilatation and wall thickness, and improve diastolic function^[15]. QT interval prolongation may be improved by beta-blockers, which also lower portal pressure and reduce the hyperdynamic load, but their effect on contractile dysfunction and mortality should be the focus of further studies^[15,105].

Liver transplantation is currently the only proven treatment for patients with cirrhotic cardiomyopathy^[11]

Table 2 Atrial fibrillation in patients with liver cirrhosis

Higher prevalence due to	Lower prevalence due to
Enlarged left atria (cirrhotic cardiomyopathy)	Accumulation of antiarrhythmic and anti-inflammatory substances
Electrolyte imbalances	Low prevalence of hypertension
Hepatorenal syndrome	Medication: diuretics, beta-blockers, ACE-inhibitors, statins
Serum bile acid concentration	Downregulation of beta-adrenergic receptors in the myocardium
Metabolic abnormalities	
Inflammatory syndrome	
Atrial interstitial fibrosis	

ACE: Angiotensin-converting enzyme.

Table 3 Risk factors for arrhythmias after liver transplantation

Risk factor
Stress of major surgery
Advanced age
Comorbidities: low blood pressure, anemia, limitation of the cardiac reserve
Hydroelectrolytic and acid-base imbalances
Hypothermia
Secondary development of hypertension, diabetes mellitus, obesity

and can improve cardiac hypertrophy, diastolic and systolic function, and autonomic dysfunction^[1,2,14,15,29,41,106,107]. The prolonged QT interval reverses in approximately half of the patients after liver transplantation, likely a consequence of diminished portosystemic shunting, but can also be prolonged^[27,108]. Total cardiac events after liver transplantation, particularly arrhythmias, and post-transplant mortality are associated with prolonged QTc and the presence of a Q wave^[19]. A prolonged QTc interval also predicts post-transplant atrial arrhythmias^[19] and peri-transplant heart failure^[109]. However, liver transplantation is a stressful event for the cardiovascular system of the patients with advanced liver disease, considering also the advanced age and comorbidities^[110]. Furthermore, liver transplantation highlights the limitation of the cardiac reserve, even in patients with no previous history of cardiac disease^[15]. Although autonomic dysfunction, measured by heart rate variability, is partially corrected 2-6 years after liver transplantation, parasympathetic impairment is not improved^[107].

Considering the high prevalence of cirrhotic cardiomyopathy and coronary heart disease and the high perioperative mortality, a careful cardiac evaluation of patients with liver cirrhosis is required before liver transplantation, including electrocardiography, cardiopulmonary exercise testing, dobutamine stress echocardiography, coronary angiography and myocardial perfusion imaging, and coronary multidetector computed tomography angiography^[1,105,111]. Post-transplant reperfusion may result in cardiac death due to arrhythmias, acute heart failure, and myocardial infarction^[15,110,112]. Risk factors for arrhythmia occurring during reperfusion of the graft are severe hydroelectrolytic and acid-base imbalances and hypothermia^[112]. The most important risk factors

for arrhythmias after liver transplantation are included in Table 3. Zaballos *et al*^[112] reported the case of a man with severe hemodynamic alterations who developed atrioventricular re-entry tachycardia related to dual nodal conduction during liver transplantation. Kobayashi *et al*^[93] reported complete atrioventricular block and cardiac arrest with diffuse myocardial abscesses after liver transplantation in a woman with liver cirrhosis and hepatocellular carcinoma, which required resuscitation and temporary cardiac pacing. Chin *et al*^[113] described a case of torsade de pointes and a prolonged QTc after liver transplantation in a 39-year-old male patient with hepatitis B-related cirrhosis, which was due to low hematocrit and a low arterial blood pressure, demonstrating the importance of an optimal coronary perfusion to prevent sudden cardiac death. Cardiovascular disease also contributes to late mortality after transplantation, due to the secondary development of hypertension, hyperlipidemia, diabetes and obesity from chronic immunosuppression^[82].

CONCLUSION

The latency of cirrhotic cardiomyopathy requires careful assessment of arrhythmia risk in cirrhotic patients. To evaluate the predictive value of ventricular repolarization indices in liver cirrhosis, further follow-up studies are needed. In particular, future studies should focus on the relationships between arrhythmia risk and structural heterogeneity of the cirrhotic heart, markers of inflammation, fibrosis and immunologic syndromes, and biomarkers of liver cell death and active infection. Close monitoring of cirrhotic patients is needed for arrhythmias, particularly when QT interval-prolonging drugs are given, or if electrolyte imbalances or hepatorenal syndrome appear. Arrhythmia risk may persist after liver transplantation due to possible QT interval prolongation, persistence of the parasympathetic impairment, post-transplant reperfusion and chronic immunosuppression, as well as consideration of the fact that the transplant itself is a stressful event for the cardiovascular system.

REFERENCES

- 1 Møller S, Bernardi M. Interactions of the heart and the liver. *Eur*

- Heart J* 2013; **34**: 2804-2811 [PMID: 23853073 DOI: 10.1093/eurheartj/eh246]
- 2 **Fouad YM**, Yehia R. Hepato-cardiac disorders. *World J Hepatol* 2014; **6**: 41-54 [PMID: 24653793 DOI: 10.4254/wjh.v6.i1.41]
 - 3 **Al Hamoudi W**, Lee SS. Cirrhotic cardiomyopathy. *Ann Hepatol* 2006; **5**: 132-139 [PMID: 17060868]
 - 4 **Mozos I**. Ventricular arrhythmia risk in noncardiac diseases. In: Aronow WS, editor. Cardiac arrhythmias. Mechanisms, pathophysiology and treatment. Croatia: In Tech, 2014: 89-109
 - 5 **Wiegand J**, Berg T. The etiology, diagnosis and prevention of liver cirrhosis: part 1 of a series on liver cirrhosis. *Dtsch Arztebl Int* 2013; **110**: 85-91 [PMID: 23451000 DOI: 10.3238/arztebl.2013.0085]
 - 6 **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]
 - 7 **Boyer TD**, Manns MP, Sanyal AJ. Zakim and Boyer's Hepatology. A Textbook of Liver Disease. Philadelphia: Elsevier, Saunders, 2012
 - 8 **Zaky A**, Lang JD. Cirrhosis-associated cardiomyopathy. *J Anesth Clin Res* 2012; **3**: 266 [DOI: 10.4172/2155-6148.1000266]
 - 9 **Baik SK**, Fouad TR, Lee SS. Cirrhotic cardiomyopathy. *Orphanet J Rare Dis* 2007; **2**: 15 [PMID: 17389039 DOI: 10.1186/1750-1172-2-15]
 - 10 **Mozos I**, Costea C, Serban C, Susan L. Factors associated with a prolonged QT interval in liver cirrhosis patients. *J Electrocardiol* 2011; **44**: 105-108 [PMID: 21146831 DOI: 10.1016/j.jelectrocard.2010.10.034]
 - 11 **Wiese S**, Hove JD, Bendtsen F, Møller S. Cirrhotic cardiomyopathy: pathogenesis and clinical relevance. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 177-186 [PMID: 24217347 DOI: 10.1038/nrgastro.2013.210]
 - 12 **Merli M**, Calicchia A, Ruffa A, Pellicori P, Riggio O, Giusto M, Gaudio C, Torromeo C. Cardiac dysfunction in cirrhosis is not associated with the severity of liver disease. *Eur J Intern Med* 2013; **24**: 172-176 [PMID: 22958907 DOI: 10.1016/j.ejim.2012.08.007]
 - 13 **Moaref A**, Zamirani M, Yazdani M, Salehi O, Sayadi M, Aghasadeghi K. The Correlation between Echocardiographic Findings and QT Interval in Cirrhotic Patients. *Int Cardiovasc Res J* 2014; **8**: 39-43 [PMID: 24936479]
 - 14 **Huffman C**, Wagman G, Fudim M, Zolty R, Vittorio T. Reversible cardiomyopathies--a review. *Transplant Proc* 2010; **42**: 3673-3678 [PMID: 21094837 DOI: 10.1016/j.transproceed.2010.08.034]
 - 15 **Yang YY**, Lin HC. The heart: pathophysiology and clinical implications of cirrhotic cardiomyopathy. *J Chin Med Assoc* 2012; **75**: 619-623 [PMID: 23245476 DOI: 10.1016/j.jcma.2012.08.015]
 - 16 **Wong F**. Cirrhotic cardiomyopathy. *Hepatol Int* 2009; **3**: 294-304 [PMID: 19669380 DOI: 10.1007/s12072-008-9109-7]
 - 17 **Bernardi M**, Calandra S, Colantoni A, Trevisani F, Raimondo ML, Sica G, Schepis F, Mandini M, Simoni P, Contin M, Raimondo G. Q-T interval prolongation in cirrhosis: prevalence, relationship with severity, and etiology of the disease and possible pathogenetic factors. *Hepatology* 1998; **27**: 28-34 [PMID: 9425913]
 - 18 **Zardi EM**, Abbate A, Zardi DM, Dobrina A, Margiotta D, Van Tassel BW, Afeltra A, Sanyal AJ. Cirrhotic cardiomyopathy. *J Am Coll Cardiol* 2010; **56**: 539-549 [PMID: 20688208 DOI: 10.1016/j.jacc.2009.12.075]
 - 19 **Josefsson A**, Fu M, Björnsson E, Kalaitzakis E. Prevalence of pre-transplant electrocardiographic abnormalities and post-transplant cardiac events in patients with liver cirrhosis. *BMC Gastroenterol* 2014; **14**: 65 [PMID: 24708568 DOI: 10.1186/1471-230X-14-65]
 - 20 **Hansen S**, Møller S, Bendtsen F, Jensen G, Henriksen JH. Diurnal variation and dispersion in QT interval in cirrhosis: relation to haemodynamic changes. *J Hepatol* 2007; **47**: 373-380 [PMID: 17459513 DOI: 10.1016/j.jhep.2007.03.013]
 - 21 **Kowalski HJ**, Abelmann WH. The cardiac output at rest in Laennec's cirrhosis. *J Clin Invest* 1953; **32**: 1025-1033 [PMID: 13096569 DOI: 10.1172/JCI102813]
 - 22 **Genovesi S**, Prata Pizzala DM, Pozzi M, Ratti L, Milanese M, Pieruzzi F, Vincenti A, Stella A, Mancina G, Stramba-Badiale M. QT interval prolongation and decreased heart rate variability in cirrhotic patients: relevance of hepatic venous pressure gradient and serum calcium. *Clin Sci (Lond)* 2009; **116**: 851-859 [PMID: 19076059 DOI: 10.1042/CS20080325]
 - 23 **Bernardi M**, Maggioli C, Dibra V, Zaccherini G. QT interval prolongation in liver cirrhosis: innocent bystander or serious threat? *Expert Rev Gastroenterol Hepatol* 2012; **6**: 57-66 [PMID: 22149582 DOI: 10.1586/egh.11.86]
 - 24 **Akiyama T**, Batchelder J, Worsman J, Moses HW, Jedlinski M. Hypocalcemic Torsades de Pointes. *J Electrocardiol* 1989; **22**: 89-92 [PMID: 2921582]
 - 25 **Del Rosario ME**, Weachter R, Flaker GC. Drug-induced QT prolongation and sudden death. *Mo Med* 2010; **107**: 53-58 [PMID: 2022297]
 - 26 **Ward CA**, Ma Z, Lee SS, Giles WR. Potassium currents in atrial and ventricular myocytes from a rat model of cirrhosis. *Am J Physiol* 1997; **273**: G537-G544 [PMID: 9277435]
 - 27 **Adigun AQ**, Pinto AG, Flockhart DA, Gorski JC, Li L, Hall SD, Chalasani N. Effect of cirrhosis and liver transplantation on the gender difference in QT interval. *Am J Cardiol* 2005; **95**: 691-694 [PMID: 15721125 DOI: 10.1016/j.amjcard.2004.10.054]
 - 28 **Day CP**, James OF, Butler TJ, Campbell RW. QT prolongation and sudden cardiac death in patients with alcoholic liver disease. *Lancet* 1993; **341**: 1423-1428 [PMID: 8099138]
 - 29 **Finucci G**, Lunardi F, Sacerdoti D, Volpin R, Bortoluzzi A, Bombonato G, Angeli P, Gatta A. Q-T interval prolongation in liver cirrhosis. Reversibility after orthotopic liver transplantation. *Jpn Heart J* 1998; **39**: 321-329 [PMID: 9711183]
 - 30 **Luca C**. Electrophysiological properties of right heart and atrioventricular conducting system in patients with alcoholic cardiomyopathy. *Br Heart J* 1979; **42**: 274-281 [PMID: 508449]
 - 31 **Kupari M**, Koskinen P. Alcohol, cardiac arrhythmias and sudden death. *Novartis Found Symp* 1998; **216**: 68-79; discussion 79-85 [PMID: 9949788]
 - 32 **Moushmoush B**, Abi-Mansour P. Alcohol and the heart. The long-term effects of alcohol on the cardiovascular system. *Arch Intern Med* 1991; **151**: 36-42 [PMID: 1985607 DOI: 10.1001/archinte.1991.00400010060007]
 - 33 **Lorsheyd A**, de Lange DW, Hijmering ML, Cramer MJ, van de Wiel A. PR and QTc interval prolongation on the electrocardiogram after binge drinking in healthy individuals. *Neth J Med* 2005; **63**: 59-63 [PMID: 15766009]
 - 34 **Mozos I**, Serban C, Mihaescu R. Late ventricular potentials in cardiac and extracardiac diseases. In: Breijo-Marquez FR, editor. Cardiac arrhythmias-New considerations, Croatia: In Tech, 2012: 227-256
 - 35 **Zipes DP**, Camm AJ, Borggrefe M, Buxton AE, Chaitman B, Fromer M, Gregoratos G, Klein G, Moss AJ, Myerburg RJ, Priori SG, Quinones MA, Roden DM, Silka MJ, Tracy C, Priori SG, Blanc JJ, Budaj A, Camm AJ, Dean V, Deckers JW, Despres C, Dickstein K, Lekakis J, McGregor K, Metra M, Morais J, Osterspey A, Tamargo JL, Zamorano JL, Smith SC, Jacobs AK, Adams CD, Antman EM, Anderson JL, Hunt SA, Halperin JL, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Develop guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death) developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. *Europace* 2006; **8**: 746-837 [PMID: 16935866 DOI: 10.1093/europace/eul108]
 - 36 **Priori SG**, Aliot E, Blomstrom-Lundqvist C, Bossaert L, Breithardt G, Brugada P, Camm AJ, Cappato R, Cobbe SM, Di Mario C, Maron BJ, McKenna WJ, Pedersen AK, Ravens U, Schwartz PJ, Trusz-Gluza M, Vardas P, Wellens HJ, Zipes DP. Task Force on Sudden Cardiac Death of the European Society of Cardiology. *Eur Heart J* 2001; **22**: 1374-1450 [PMID: 11482917]

- DOI: 10.1053/euhj.2001.2824]
- 37 **de Vreede-Swagemakers JJ**, Gorgels AP, Weijnenberg MP, Dubois-Arbouw WI, Golombek B, van Ree JW, Knottnerus A, Wellens HJ. Risk indicators for out-of-hospital cardiac arrest in patients with coronary artery disease. *J Clin Epidemiol* 1999; **52**: 601-607 [PMID: 10391652]
 - 38 **di Giuseppe R**, de Lorgeril M, Salen P, Laporte F, Di Castelnuovo A, Krogh V, Siani A, Arnout J, Cappuccio FP, van Dongen M, Donati MB, de Gaetano G, Iacoviello L. Alcohol consumption and n-3 polyunsaturated fatty acids in healthy men and women from 3 European populations. *Am J Clin Nutr* 2009; **89**: 354-362 [PMID: 19056552 DOI: 10.3945/ajcn.2008.26661]
 - 39 **Kempler P**, Szalay F, Váradi A, Keresztes K, Kádár E, Tanczos E, Petrik J. Prolongation of the QTc-interval reflects the severity of autonomic neuropathy in primary biliary cirrhosis and in other non-alcoholic liver diseases. *Z Gastroenterol* 1993; **31** Suppl 2: 96-98 [PMID: 7483730]
 - 40 **Henriksen JH**, Fuglsang S, Bendtsen F, Christensen E, Møller S. Dyssynchronous electrical and mechanical systole in patients with cirrhosis. *J Hepatol* 2002; **36**: 513-520 [PMID: 11943423 DOI: 10.1016/S0168-8278(02)00010-7]
 - 41 **Zamirian M**, Tavassoli M, Aghasadeghi K. Corrected QT interval and QT dispersion in cirrhotic patients before and after liver transplantation. *Arch Iran Med* 2012; **15**: 375-377 [PMID: 22642249]
 - 42 **Fishberger SB**, Pittman NS, Rossi AF. Prolongation of the QT interval in children with liver failure. *Clin Cardiol* 1999; **22**: 658-660 [PMID: 10526691]
 - 43 **Arikan C**, Kilic M, Tumgor G, Levent E, Yuksekkaya HA, Yagci RV, Aydogdu S. Impact of liver transplantation on rate-corrected QT interval and myocardial function in children with chronic liver disease*. *Pediatr Transplant* 2009; **13**: 300-306 [PMID: 18537904 DOI: 10.1111/j.1399-3046.2008.00909.x]
 - 44 **Ytting H**, Henriksen JH, Fuglsang S, Bendtsen F, Møller S. Prolonged Q-T(c) interval in mild portal hypertensive cirrhosis. *J Hepatol* 2005; **43**: 637-644 [PMID: 16083986 DOI: 10.1016/j.jhep.2005.04.015]
 - 45 **Faigel DO**, Metz DC, Kochman ML. Torsade de pointes complicating the treatment of bleeding esophageal varices: association with neuroleptics, vasopressin, and electrolyte imbalance. *Am J Gastroenterol* 1995; **90**: 822-824 [PMID: 7733096]
 - 46 **Henriksen JH**, Gülberg V, Fuglsang S, Schifter S, Bendtsen F, Gerbes AL, Møller S. Q-T interval (QT(C)) in patients with cirrhosis: relation to vasoactive peptides and heart rate. *Scand J Clin Lab Invest* 2007; **67**: 643-653 [PMID: 17852825 DOI: 10.1080/00365510601182634]
 - 47 **Camm AJ**, Yap YG, Malik M. Acquired long QT syndrome. Wiley Online Library, 2007 [DOI: 10.1002/9780470994771.ch11]
 - 48 **Kosar F**, Ates F, Sahin I, Karıncaoglu M, Yildirim B. QT interval analysis in patients with chronic liver disease: a prospective study. *Angiology* 2007; **58**: 218-224 [PMID: 17495272 DOI: 10.1177/0003319707300368]
 - 49 **Trevisani F**, Di Micoli A, Zambruni A, Biselli M, Santi V, Erroi V, Lenzi B, Caraceni P, Domenicali M, Cavazza M, Bernardi M. QT interval prolongation by acute gastrointestinal bleeding in patients with cirrhosis. *Liver Int* 2012; **32**: 1510-1515 [PMID: 22776742 DOI: 10.1111/j.1478-3231.2012.02847.x]
 - 50 **Albarmawi A**, Czock D, Gauss A, Ehehalt R, Lorenzo Bermejo J, Burhenne J, Ganten TM, Sauer P, Haefeli WE. CYP3A activity in severe liver cirrhosis correlates with Child-Pugh and model for end-stage liver disease (MELD) scores. *Br J Clin Pharmacol* 2014; **77**: 160-169 [PMID: 23772874 DOI: 10.1111/bcp.12182]
 - 51 **Vuppalanchi R**, Liang T, Goswami CP, Nalamasu R, Li L, Jones D, Wei R, Liu W, Sarasani V, Janga SC, Chalasani N. Relationship between differential hepatic microRNA expression and decreased hepatic cytochrome P450 3A activity in cirrhosis. *PLoS One* 2013; **8**: e74471 [PMID: 24058572 DOI: 10.1371/journal.pone.0074471]
 - 52 **Chalasani N**, Gorski JC, Patel NH, Hall SD, Galinsky RE. Hepatic and intestinal cytochrome P450 3A activity in cirrhosis: effects of transjugular intrahepatic portosystemic shunts. *Hepatology* 2001; **34**: 1103-1108 [PMID: 11731998]
 - 53 **Frye RF**, Zgheib NK, Matzke GR, Chaves-Gnecco D, Rabinovitz M, Shaikh OS, Branch RA. Liver disease selectively modulates cytochrome P450-mediated metabolism. *Clin Pharmacol Ther* 2006; **80**: 235-245 [PMID: 16952490 DOI: 10.1016/j.clpt.2006.05.006]
 - 54 **Vuppalanchi R**, Juluri R, Ghabril M, Kim S, Thong N, Gorski JC, Chalasani N, Hall SD. Drug-induced QT prolongation in cirrhotic patients with transjugular intrahepatic portosystemic shunt. *J Clin Gastroenterol* 2011; **45**: 638-642 [PMID: 20962670 DOI: 10.1097/MCG.0b013e3181f8c522]
 - 55 **Werner CR**, Riessen R, Gregor M, Bitzer M. [Unexpected complication following esophageal variceal hemorrhage - Case 2/2011]. *Dtsch Med Wochenschr* 2011; **136**: 217 [PMID: 21271486 DOI: 10.1055/s-0030-1247621]
 - 56 **Stanek EJ**, Simko RJ, DeNofrio D, Pavri BB. Prolonged quinidine half-life with associated toxicity in a patient with hepatic failure. *Pharmacotherapy* 1997; **17**: 622-625 [PMID: 9165569 DOI: 10.1002/j.1875-9114.1997.tb03075.x]
 - 57 **Barre J**, Mallat A, Rosenbaum J, Deforges L, Houin G, Dhumeaux D, Tillement JP. Pharmacokinetics of erythromycin in patients with severe cirrhosis. Respective influence of decreased serum binding and impaired liver metabolic capacity. *Br J Clin Pharmacol* 1987; **23**: 753-757 [PMID: 3606934]
 - 58 **Chung EJ**, Jeon YS, Kim HJ, Lee KH, Lee JW, Han KA, Jung SH. Torsade de pointes in liver transplantation recipient after induction of general anesthesia: a case report. *Korean J Anesthesiol* 2014; **66**: 80-84 [PMID: 24567820 DOI: 10.4097/kjae.2014.66.1.80]
 - 59 **Lehmann M**, Bruns T, Herrmann A, Fritzenwanger M, Stallmach A. [54-year-old male with hepatic cirrhosis and therapy-associated torsade de pointes tachycardia]. *Internist (Berl)* 2011; **52**: 445-48, 450 [PMID: 20938628 DOI: 10.1007/s00108-010-2667-5]
 - 60 **Castro Hevia J**, Antzelevitch C, Tornés Bärzaga F, Dorantes Sánchez M, Dorticós Balea F, Zayas Molina R, Quiñones Pérez MA, Fayad Rodríguez Y. Tpeak-Tend and Tpeak-Tend dispersion as risk factors for ventricular tachycardia/ventricular fibrillation in patients with the Brugada syndrome. *J Am Coll Cardiol* 2006; **47**: 1828-1834 [PMID: 16682308 DOI: 10.1016/j.jacc.2005.12.049]
 - 61 **Antzelevitch C**, Sicouri S, Di Diego JM, Burashnikov A, Viskin S, Shimizu W, Yan GX, Kowey P, Zhang L. Does Tpeak-Tend provide an index of transmural dispersion of repolarization? *Heart Rhythm* 2007; **4**: 1114-1116; author reply 1116-1119 [PMID: 17675094 DOI: 10.1016/j.hrthm.2007.05.028]
 - 62 **Arteyeva NV**, Goshka SL, Sedova KA, Bernikova OG, Azarov JE. What does the T(peak)-T(end) interval reflect? An experimental and model study. *J Electrocardiol* 2013; **46**: 296.e1-296.e8 [PMID: 23473669 DOI: 10.1016/j.jelectrocard.2013.02.001]
 - 63 **Kilicaslan F**, Tokatli A, Ozdag F, Uzun M, Uz O, Isilak Z, Yiginer O, Yalcin M, Guney MS, Cebeci BS. Tp-e interval, Tp-e/QT ratio, and Tp-e/QTc ratio are prolonged in patients with moderate and severe obstructive sleep apnea. *Pacing Clin Electrophysiol* 2012; **35**: 966-972 [PMID: 22671991 DOI: 10.1111/j.1540-8159.2012.03439.x]
 - 64 **Demir C**, Demir M. Evaluation of Tp-e interval and Tp-e/QT ratio in patients with chronic hepatitis B. *Prague Med Rep* 2013; **114**: 239-245 [PMID: 24485341]
 - 65 **Zambruni A**, Trevisani F, Caraceni P, Bernardi M. Cardiac electrophysiological abnormalities in patients with cirrhosis. *J Hepatol* 2006; **44**: 994-1002 [PMID: 16510203 DOI: 10.1016/j.jhep.2005.10.034]
 - 66 **Møller S**, Hove JD, Diken U, Bendtsen F. New insights into cirrhotic cardiomyopathy. *Int J Cardiol* 2013; **167**: 1101-1108 [PMID: 23041091 DOI: 10.1016/j.ijcard.2012.09.089]
 - 67 **Benchimol Barbosa PR**, Sousa MO, Barbosa EC, Bomfim Ade S, Ginefra P, Nadal J. Analysis of the prevalence of ventricular late potentials in the late phase of myocardial infarction based on the site of infarction. *Arq Bras Cardiol* 2002; **78**: 352-363 [PMID: 12011951 DOI: 10.1590/S0066-782X2002000400002]
 - 68 **Pochmalicki G**, Genest M, Jibril H. Late ventricular potentials and heavy drinking. *Heart* 1997; **78**: 163-165 [PMID: 9326991]
 - 69 **Hayashi J**, Kashiwagi S, Okeda T, Okamura H, Ishibashi H,

- Hiramatsu Y, Fujino T. Electrocardiographic changes related to hypersecretion of catecholamine in a patient with fulminant hepatitis. *Jpn J Med* 1988; **27**: 187-190 [PMID: 3418984 DOI: 10.2169/internalmedicine1962.27.187]
- 70 Adler R, Takahashi M, Wright HT. Acute pericarditis associated with hepatitis B infection. *Pediatrics* 1978; **61**: 716-719 [PMID: 149291]
- 71 Ursell PC, Habib A, Sharma P, Mesa-Tejada R, Lefkowitz JH, Fenoglio JJ. Hepatitis B virus and myocarditis. *Hum Pathol* 1984; **15**: 481-484 [PMID: 6373562]
- 72 Matsumori A, Sasayama S. Newer aspects of pathogenesis of heart failure: hepatitis C virus infection in myocarditis and cardiomyopathy. *J Card Fail* 1996; **2**: S187-S194 [PMID: 8951578]
- 73 Rong Q, Huang J, Su E, Li J, Li J, Zhang L, Cao K. Infection of hepatitis B virus in extrahepatic endothelial tissues mediated by endothelial progenitor cells. *Virol J* 2007; **4**: 36 [PMID: 17407553 DOI: 10.1186/1743-422X-4-36]
- 74 Matsumori A, Ohashi N, Hasegawa K, Sasayama S, Eto T, Imaizumi T, Izumi T, Kawamura K, Kawana M, Kimura A, Kitabatake A, Matsuzaki M, Nagai R, Tanaka H, Hiroe M, Hori M, Inoko H, Seko Y, Sekiguchi M, Shimotohno K, Sugishita Y, Takeda N, Takihara K, Tanaka M, Yokoyama M. Hepatitis C virus infection and heart diseases: a multicenter study in Japan. *Jpn Circ J* 1998; **62**: 389-391 [PMID: 9626910 DOI: 10.1253/jcj.62.389]
- 75 Matsumori A, Matoba Y, Sasayama S. Dilated cardiomyopathy associated with hepatitis C virus infection. *Circulation* 1995; **92**: 2519-2525 [PMID: 7586353 DOI: 10.1161/01.CIR.92.9.2519]
- 76 Eguchi A, Wree A, Feldstein AE. Biomarkers of liver cell death. *J Hepatol* 2014; **60**: 1063-1074 [PMID: 24412608 DOI: 10.1016/j.jhep.2013.12.026]
- 77 Li F, Zhu CL, Zhang H, Huang H, Wei Q, Zhu X, Cheng XY. Role of hyaluronic acid and laminin as serum markers for predicting significant fibrosis in patients with chronic hepatitis B. *Braz J Infect Dis* 2012; **16**: 9-14 [PMID: 22358349]
- 78 Licata A, Corrao S, Petta S, Genco C, Cardillo M, Calvaruso V, Cabibbo G, Massenti F, Cammà C, Licata G, Craxi A. NT pro BNP plasma level and atrial volume are linked to the severity of liver cirrhosis. *PLoS One* 2013; **8**: e68364 [PMID: 23940514 DOI: 10.1371/journal.pone.0068364]
- 79 Panagopoulou V, Devereux S, Kossyvakis C, Raisakis K, Giannopoulos G, Bouras G, Pyrgakis V, Cleman MW. NTproBNP: an important biomarker in cardiac diseases. *Curr Top Med Chem* 2013; **13**: 82-94 [PMID: 23470072 DOI: 10.2174/1568026611313020002]
- 80 Wong F, Siu S, Liu P, Blendis LM. Brain natriuretic peptide: is it a predictor of cardiomyopathy in cirrhosis? *Clin Sci (Lond)* 2001; **101**: 621-628 [PMID: 11724649]
- 81 Henriksen JH, Gotze JP, Fuglsang S, Christensen E, Bendtsen F, Møller S. Increased circulating pro-brain natriuretic peptide (proBNP) and brain natriuretic peptide (BNP) in patients with cirrhosis: relation to cardiovascular dysfunction and severity of disease. *Gut* 2003; **52**: 1511-1517 [PMID: 12970147]
- 82 Garg A, Armstrong WF. Echocardiography in liver transplant candidates. *JACC Cardiovasc Imaging* 2013; **6**: 105-119 [PMID: 23328568 DOI: 10.1016/j.jcmg.2012.11.002]
- 83 Keffe BG, Valantine H, Keffe EB. Detection and treatment of coronary artery disease in liver transplant candidates. *Liver Transpl* 2001; **7**: 755-761 [PMID: 11552207 DOI: 10.1053/jlts.2001.26063]
- 84 Kalaitzakis E, Rosengren A, Skommevik T, Björnsson E. Coronary artery disease in patients with liver cirrhosis. *Dig Dis Sci* 2010; **55**: 467-475 [PMID: 19242795 DOI: 10.1007/s10620-009-0738-z]
- 85 Kadayifci A, Tan V, Ursell PC, Merriman RB, Bass NM. Clinical and pathologic risk factors for atherosclerosis in cirrhosis: a comparison between NASH-related cirrhosis and cirrhosis due to other aetiologies. *J Hepatol* 2008; **49**: 595-599 [PMID: 18662837 DOI: 10.1016/j.jhep.2008.05.024]
- 86 Ruiz-del-Arbol L, Monescillo A, Arocena C, Valer P, Ginès P, Moreira V, Milicua JM, Jiménez W, Arroyo V. Circulatory function and hepatorenal syndrome in cirrhosis. *Hepatology* 2005; **42**: 439-447 [PMID: 15977202 DOI: 10.1002/hep.20766]
- 87 Mozos I. Laboratory markers of ventricular arrhythmia risk in renal failure. *Biomed Res Int* 2014; **2014**: 509204 [PMID: 24982887]
- 88 Binah O, Rubinstein I, Bomzon A, Better OS. Effects of bile acids on ventricular muscle contraction and electrophysiological properties: studies in rat papillary muscle and isolated ventricular myocytes. *Naunyn Schmiedeberg's Arch Pharmacol* 1987; **335**: 160-165 [PMID: 3561530]
- 89 Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, Fukusumi S, Habata Y, Itoh T, Shintani Y, Hinuma S, Fujisawa Y, Fujino M. A G protein-coupled receptor responsive to bile acids. *J Biol Chem* 2003; **278**: 9435-9440 [PMID: 12524422]
- 90 Desai MS, Penny DJ. Bile acids induce arrhythmias: old metabolite, new tricks. *Heart* 2013; **99**: 1629-1630 [PMID: 23969477 DOI: 10.1136/heartjnl-2013-304546]
- 91 Rainer PP, Primessnig U, Harenkamp S, Doleschal B, Wallner M, Fauler G, Stojakovic T, Wachter R, Yates A, Groschner K, Trauner M, Pieske BM, von Lewinski D. Bile acids induce arrhythmias in human atrial myocardium—implications for altered serum bile acid composition in patients with atrial fibrillation. *Heart* 2013; **99**: 1685-1692 [PMID: 23894089 DOI: 10.1136/heartjnl-2013-304163]
- 92 Gundling F, Schmidler F, Zelihic E, Seidl H, Haller B, Ronel J, Löffler N, Schepp W. [Frequency of cardiac arrhythmia in patients with liver cirrhosis and evaluation of associated factors]. *Z Gastroenterol* 2012; **50**: 1149-1155 [PMID: 23150106 DOI: 10.1055/s-0032-1313182]
- 93 Kobayashi T, Sato Y, Yamamoto S, Oya H, Takeishi T, Kokai H, Hatakeyama K. Temporary cardiac pacing for fatal arrhythmia in living-donor liver transplantation: three case reports. *Transplant Proc* 2008; **40**: 2818-2820 [PMID: 18929869 DOI: 10.1016/j.transproceed.2008.07.018]
- 94 Ballestri S, Lonardo A, Bonapace S, Byrne CD, Loria P, Targher G. Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 1724-1745 [PMID: 24587651 DOI: 10.3748/wjg.v20.i7.1724]
- 95 Yap YG. Inflammation and atrial fibrillation: cause or parphenomenon? *Europace* 2009; **11**: 980-981 [PMID: 19635815 DOI: 10.1093/europace/eup191]
- 96 Zamirian M, Sarmadi T, Aghasadeghi K, Kazemi MB. Liver cirrhosis prevents atrial fibrillation: A reality or just an illusion? *J Cardiovasc Dis Res* 2012; **3**: 109-112 [PMID: 22629027 DOI: 10.4103/0975-3583.95363]
- 97 Milliez P, Deangelis N, Rucker-Martin C, Leenhardt A, Vicaut E, Robidel E, Beaufrils P, Delcayre C, Hatem SN. Spironolactone reduces fibrosis of dilated atria during heart failure in rats with myocardial infarction. *Eur Heart J* 2005; **26**: 2193-2199 [PMID: 16141258 DOI: 10.1093/eurheartj/ehi478]
- 98 Wong KY, Wong SY, McSwiggan S, Ogston SA, Sze KY, MacWalter RS, Struthers AD. Myocardial fibrosis and QTc are reduced following treatment with spironolactone or amiloride in stroke survivors: a randomised placebo-controlled cross-over trial. *Int J Cardiol* 2013; **168**: 5229-5233 [PMID: 23993727 DOI: 10.1016/j.ijcard.2013.08.027]
- 99 Sersté T, Melot C, Francoz C, Durand F, Rautou PE, Valla D, Moreau R, Lebre C. Deleterious effects of beta-blockers on survival in patients with cirrhosis and refractory ascites. *Hepatology* 2010; **52**: 1017-1022 [PMID: 20583214 DOI: 10.1002/hep.23775]
- 100 Ge PS, Runyon BA. The changing role of beta-blocker therapy in patients with cirrhosis. *J Hepatol* 2014; **60**: 643-653 [PMID: 24076364 DOI: 10.1016/j.jhep.2013.09.016]
- 101 Sanders P, Morton JB, Davidson NC, Spence SJ, Vohra JK, Sparks PB, Kalman JM. Electrical remodeling of the atria in congestive heart failure: electrophysiological and electroanatomic mapping in humans. *Circulation* 2003; **108**: 1461-1468 [PMID: 12952837 DOI: 10.1161/01.CIR.0000090688.49283.67]
- 102 Yu M, Zheng Y, Sun HX, Yu DJ. Inhibitory effects of enalaprilat on rat cardiac fibroblast proliferation via ROS/P38MAPK/TGF-β1

- signaling pathway. *Molecules* 2012; **17**: 2738-2751 [PMID: 22395404 DOI: 10.3390/molecules17032738]
- 103 **Schaer BA**, Schneider C, Jick SS, Conen D, Osswald S, Meier CR. Risk for incident atrial fibrillation in patients who receive antihypertensive drugs: a nested case-control study. *Ann Intern Med* 2010; **152**: 78-84 [PMID: 20083826 DOI: 10.7326/0003-4819-152-2-201001190-00005]
- 104 **Tousoulis D**, Oikonomou E, Siasos G, Stefanadis C. Statins in heart failure--With preserved and reduced ejection fraction. An update. *Pharmacol Ther* 2014; **141**: 79-91 [PMID: 24022031 DOI: 10.1016/j.pharmthera.2013.09.001]
- 105 **Møller S**, Dümcke CW, Krag A. The heart and the liver. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 51-64 [PMID: 19210113 DOI: 10.1586/17474124.3.1.51]
- 106 **Bal JS**, Thuluvath PJ. Prolongation of QTc interval: relationship with etiology and severity of liver disease, mortality and liver transplantation. *Liver Int* 2003; **23**: 243-248 [PMID: 12895263 DOI: 10.1034/j.1600-0676.2003.00833.x]
- 107 **Baratta L**, Tubani L, Merli M, Labbadia F, Facchini D, De Marco R, Rossi M, Attili AF, Berloco P, Ginanni Corradini S. Long-term effect of liver transplantation on cirrhotic autonomic cardiac dysfunction. *Dig Liver Dis* 2010; **42**: 131-136 [PMID: 19540819 DOI: 10.1016/j.dld.2009.05.009]
- 108 **Carey EJ**, Gautam M, Ingall T, Douglas DD. The effect of liver transplantation on autonomic dysfunction in patients with end-stage liver disease. *Liver Transpl* 2008; **14**: 235-239 [PMID: 18236403 DOI: 10.1002/lt.21350]
- 109 **Josefsson A**, Fu M, Allayhari P, Björnsson E, Castedal M, Olausson M, Kalaitzakis E. Impact of peri-transplant heart failure & left-ventricular diastolic dysfunction on outcomes following liver transplantation. *Liver Int* 2012; **32**: 1262-1269 [PMID: 22621679 DOI: 10.1111/j.1478-3231.2012.02818.x]
- 110 **Rugină M**, Predescu L, Sălăgean M, Gheorghe L, Gheorghe C, Tulbure D, Popescu I, Bubenek-Turconi S. Pre-liver transplantation, cardiac assessment. *Chirurgia (Bucur)* 2012; **107**: 283-290 [PMID: 22844825]
- 111 **Keeling AN**, Flaherty JD, Davarpanah AH, Ambrosy A, Farrelly CT, Harinstein ME, Flamm SL, Abecassis MI, Skaro AI, Carr JC, Gheorghide M. Coronary multidetector computed tomographic angiography to evaluate coronary artery disease in liver transplant candidates: methods, feasibility and initial experience. *J Cardiovasc Med (Hagerstown)* 2011; **12**: 460-468 [PMID: 21610507 DOI: 10.2459/JCM.0b013e3283483916]
- 112 **Zaballos M**, Jimeno C, Jiménez C, Fraile JR, Almendral E. [Dual atrioventricular nodal conduction and arrhythmia with severe hemodynamic alterations during liver retransplantation]. *Rev Esp Anestesiol Reanim* 2005; **52**: 355-358 [PMID: 16038175]
- 113 **Chin JH**, Park JY, Kim YK, Kim SH, Kong YG, Park PH, Hwang GS. Torsades de pointes triggered by severe diastolic hypotension with low hematocrit in the neohepatic stage of liver transplantation: a case report. *Transplant Proc* 2010; **42**: 1959-1962 [PMID: 20620555 DOI: 10.1016/j.transproceed.2010.02.093]

P- Reviewer: Sirin G, Sunami Y, Xiong XJ, Wang CX **S- Editor:** Qi Y
L- Editor: A **E- Editor:** Liu SQ



Recent advances in multidisciplinary management of hepatocellular carcinoma

Asmaa I Gomaa, Imam Waked

Asmaa I Gomaa, Imam Waked, Hepatology Department, National Liver Institute, Menoufiya University, Shebeen El-Kom 35111, Egypt

Author contributions: Gomaa AI and Waked I reviewed the literature and wrote the manuscript.

Conflict-of-interest: None related to this work; Waked I is speaker for Roche HL, MSD, BMS, and Gilead; advisory board for Janssen, Roche HL, and MSD; investigator for Roche HL, BMS, Bayer, Janssen, AbbVie, and Gilead.

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

Correspondence to: Asmaa I Gomaa, MD, Hepatology Department, National Liver Institute, Menoufiya University, Gamal Abd El-Nasir, Shebeen El-Kom 35111,

Egypt. aibrahim@liver-eg.org

Telephone: +20-100-6157160

Fax: +20-48-2234586

Received: August 28, 2014

Peer-review started: August 28, 2014

First decision: November 27, 2014

Revised: December 17, 2014

Accepted: January 15, 2015

Article in press: January 19, 2015

Published online: April 8, 2015

Abstract

The incidence of hepatocellular carcinoma (HCC) is increasing, and it is currently the second leading cause of cancer-related death worldwide. Potentially curative treatment options for HCC include resection, transplantation, and percutaneous ablation, whereas palliative treatments include trans-arterial chemoembolization (TACE), radioembolization, and systemic treatments. Due to the diversity of available treatment options and patients' presentations, a multidisciplinary

team should decide clinical management of HCC, according to tumor characteristics and stage of liver disease. Potentially curative treatments are suitable for very-early- and early-stage HCC. However, the vast majority of HCC patients are diagnosed in later stages, where the tumor characteristics or progress of liver disease prevent curative interventions. For patients with intermediate-stage HCC, TACE and radioembolization improve survival and are being evaluated in addition to potentially curative therapies or with systemic targeted therapy. There is currently no effective systemic chemotherapy, immunologic, or hormonal therapy for HCC, and sorafenib is the only approved molecular-targeted treatment for advanced HCC. Other targeted agents are under investigation; trials comparing new agents in combination with sorafenib are ongoing. Combinations of systemic targeted therapies with local treatments are being evaluated for further improvements in HCC patient outcomes. This article provides an updated and comprehensive overview of the current standards and trends in the treatment of HCC.

Key words: Hepatocellular carcinoma; Molecular targeted agents; Radiofrequency ablation; Sorafenib; Trans-arterial chemoembolization

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: This article reviews the available treatment options for hepatocellular carcinoma. The recent clinical trials of molecular-targeted therapies, as single agents or in combination with other treatments, are reviewed, and some future study directions are addressed. The importance of a multidisciplinary approach to management is highlighted.

Gomaa AI, Waked I. Recent advances in multidisciplinary management of hepatocellular carcinoma. *World J Hepatol* 2015; 7(4): 673-687 Available from: URL: <http://www.wjgnet.com>

INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is increasing, and is currently the second leading cause of cancer-related death worldwide, accounting for approximately 800000 deaths every year^[1]. Clinical management of HCC is tailored according to tumor characteristics, stage of liver disease, and condition of the patients (age, performance status, and presence or absence of comorbidities). The American Association for the Study of Liver Diseases^[2] and the European Association for the Study of the Liver (EASL)^[3] endorse the use of Barcelona clinic liver cancer (BCLC) staging for the classification and management of patients with HCC. Therapeutic options are stage dependent and can be classified into three categories: curative, palliative, and symptomatic. However, curative treatment options, including resection and percutaneous ablation, are only suitable for early-stage tumors, and are associated with five-year survival rates of up to 75%^[4].

Recently, treatment indications have been refined; patients who are not candidates for the first-line therapy for their stage can be shifted to the treatment option for the next BCLC stage (treatment stage migration concept)^[3,5]. Trans-arterial chemoembolization (TACE) can be performed at an early stage in patients for whom radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI) cannot be performed because of tumor location (proximity to a gallbladder, biliary tree, or blood vessel), unresectability of the tumor, failed prior curative treatments, or medical comorbidities^[6].

The presentations of HCC are variable within each patient. Although the management guidelines for HCC recommend monotherapies as a treatment option, combined or sequential treatment modalities are effective in improving the outcome of patients with HCC. In practice, a multi-modal approach combining various treatments is used, and a multidisciplinary team, where the roles are intertwined and complimentary, should be involved in the management of every case^[7,8].

SURGICAL RESECTION

Surgical resection is the recommended treatment for patients with a single nodule, preserved liver function, and good performance status. It is associated with five-year survival rates up to 70%^[9] and a 2%-3% perioperative mortality in cirrhotic patients. Some centers report five-year survival rates above 50% in patients undergoing resection for multiple tumors fulfilling Milan criteria (up to three nodules, each ≤ 3 cm), who are not suitable for transplantation^[10], and resection in patients with more advanced stages of HCC has been reported with acceptable outcomes^[11].

The minimal critical remnant liver volume for safe resection is approximately 25% (15%-40%) for patients without cirrhosis and 50% (25%-90%) for patients with cirrhotic livers. Preoperative portal vein embolization is occasionally performed when the estimated remnant liver volume is less than the minimal requirement^[12], aimed at diverting portal flow, with its content of growth factors, to the non-tumorous lobe to sufficiently increase its size to permit resection. However, the effectiveness of portal vein embolization in cases of HCC with a cirrhotic liver has not been sufficiently tested in large controlled studies^[3].

Portal hypertension in cirrhotic patients is considered a relative contraindication for surgical resection, and a hepatic venous pressure gradient > 10 mmHg is reportedly the best predictor of postoperative liver decompensation and poor long-term outcome in compensated cirrhotic patients undergoing resection^[2,13]. In practice, resection for patients with significant portal hypertension is still a subject of debate. Similarly, the presence of splenomegaly (major diameter > 12 cm) or esophageal varices with a platelet count of $< 100000/\text{mm}^3$ was correlated with hepatic venous pressure gradient, postoperative decompensation, and poor survival^[14]. However, Cucchetti *et al.*^[15] reported that patients with the same model for end-stage liver disease (MELD) score and extent of hepatectomy had similar outcomes regardless of portal hypertension.

Resection has been refined with the use of the RFA-based resection device, the Habib 4X sealer (a new bipolar RF device designed specifically for liver resection). It releases controlled RF energy between two pairs of electrodes, producing a plane of coagulative necrosis along the intended line of parenchymal resection, avoiding over-coagulation of liver parenchyma. The heat produced seals biliary and blood vessels, resulting in minimal blood loss. With this device, morbidity and mortality rates are superior to other methods of liver resection^[16].

Laparoscopic resection, though a more sophisticated surgical procedure, is associated with reduced operative and postoperative morbidities^[17]. A recent meta-analysis showed that laparoscopic hepatectomy decreases blood loss, transfusion requirement, postoperative morbidity, recovery time, and hospital stay compared to open hepatectomy, with no difference in recurrence or survival^[18]. However, no randomized controlled trials (RCTs) were reported in this meta-analysis.

An important postoperative concern is the high risk of HCC recurrence. Five-year recurrence rates of 68% have been reported after liver resection of very-early-stage HCC. The presence of satellite nodules, cirrhosis, the use of non-anatomic resection, and elevated α -fetoprotein (AFP) levels are independently associated with tumor recurrence^[14,19]. Late recurrence can be predicted using molecular biomarkers and gene signatures^[20] that are used for the selection of patients amenable to hepatic resection. The 5-gene score, based on combined expression levels of *HN1*, *RAN*,

RAMP3, *KRT19* and *TAF9*, was associated with disease-specific survival^[20].

LIVER TRANSPLANTATION

Liver transplantation (LTx) is the best treatment option for patients with decompensated cirrhosis. HCC is the only solid tumor where transplantation plays an important role in management, due to the fact that it allows removal of the primary tumor and treats hepatic insufficiency^[21]. The main obstacles for HCC patients amenable to LTx are the organ shortage and the long waiting time for transplantation. Increasing the donor pool by live donation, using bridging therapy, and applying prioritization policies can help overcome this problem^[22]. A MELD exception was developed to assign extra points to HCC patients due to their high dropout rate and mortality while on the waiting list. However, no extra points are assigned to patients with compensated cirrhosis and small HCC tumors (< 2 cm) because of the improved survival with local ablation^[3]. In practice, LTx is recommended for patients with tumors within the Milan criteria (a single lesion \leq 5 cm, or up to three lesions \leq 3 cm each)^[23]. Restriction of LTx to patients within the Milan criteria results in a five-year overall survival rate of 75%, with a risk of recurrence < 15%^[24]. The perioperative mortality and one-year mortality are approximately 3 and \leq 10%, respectively. For patients with early-stage HCC, LTx offers the best chance of survival (106 mo), compared with surgical resection (52 mo), RFA (62 mo), PEI (44 mo), and TACE (34 mo)^[25].

A systematic review of 90 studies over 15 years, including 17780 patients, identified the Milan criteria as an independent prognostic factor for outcome after LTx, with five-year survival rates comparable to non-HCC patients (65%-78%)^[24]. An expansion of the Milan criteria to "up-to-seven" criteria (the sum of the size of the largest tumor and the number of tumors in patients without microvascular invasion) was proposed^[23] and externally validated in an independent series^[26], but requires larger prospective validation studies^[3]. Although listing criteria for LTx currently depend on tumor number and size, the use of molecular markers and gene signatures for determining tumor behavior are under development^[27].

The presence of vascular invasion, high AFP level, and transplant waiting time of more than 6 mo, are considered accurate predictive factors for poor survival and recurrence risk. Increased AFP was associated with higher risk of progression and dropout while waiting for a transplant^[28,29], and a steady increase of AFP > 15 ng/mL per month was considered the most significant prognostic determinant^[30]. In a large French multicenter study, incorporation of AFP in a prognostic score model for post-LTx outcome significantly improved the predictive performance of the Milan criteria in prioritization for LTx^[29]. Moreover, adding AFP > 400 to a total tumor volume of 115 cm as a cutoff improved

prognosis prediction in an analysis of data of 6478 patients from the Scientific Registry of Transplant Recipients, and performed better than tumor size and number characteristics for predicting post-LTx prognosis^[31].

LOCAL ABLATIVE THERAPY

Tumor ablation can be obtained using either chemical (alcohol and acetic acid) or physical (heating or cooling) methods. The first technique used to locally treat HCC was PEI^[32], which involves the intralesional injection of absolute alcohol. Temperature ablative techniques have advanced, including heating techniques such as RFA^[33], microwave ablation (MWA)^[34], laser ablation^[35], and cryoablation^[36].

PEI

PEI is indicated for the treatment of nodular HCC \leq 5 cm in diameter, and achieves complete necrosis in 90% of tumors < 2 cm, 70% in those 2- < 3 cm, and 50% in those between 3 and 5 cm^[37]. Patient outcome was improved with the use of a specific needle with three retractable prongs, achieving an 80%-90% rate of sustained complete response in tumors < 4 cm^[38]. The major limitation of PEI is the high incidence of local recurrence (33%-43%).

RFA

RFA is superior to other local ablative therapies, and is currently the most commonly used ablative method, replacing PEI as the locoregional therapy of choice for early HCC^[37]. RFA is considered the standard of care for patients with very early- and early-stage tumors, as well as those not suitable for or that refuse surgery. RFA is recommended as the main ablative therapy for tumors < 5 cm, whereas PEI is recommended in cases where RFA is not technically feasible^[3].

In a cohort study, complete ablation was achieved in more than 90% of cases, with a local recurrence rate of < 1% and five-year survival rate ranging from 40% to 70% for lesions < 2 cm in diameter^[39]. Three independent meta-analyses, including five RCTs, showed better results regarding local tumor control and survival benefits in patients treated with RFA, compared to ablation with PEI. In addition, patients with tumors 2-5 cm had better survivals if treated by RFA rather than by PEI^[40-42].

Some groups have suggested that RFA should be considered as a first-line therapy, even when resection is possible, because it is associated with fewer side effects^[39]. The main advantages compared to surgical intervention are that it is less invasive and provides an increased possibility for parenchymal sparing^[39,43]. Whether surgical resection for very early HCC is superior to RFA remains controversial. Whereas a Markov model analysis indicated that surgical resection was preferable to RFA in terms of overall survival^[44], Peng *et al.*^[45] reported that RFA was better. A survey in Japan

including 1235 patients with very early HCC (≤ 2 cm) who underwent resection and 1315 patients who received RFA showed no significant difference in overall survival between the two groups (one-year, 98% vs 99%; two-year, 94% vs 95%), over a median follow-up of 37 mo^[46]. However, the disease-free survival rate was significantly better after resection than after RFA (one-year, 91% vs 84%; two-year, 70% vs 58%; $P < 0.001$). Similarly, Wang *et al.*^[47] suggested that surgical resection was equivalent to RFA in terms of overall survival, and was associated with better disease-free survival.

The size limitation of RFA has been overcome with the use of expandable tipped or cool-tip electrodes, allowing effective ablation of areas ≥ 5 cm in diameter^[48]. However, RCTs with a large sample size are needed before ablation therapy can be confirmed as an alternative to surgery for potentially resectable HCC.

Other ablative therapies

MWA is an alternative to RFA for thermal ablation of HCC. Only one RCT^[49] compared the effectiveness of MWA to RFA, which revealed a tendency to favor RFA with respect to rates of local recurrence and complications, likely due to the small volume of coagulation obtained with a single probe insertion^[50]. However, newer devices may have overcome this limitation. One advantage of MWA over RFA is that treatment outcome is not affected by the heat-sink effect of vessels in proximity to the tumor^[49].

Laser ablation refers to thermal tissue destruction by conversion of absorbed light into heat^[51]. The only randomized prospective study comparing laser ablation with RFA reported no significant difference in overall survival rates, with cumulative rates of 91.8%, 59.0% and 28.4% at one, three and five years respectively, without significant complications. However, a significantly better survival rate was reported for RFA in patients with Child-Pugh A stage disease^[52].

Cryoablation uses the extreme cold of liquid nitrogen or argon gas to destroy abnormal tissue^[53]. Cryoablation showed better local control than RFA or MWA for tumors > 2 cm^[54]. A multicenter RCT in China that included 360 patients with one or two tumors < 4 cm in diameter found that cryoablation is safe and as effective as RFA, with a similar five-year survival^[55].

TACE

HCC receives 90% of its blood supply from the hepatic artery and only 10% from the portal vein^[56]. Thus, the purpose of trans-arterial therapy is to block the blood supply and induce tumor necrosis, without significantly affecting hepatic blood supply^[57]. Trans-arterial therapies include TACE, trans-arterial embolization, trans-arterial chemotherapy, and trans-arterial radioembolization^[57,58].

TACE is currently the standard of care for patients with compensated liver function and large multifocal

lesions without evidence of vascular invasion or extra-hepatic spread^[3]. In Japan, TACE is recommended even for HCC patients with vascular invasion if radiologic portal invasion is distal to, or in the second-order branches of, the portal vein^[59]. The main contraindications to TACE are extended portal vein thrombosis, diffuse tumor, extra-hepatic spread, and decompensated liver cirrhosis^[22,60]. TACE improves survival compared to supportive care or suboptimal therapies^[61], observed as an increase in the median survival of patients with intermediate-stage HCC to 20 mo^[62]. However, a meta-analysis that included nine trials (six trials assessing TACE and three trials assessing trans-arterial embolization) has shown that trans-arterial therapy does not significantly increase survival in patients with unresectable HCC compared to controls^[63].

Proper patient selection is crucial to prevent post-TACE-induced liver failure. Patients with total bilirubin > 3 mg/dL were excluded from TACE in several studies^[64,65], and an AFP > 200 ng/mL and a MELD score > 10 were associated with greater risk of mortality^[66]. Bolondi *et al.*^[67] proposed a substaging of intermediate-stage HCC (BCLC-B) patients from B1 to B4, taking into account the tumor burden and Child-Pugh score (A5 to B9). BCLC-B includes disease ranging from variable tumor burden, which can be a multifocal HCC affecting both lobes, extending up to near replacement of the liver, and includes patients with a wide range of liver function impairment (Child-Pugh score from 5 to 9). Substaging revealed decreasing survival for higher B substages, and thus TACE was recommended for early subgroups only^[67].

Drug-eluting beads (DEB)-TACE involves the use of embolic microspheres with the ability to sequester and release chemotherapeutic agents in a controlled manner over a one-week period, which subsequently increases the local concentration of the drug with minimal systemic toxicity^[68]. A randomized phase II study (the PRECISIONV trial) reported that DEB-TACE is a valuable alternative and may be preferred over conventional TACE^[69].

Assessment of response to TACE

The use of locoregional options to induce tumor necrosis necessitated a refinement of the conventional criteria to evaluate treatment response. Extent of tumor necrosis has been correlated with outcome after ablation, TACE and systemic therapy. A modification of the response evaluation criteria in solid tumors (modified RECIST) takes into account the degree of tumor necrosis, evaluated by dynamic computed tomography or magnetic resonance imaging^[70] and has been adopted by the latest EASL guidelines for evaluating locoregional therapies for HCC^[3].

Failure of TACE

There is no established definition for TACE refractoriness, nor is there a consensus for when to consider TACE failure and refer the patient to an alternative

therapy. Despite the absence of solid evidence, however, panels of experts have proposed treatment migration to sorafenib (downward treatment stage migration) for intermediate-stage patients if they demonstrate disease progression or poor tolerance after first or second TACE^[71,72]. The current EASL guidelines recommend switching to sorafenib if intermediate-stage patients are non-responsive to at least two cycles of TACE^[3].

Repetition of TACE should be considered based on evidence using mRECIST and the risk of adverse events. The response to the first TACE and its effect on the underlying liver disease help in identifying patients at risk of adverse outcome with repeated TACE. Sieghart *et al.*^[73] conducted a multivariate analysis to investigate TACE repeated for a second or third session and identified three prognostic factors: increase in aspartate aminotransferase by > 25%, increase in Child-Pugh score, and absence of tumor response. These factors were incorporated into an "ART" score, and patients with an ART score of 0-1.5 points benefitted from a second TACE, whereas those with a score \geq 2.5 did not^[74].

RADIOEMBOLIZATION

Radioembolization, or selective internal radiation therapy (SIRT), has recently emerged as a therapeutic option for intermediate-stage HCC. Unlike TACE, SIRT delivers local radiation to the tumor or liver tissue without causing ischemia. β radiation from radioactive yttrium-loaded glass or resin microspheres is applied to the tumor through the arteries that feed it, so that tumor nodules are treated irrespective of their number, size, or location^[75]. The procedure is well tolerated with survival rates similar to TACE. Moreover, it is as safe and effective as sorafenib in patients with more advanced-stage HCC, including patients with portal vein thrombosis and large tumor burden^[76-79].

In a study comparing radioembolization to TACE, radioembolization was associated with fewer side effects, better response rate, and longer time to progression (13.3 mo vs 8.4 mo), without difference in median survival time (20.5 mo vs 17.5 mo)^[80]. Another study reported similar safety profile and response rates^[76]. However, the cost associated with radioembolization may limit the applicability of this technique.

Stereotactic radiotherapy (SRT) allows the delivery of a high dose of radiation in a single (radio-surgery) or limited number (hypo-fractionation) of sessions, while sparing surrounding structures and healthy tissue^[81]. Blomgren *et al.*^[82] first introduced SRT for liver tumors in 1995, with treatment doses ranging from 15 to 45 Gy, in one to five fractions. In a phase I / II study using a single dose ranging from 14 to 26 Gy, the treatment was well tolerated in all patients, with no major side effects^[83], and the tumor control rate at 6 wk was 98%^[84].

The CyberKnife Radio-surgery System is able to deliver very high doses of radiation to both primary and metastatic liver tumors with extreme accuracy, and

treatments can be completed in one to five sessions. Louis *et al.*^[81] treated 25 patients with CyberKnife stereotactic radiotherapy using respiratory motion tracking, which enables the radiation beam to track tumor movement in real time and allows patients to breathe normally during their treatment sessions. The actuarial one- and two-year local control rates were 95%, and the one- and two-year survival rates were 79% and 52% respectively, with good clinical tolerance. CyberKnife and SRT (though currently still very expensive) offer a local therapy for HCC patients who are not eligible for surgery, embolization, chemotherapy or radiofrequency ablation, without significant complications.

SYSTEMIC CHEMOTHERAPY

HCC is among the most chemoresistant tumors, and until 2007, no systemic chemotherapy was recommended for patients with advanced tumors^[3]. Systemic chemotherapy with cytotoxic agents, such as doxorubicin, gemcitabine, cisplatin, 5-fluorouracil or combined regimens for palliative care, was associated with low response rates (< 10%) with only marginal improvements in survival^[85]. Moreover, these drugs are poorly tolerated in patients with underlying liver cirrhosis^[85-87].

Interferon (IFN) therapy^[87], anti-androgens, or tamoxifen^[88] used in the treatment of advanced HCC show contradictory results without obvious benefit. A meta-analysis of seven RCTs, including 898 patients, evaluated tamoxifen vs conservative management and showed neither anti-tumor effects nor survival benefits for tamoxifen^[89]. Subsequent large RCTs reported negative results in terms of survival^[90,91].

Cisplatin, IFN, doxorubicin, and fluorouracil (PIAF) used in combination showed promising activity in a phase II study^[92]. A randomized phase III study including 188 patients with HCC was conducted to investigate the effect of PIAF combination compared to doxorubicin alone^[87]. The median survival rate of the PIAF group did not significantly differ from the doxorubicin group (8.67 mo vs 6.83 mo), and patients treated with the PIAF regimen experienced a significantly higher rate of myelotoxicity.

TARGETED THERAPY FOR HCC

Hepatocarcinogenesis is associated with epigenetic and genetic alterations that eventually lead to uncontrolled growth of hepatocytes. Signal transduction pathways, oncogenes, and growth factors and their receptors are considered new potential therapeutic targets for systemic targeted therapies, limiting widespread systemic toxicity^[93]. Several targeted agents are currently in clinical development.

Sorafenib

Sorafenib is an orally administered multikinase inhibitor with antiproliferative and antiangiogenic activity^[94]. Sorafenib mediates downregulation of anti-apoptotic

Table 1 Phase III trials of some systemic targeted agents in advanced hepatocellular carcinoma

Ref.	Study design	Patients, <i>n</i>	Overall survival, mo
Zhu <i>et al</i> ^[109] (SEARCH trial)	Sorafenib <i>vs</i> sorafenib + erlotinib	358 <i>vs</i> 362	Sorafenib: 8.5 Sorafenib + erlotinib: 9.5
Cheng <i>et al</i> ^[105] (SUN1170 trial)	Sorafenib <i>vs</i> sunitinib	544 <i>vs</i> 530	Sorafenib: 10.2 Sunitinib: 7.9
Cainap <i>et al</i> ^[106] (LIGHT trial)	Sorafenib <i>vs</i> linifanib	517 <i>vs</i> 518	Sorafenib: 9.8 Linifanib: 9.1
Johnson <i>et al</i> ^[107] (BRISK-FL trial)	Sorafenib <i>vs</i> brivanib	578 <i>vs</i> 577	Sorafenib: 9.9 Brivanib: 9.5
Llovet <i>et al</i> ^[108] (BRISK-PS trial)	Brivanib <i>vs</i> placebo	263 <i>vs</i> 132	Brivanib: 9.4 Placebo: 8.3

proteins, leading to enhanced cytotoxicity of HCC cells to tumor necrosis factor-related apoptosis inducing ligand^[95]. Two phase III randomized placebo-controlled trials, the SHARP multicenter trial^[96] and the Asia-Pacific trial^[97], reported improved overall survival and better outcome for patients who received sorafenib, which was generally well tolerated with mild toxicity. The two most common grade 3 adverse reactions with sorafenib were the hand-foot-skin reaction (8%) and diarrhea (8%). The overall incidence of serious adverse events in the sorafenib and placebo groups was comparable (52% and 54%, respectively).

Based on these findings, sorafenib was approved for treatment of advanced HCC, including patients with unresectable Child-Pugh A or B HCC with performance status 0-2 and vascular invasion or distant metastasis^[3], as well as for patients intolerant to TACE or in whom the procedure is technically difficult^[98,99]. However, the prognosis for patients with this stage of HCC is still poor, with a median overall survival rate of 6.5-10.7 mo^[96]. In addition, Cammà *et al*^[100] recently concluded that sorafenib at full dose was not a cost-effective treatment compared to best supportive care in intermediate- and advanced-stage HCC.

Sorafenib is currently being tested as an adjuvant after resection, with local ablation for early-stage HCC, in combination with chemoembolization for intermediate stages^[101], in combination with erlotinib or systemic doxorubicin in advanced stages. Additionally, sorafenib was effective as a first-line treatment in Child-Pugh B patients with lower survival^[3]. In a large retrospective study, the median survival with sorafenib was 5.5 mo compared to 11.3 mo for Child-Pugh A patients^[102]. The prospective GIDEON trial confirmed that the median overall survival was shorter in Child-Pugh class B patients (5.2 mo *vs* 13.6 mo in Child A), although the time to progression was similar across subgroups. Serious adverse events were more common in Child-Pugh class B patients^[103,104].

Other molecular targeted agents

The antiangiogenic tyrosine kinase inhibitors, sunitinib^[105], linifanib^[106], brivanib^[107,108], or the combination of sorafenib with erlotinib^[109] are not superior to

sorafenib in sorafenib-naïve advanced HCC patients, or as a second-line therapy^[110] (Table 1). This may be due to the fact that inhibition of a single signaling pathway can induce feedback activation of other pathways. Therefore, combination therapies may demonstrate beneficial synergistic activity^[111].

Many molecular-targeted agents other than sorafenib, used in combination or with sorafenib, are in different stages of clinical development, with encouraging results from phase I - II studies^[112-115]. The first phase III study of combination therapy in advanced HCC was SEARCH, a randomized trial testing sorafenib with the epithelial growth factor tyrosine kinase inhibitor erlotinib, which found no survival benefit over sorafenib alone^[109].

PREVENTION OF HCC RECURRENCE

Persistence of chronic viral hepatitis in patients treated for HCC is associated with increased rates of recurrence and poor survival, thus control of hepatitis C virus (HCV) replication is an important factor for infected patients. IFN therapy following successful ablation of HCC was shown to be safe and lead to a reduction in recurrence, and patients who continued IFN therapy after tumor ablation had better survival^[116]. Long-term, intermittent standard IFN therapy successfully delayed recurrence of HCC after RFA, PEI, and surgical resection^[117]. A meta-analysis evaluating the effect of adjuvant standard IFN treatment following resections showed significant improvement in three-year recurrence-free survival (54% *vs* 30%)^[118], and other studies have shown similar results^[3,119,120]. The use of pegylated-IFN was more effective, and postoperative administration in combination with ribavirin for ≥ 16 wk was associated with reduced recurrence of HCC in patients with HCV infection^[121]. Further improvement in prognosis may be expected with the higher efficacy of direct antiviral therapy.

Patients with hepatitis B virus (HBV)-related HCC, even after successful treatment of the initial tumor, usually have multiple recurrences or metastases. High viral load is one of the most important risk factors for HCC development and recurrence following surgical

resection^[122]. Similar to HCV, antiviral therapy for HBV following curative HCC ablation improved patient survival and decreased HCC recurrence. In their study, Hann *et al.*^[123] followed patients for 12 years who underwent local tumor ablation with or without concomitant antiviral therapy with lamivudine. Although initially there was no difference between the treatment groups with respect to tumor size (all ≤ 7 cm), levels of AFP and albumin, antiviral therapy was significantly associated with increased median survival (36 mo vs 16 mo)^[123].

No other modality has demonstrated equivalent effectiveness for decreasing recurrence after curative treatment of HCC as antiviral therapy has for viral hepatitis-related tumors. Chemoembolization^[124], internal radiation^[125,126], immune therapies^[127], retinoids^[128], and the heparanase inhibitor PI-88^[129] have been investigated as methods of reducing postoperative recurrence; however, none can be recommended as a preoperative/postoperative adjuvant/neo-adjuvant therapy for improving prognosis and diminishing the incidence of recurrence following curative therapy.

MULTIDISCIPLINARY TEAM

HCC has diverse presentations that are compounded by the status of liver disease, and the multiple treatment options available make choosing the first line of treatment for a given patient a difficult task. Treatment of HCC patients should be undertaken by a multidisciplinary team that includes all the specialties involved in delivering the different therapies. In addition, simultaneous or sequential multi-modal therapies for patients with HCC show promise for improving patient outcome, further emphasizing the importance of a multidisciplinary approach to HCC management.

The multidisciplinary team should include hepatologists, medical and surgical oncologists, transplant surgeons, diagnostic and interventional radiologists, radiation oncologists, and pathologists^[130]. All members should play an active role, as their expertise is required to provide optimal care for patients with HCC. The hepatologist should assess underlying liver disease, identify patients at risk for HCC, and monitor for early detection. Hepatologists are essential for managing liver disease and its complications, arranging for and monitoring treatment, and referring eligible patients for LTx. Oncologists are responsible for assigning systemic or targeted therapy as initial treatment or adjuvant therapy, and for managing associated side effects. The diagnostic radiologist makes and confirms the diagnosis, stages the tumor, its spread and vascular invasion, and assesses the radiologic response to treatment. The interventional radiologist delivers ablative therapy in early stages, and palliative therapy for intermediate-stage tumors. The hepatobiliary surgeon evaluates for and performs resection or transplantation. The pathologist assesses the grade of tumor differentiation,

stage of progression, and evaluates tissue markers. This multidisciplinary team also involves nurses, supportive care specialists, and palliative physicians^[130].

MULTI-MODAL THERAPIES

With the multidisciplinary approach, various treatments are being delivered simultaneously or sequentially, as first- or second-line therapies, to improve patient outcome.

Transplantation and locoregional treatment

Patients whose tumors exceed the Milan criteria can undergo locoregional treatment (TACE or RFA) to down-stage the tumor to within the Milan criteria to allow LTx. Two prospective studies showed similar survival after LTx for patients with successfully down-staged HCC compared with those who initially met the Milan criteria^[131,132].

Neo-adjuvant therapies for patients while on the waiting list are used in most centers. Systemic and interventional treatments are used to bridge patients in order to control disease and prevent tumor progression when the waiting time exceeds 6 mo^[133,134]. Percutaneous treatments are more cost-effective than surgical resection^[135]. Moreover, a poor response to TACE before transplantation is an indicator of post-transplantation recurrence^[136].

Surgery and sorafenib

Sorafenib following curative surgery in a phase II trial including 30 patients resulted in a lower tumor recurrence rate compared to surgery alone (33.3% vs 73.6%)^[137].

TACE and ablative therapy

Combining PEI with TACE has been shown to be effective for unresectable HCC^[138]. The three-year survival rate was longer in patients with large and unresectable HCC treated with a combination of TACE and PEI than with TACE alone (22% vs 4%, respectively).

Combining RFA with TACE was evaluated in a RCT for patients with tumors between 3 and 5 cm^[139]. The local tumor progression rate was significantly lower with combined treatment compared to RFA only (6% vs 39%).

Sorafenib and locoregional treatment

There are more than 20 clinical trials in progress evaluating locoregional treatments combined with molecular-targeted agents, and some have demonstrated promising results^[140-142]. A large phase III, randomized, placebo-controlled trial (the STORM trial) evaluating sorafenib as an adjuvant therapy after curative treatment (resection or local ablation) is ongoing^[143].

Sorafenib and TACE

Following TACE, the tumor microenvironment becomes

Table 2 Clinical studies on combined sorafenib and transcatheter arterial chemoembolization for intermediate and advanced hepatocellular carcinoma

Ref.	Study design	Timing of sorafenib	Patients, <i>n</i>	BCLC stage	Child-Pugh class	Primary endpoint results
Kudo <i>et al</i> ^[155]	Sorafenib + TACE <i>vs</i> TACE	Sequential	229 <i>vs</i> 229	B	A	TTP (5.4 mo <i>vs</i> 3.7 mo)
Lencioni <i>et al</i> ^[151]	Sorafenib + DEB-TACE <i>vs</i> DEB-TACE + placebo (SPACE trial)	Continuous	154 <i>vs</i> 153	B	A	TTP (169 d <i>vs</i> 166 d; <i>P</i> = 0.072)
Martin <i>et al</i> ^[158]	Sorafenib + DEB-TACE <i>vs</i> DEB-TACE	NR	30 <i>vs</i> 120	B, C	B	OS (12 mo <i>vs</i> 10 mo)
Sansonno <i>et al</i> ^[154]	Sorafenib + TACE <i>vs</i> TACE	Sequential	40 <i>vs</i> 40	B	A	TTP (9.2 mo <i>vs</i> 4.9 mo)
Han <i>et al</i> ^[156]	Sorafenib + TACE	Sequential	63	A, B, C	A	TTP (10.6 mo)
(subgroup analysis of START)						OS (16.5 mo)
Chung <i>et al</i> ^[150]	Sorafenib + TACE	Sequential	63	A, B, C	A	DCR (52%)
(subgroup analysis of START)						
Park <i>et al</i> ^[149]	Sorafenib + TACE	Interrupted	50	B, C	A, B	TTP (7.1 mo)
(COISUN Korea)						PFS (52% at 6 mo)
Pawlik <i>et al</i> ^[148]	Sorafenib + DEB-TACE	Continuous	35	B, C	A, B	DCR (95%)
						OR (58%)
Cabrera <i>et al</i> ^[153]	Sorafenib + DEB-TACE or Y-90	Continuous	47	B, C	A, B	At 6 mo
						DCR (68%)
						OS (8.5 mo)

BCLC: Barcelona clinic liver cancer; TACE: Transcatheter arterial chemoembolization; DEB: Drug-eluting beads; NR: Not recorded; TTP: Time to progression; DCR: Disease control rate; OR: Objective response; OS: Overall survival; PFS: Progression-free survival.

unbalanced due to increased hypoxia, leading to upregulation of hypoxia inducible factor-1, which in turn upregulates vascular endothelial and platelet-derived growth factors, thus increasing tumor angiogenesis^[144,145]. Studies have shown a significant association between poor prognosis after TACE and risk of extrahepatic metastasis with upregulation of vascular endothelial growth factor^[146,147]. Efforts to improve the outcome of TACE include the use of adjuvant or concurrent antiangiogenic agents to block the neovascularization^[142].

Sorafenib can be used a few days to weeks after the first TACE (sequential introduction) or started prior to the planned TACE and only interrupted for a few days around the time of the procedure (interrupted scheduling). Studies that evaluated the effects of sequential sorafenib treatment after TACE revealed inconsistent results. In phase II studies, sorafenib concomitant with TACE or DEB-TACE was well tolerated and effective in unresectable HCC^[148-151]. Synchronous therapy with sorafenib and TACE has also been retrospectively analyzed: the median overall survival for the combined sorafenib and TACE was 27 mo compared to 17 mo for TACE alone^[152].

Several prospective controlled studies have evaluated the efficacy of combination treatment^[153-158] (Table 2). However, there is a diversity of study designs, including various primary endpoints, patient populations, TACE procedures, timing of randomization and drug administration, which may account for the observed conflicting results^[157]. Overall, the results of combined TACE and sorafenib in intermediate- and advanced-stage HCC appear promising. The results of ongoing trials will define the role of this combination in clinical practice, whether it can overcome TACE refractoriness in intermediate-stage HCC patients, and whether it will have an additive role for advanced-stage HCC

treatment.

Sorafenib and radioembolization

Several ongoing clinical trials are evaluating the combination of radioembolization and sorafenib in patients with HCC. A retrospective analysis of Child-Pugh class A and B HCC patients who received sorafenib first, followed by yttrium-90, then resumed sorafenib post-treatment, showed that the overall survival was higher than has been previously reported for sorafenib alone^[159]. Further prospective studies are being conducted to evaluate this combination.

Sorafenib and systemic chemotherapy

Several combinations of sorafenib with systemic chemotherapeutic agents have been evaluated, including sorafenib with doxorubicin^[160], octreotide^[161], oxaliplatin^[162], 5-fluorouracil^[163], S-1 fluoropyrimidines^[164], PR-104^[165], tegafur/uracil^[166], cisplatin and gemcitabine^[167], and AVE 1642 (a human monoclonal antibody inhibiting the insulin-like growth factor-1 receptor)^[168] (Table 3). Other ongoing phase II trials include the combination of sorafenib with gemcitabine/oxaliplatin^[169], modified FOLFOX^[170], or capecitabine/oxaliplatin^[171].

A randomized, double-blind phase II trial in advanced HCC that compared the efficacy of sorafenib and doxorubicin *vs* doxorubicin plus placebo showed encouraging results (median overall survival 13.7 mo *vs* 6.5 mo; median time to progression 6.4 mo *vs* 2.8 mo; and progression-free survival 6.0 mo *vs* 2.7 mo)^[160]. A phase III randomized study of sorafenib plus doxorubicin compared with sorafenib alone (CALGB 80802) is ongoing in patients with advanced HCC^[172].

In a systematic review of eight studies with sorafenib combined with other anti-cancer agents for therapy of

Table 3 Combined sorafenib plus systemic anticancer therapy for unresectable hepatocellular carcinoma

Ref.	Chemotherapeutic agent	Type of study	Patients, <i>n</i>	Median OS, mo	DCR, %	Median PFS, mo
Abou-Alfa <i>et al</i> ^[160]	Doxorubicin	Multicenter randomized prospective phase II	47 vs 49	13.7 vs 6.5	62	6.0 vs 2.7
Hsu <i>et al</i> ^[166]	Tegafur/uracil	Prospective phase II	53	7.4	57	3.7
Prete <i>et al</i> ^[161]	Long-acting octreotide	Prospective phase II	50	12	76	7
Abou-Alfa <i>et al</i> ^[165]	PR-104	Prospective phase I	14	NR	50	NR
Lee <i>et al</i> ^[164]	S-1 fluoropyrimidines	Prospective phase I	20	10.4	52.9	3.9
Petrini <i>et al</i> ^[163]	5-Fluorouracil	Prospective phase II	39	13.7	48.7	7.5

DCR: Disease control rate; NR: Not recorded; OS: Overall survival; PFS: Progression-free survival.

advanced HCC, the disease control rate was 50%-70%, median progression-free survival was 3.7-7.5 mo, and median overall survival was 7.4-40.1 mo^[173]. Xie *et al*^[174] performed a systematic review of 21 prospective studies with sorafenib treatment alone (seven studies) or combined with other treatment (14 studies) and found that sorafenib increased overall survival by 2.3-2.8 mo, prolonged the time to tumor progression by 1.4-2.7 mo, and increased disease control rate by 11%-19%. Advanced cirrhosis and combined treatment of sorafenib with 5-fluorouracil drugs were the major risk factors for developing adverse events.

These results are promising, and suggest that sorafenib in combination with some agents (particularly mammalian target of rapamycin inhibitors) is an effective and tolerable treatment option for advanced HCC^[171]. However, these trials included small numbers of patients, and although some reported survival advantage over sorafenib alone, combination therapy cannot be recommended for routine practice outside the setting of clinical trials. Large RCTs are needed to establish the efficacy and safety of these combination regimens.

CONCLUSION

Treatment of patients with HCC represents a major challenge in clinical practice. HCC patients require multidisciplinary clinical management and selection of tailored treatments according to disease stage, patient age, and comorbidities. Earlier diagnosis will allow therapies to be more effective, leading to a better prognosis. Several areas in management of HCC still need further evaluation, including the use of neoadjuvant/adjuvant therapies to decrease recurrence after resection or ablation, combinations of local and systemic therapies, combinations of systemic targeted therapies, and second-line therapies. Analysis of the cost-effectiveness of the treatments under investigation should also be an important consideration in future trials.

REFERENCES

- World Health Organization (WHO). Global battle against cancer won't be won with treatment alone Effective prevention measures urgently needed to prevent cancer crisis. Lyon, London: International Agency for Research on Cancer, 2014
- Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- Roskams T. Anatomic pathology of hepatocellular carcinoma: impact on prognosis and response to therapy. *Clin Liver Dis* 2011; **15**: 245-259, vii-x [PMID: 21689611 DOI: 10.1016/j.cld.2011.03.004]
- Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: 24531850 DOI: 10.1136/gutjnl-2013-306627]
- Cabibbo G, Enea M, Attanasio M, Bruix J, Craxi A, Cammà C. A meta-analysis of survival rates of untreated patients in randomized clinical trials of hepatocellular carcinoma. *Hepatology* 2010; **51**: 1274-1283 [PMID: 20112254 DOI: 10.1002/hep.23485]
- Guy J, Kelley RK, Roberts J, Kerlan R, Yao F, Terrault N. Multidisciplinary management of hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2012; **10**: 354-362 [PMID: 22083023 DOI: 10.1016/j.cgh.2011.11.008]
- Kaseb AO, Abaza YM, Roses RE. Multidisciplinary management of hepatocellular carcinoma. *Recent Results Cancer Res* 2013; **190**: 247-259 [PMID: 22941025 DOI: 10.1007/978-3-642-16037-0_16]
- Lee KK, Kim DG, Moon IS, Lee MD, Park JH. Liver transplantation versus liver resection for the treatment of hepatocellular carcinoma. *J Surg Oncol* 2010; **101**: 47-53 [PMID: 19798686 DOI: 10.1002/jso.21415]
- Mazzaferro V, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, Capussotti L, Calise F, Pellicci R, Belli G, Tagger A, Colombo M, Bonino F, Majno P, Llovet JM. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; **44**: 1543-1554 [PMID: 17133492 DOI: 10.1002/hep.21415]
- Yang T, Lin C, Zhai J, Shi S, Zhu M, Zhu N, Lu JH, Yang GS, Wu MC. Surgical resection for advanced hepatocellular carcinoma according to Barcelona Clinic Liver Cancer (BCLC) staging. *J Cancer Res Clin Oncol* 2012; **138**: 1121-1129 [PMID: 22402598 DOI: 10.1007/s00432-012-1188-0]
- Kinoshita H, Sakai K, Hirohashi K, Igawa S, Yamasaki O, Kubo S. Preoperative portal vein embolization for hepatocellular carcinoma. *World J Surg* 1986; **10**: 803-808 [PMID: 3022488 DOI: 10.1007/BF01655244]
- Bruix J, Castells A, Bosch J, Feu F, Fuster J, Garcia-Pagan JC, Visa J, Bru C, Rodés J. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 1996; **111**: 1018-1022 [PMID: 8831597 DOI: 10.1016/S0016-5085(96)70070-7]
- Roayaie S, Obeidat K, Sposito C, Mariani L, Bhoori S, Pellegrinelli A, Labow D, Llovet JM, Schwartz M, Mazzaferro V. Resection of hepatocellular cancer ≤2 cm: results from two Western centers. *Hepatology* 2013; **57**: 1426-1435 [PMID: 22576353 DOI: 10.1002/hep.24199]

- 10.1002/hep.25832]
- 15 **Cucchetti A**, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, Ramacciato G, Grazi GL, Pinna AD. Is portal hypertension a contraindication to hepatic resection? *Ann Surg* 2009; **250**: 922-928 [PMID: 19855258 DOI: 10.1097/SLA.0b013e3181b977a5]
 - 16 **Pai M**, Jiao LR, Khorsandi S, Canelo R, Spalding DR, Habib NA. Liver resection with bipolar radiofrequency device: Habib 4X. *HPB* (Oxford) 2008; **10**: 256-260 [PMID: 18773112 DOI: 10.1080/13651820802167136]
 - 17 **Gagner M**, Rogula T, Selzer D. Laparoscopic liver resection: benefits and controversies. *Surg Clin North Am* 2004; **84**: 451-462 [PMID: 15062655 DOI: 10.1016/j.suc.2003.11.002]
 - 18 **Li N**, Wu YR, Wu B, Lu MQ. Surgical and oncologic outcomes following laparoscopic versus open liver resection for hepatocellular carcinoma: A meta-analysis. *Hepatol Res* 2012; **42**: 51-59 [PMID: 21988222 DOI: 10.1111/j.1872-034X.2011.00890.x]
 - 19 **Kim BW**, Kim YB, Wang HJ, Kim MW. Risk factors for immediate post-operative fatal recurrence after curative resection of hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 99-104 [PMID: 16440425]
 - 20 **Nault JC**, De Reyniès A, Villanueva A, Calderaro J, Rebouissou S, Couchy G, Decaens T, Franco D, Imbeaud S, Rousseau F, Azoulay D, Saric J, Blanc JF, Balabaud C, Bioulac-Sage P, Laurent A, Laurent-Puig P, Llovet JM, Zucman-Rossi J. A hepatocellular carcinoma 5-gene score associated with survival of patients after liver resection. *Gastroenterology* 2013; **145**: 176-187 [PMID: 23567350 DOI: 10.1053/j.gastro.2013.03.051]
 - 21 **Mazzaferro V**, Chun YS, Poon RT, Schwartz ME, Yao FY, Marsh JW, Bhoori S, Lee SG. Liver transplantation for hepatocellular carcinoma. *Ann Surg Oncol* 2008; **15**: 1001-1007 [PMID: 18236119 DOI: 10.1245/s10434-007-9559-5]
 - 22 **de Lope CR**, Tremosini S, Forner A, Reig M, Bruix J. Management of HCC. *J Hepatol* 2012; **56** Suppl 1: S75-S87 [PMID: 22300468 DOI: 10.1016/S0168-8278(12)60009-9]
 - 23 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
 - 24 **Mazzaferro V**, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773 DOI: 10.1002/lt.22365]
 - 25 **Farinati F**, Sergio A, Baldan A, Giacomini A, Di Nolfo MA, Del Poggio P, Benvegna L, Rapaccini G, Zoli M, Borzio F, Giannini EG, Caturelli E, Trevisani F. Early and very early hepatocellular carcinoma: when and how much do staging and choice of treatment really matter? A multi-center study. *BMC Cancer* 2009; **9**: 33 [PMID: 19171074 DOI: 10.1186/1471-2407-9-33]
 - 26 **Raj A**, McCall J, Gane E. Validation of the "Metroticket" predictor in a cohort of patients transplanted for predominantly HBV-related hepatocellular carcinoma. *J Hepatol* 2011; **55**: 1063-1068 [PMID: 21354447 DOI: 10.1016/j.jhep.2011.01.052]
 - 27 **Schwartz M**, Dvorchik I, Roayaie S, Fiel MI, Finkelstein S, Marsh JW, Martignetti JA, Llovet JM. Liver transplantation for hepatocellular carcinoma: extension of indications based on molecular markers. *J Hepatol* 2008; **49**: 581-588 [PMID: 18602719 DOI: 10.1016/j.jhep.2008.03.032]
 - 28 **Toso C**, Mentha G, Majno P. Selection of patients with hepatocellular carcinoma before liver transplantation: need to combine alpha-fetoprotein with morphology? *Hepatobiliary Pancreat Dis Int* 2010; **9**: 460-461 [PMID: 20943453]
 - 29 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigsen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debette-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D. Liver transplantation for hepatocellular carcinoma: a model including α -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-984.e3; quiz e14-15 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]
 - 30 **Vibert E**, Azoulay D, Hoti E, Iacopinelli S, Samuel D, Salloum C, Lemoine A, Bismuth H, Castaing D, Adam R. Progression of α -fetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010; **10**: 129-137 [PMID: 20070666 DOI: 10.1111/j.1600-6143.2009.02750.x]
 - 31 **Toso C**, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology* 2009; **49**: 832-838 [PMID: 19152426 DOI: 10.1002/hep.22693]
 - 32 **Livraghi T**, Festi D, Monti F, Salmi A, Vettori C. US-guided percutaneous alcohol injection of small hepatic and abdominal tumors. *Radiology* 1986; **161**: 309-312 [PMID: 3020612 DOI: 10.1148/radiology.161.2.3020612]
 - 33 **Rossi S**, Di Stasi M, Buscarini E, Quaretti P, Garbagnati F, Squassante L, Paties CT, Silverman DE, Buscarini L. Percutaneous RF interstitial thermal ablation in the treatment of hepatic cancer. *AJR Am J Roentgenol* 1996; **167**: 759-768 [PMID: 8751696 DOI: 10.2214/ajr.167.3.8751696]
 - 34 **Seki T**, Wakabayashi M, Nakagawa T, Itho T, Shiro T, Kunieda K, Sato M, Uchiyama S, Inoue K. Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. *Cancer* 1994; **74**: 817-825 [PMID: 8039109]
 - 35 **Pacella CM**, Bizzarri G, Magnolfi F, Cecconi P, Caspani B, Anelli V, Bianchini A, Valle D, Pacella S, Manenti G, Rossi Z. Laser thermal ablation in the treatment of small hepatocellular carcinoma: results in 74 patients. *Radiology* 2001; **221**: 712-720 [PMID: 11719667 DOI: 10.1148/radiol.2213001501]
 - 36 **Ross WB**, Horton M, Bertolino P, Morris DL. Cryotherapy of liver tumours--a practical guide. *HPB Surg* 1995; **8**: 167-173 [PMID: 7547619]
 - 37 **Lencioni R**. Loco-regional treatment of hepatocellular carcinoma. *Hepatology* 2010; **52**: 762-773 [PMID: 20564355 DOI: 10.1002/hep.23725]
 - 38 **Kuang M**, Lu MD, Xie XY, Xu HX, Xu ZF, Liu GJ, Yin XY, Huang JF, Lencioni R. Ethanol ablation of hepatocellular carcinoma Up to 5.0 cm by using a multipronged injection needle with high-dose strategy. *Radiology* 2009; **253**: 552-561 [PMID: 19709992 DOI: 10.1148/radiol.2532082021]
 - 39 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357 DOI: 10.1002/hep.21933]
 - 40 **Orlando A**, Leandro G, Olivo M, Andriulli A, Cottone M. Radiofrequency thermal ablation vs. percutaneous ethanol injection for small hepatocellular carcinoma in cirrhosis: meta-analysis of randomized controlled trials. *Am J Gastroenterol* 2009; **104**: 514-524 [PMID: 19174803 DOI: 10.1038/ajg.2008.80]
 - 41 **Cho YK**, Kim JK, Kim MY, Rhim H, Han JK. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* 2009; **49**: 453-459 [PMID: 19065676 DOI: 10.1002/hep.22648]
 - 42 **Shen A**, Zhang H, Tang C, Chen Y, Wang Y, Zhang C, Wu Z. Systematic review of radiofrequency ablation versus percutaneous ethanol injection for small hepatocellular carcinoma up to 3 cm. *J Gastroenterol Hepatol* 2013; **28**: 793-800 [PMID: 23432154 DOI: 10.1111/jgh.12162]
 - 43 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328 [PMID:

- 16495695]
- 44 **Cho YK**, Kim JK, Kim WT, Chung JW. Hepatic resection versus radiofrequency ablation for very early stage hepatocellular carcinoma: a Markov model analysis. *Hepatology* 2010; **51**: 1284-1290 [PMID: 20099299 DOI: 10.1002/hep.23466]
 - 45 **Peng ZW**, Lin XJ, Zhang YJ, Liang HH, Guo RP, Shi M, Chen MS. Radiofrequency ablation versus hepatic resection for the treatment of hepatocellular carcinomas 2 cm or smaller: a retrospective comparative study. *Radiology* 2012; **262**: 1022-1033 [PMID: 22357902 DOI: 10.1148/radiol.11110817]
 - 46 **Takayama T**, Makuuchi M, Kojiro M, Lauwers GY, Adams RB, Wilson SR, Jang HJ, Charnsangavej C, Taouli B. Early hepatocellular carcinoma: pathology, imaging, and therapy. *Ann Surg Oncol* 2008; **15**: 972-978 [PMID: 18236118 DOI: 10.1245/s10434-007-9685-0]
 - 47 **Wang JH**, Wang CC, Hung CH, Chen CL, Lu SN. Survival comparison between surgical resection and radiofrequency ablation for patients in BCLC very early/early stage hepatocellular carcinoma. *J Hepatol* 2012; **56**: 412-418 [PMID: 21756858 DOI: 10.1016/j.jhep.2011.05.020]
 - 48 **Cucchetti A**, Piscaglia F, Cescon M, Colecchia A, Ercolani G, Bolondi L, Pinna AD. Cost-effectiveness of hepatic resection versus percutaneous radiofrequency ablation for early hepatocellular carcinoma. *J Hepatol* 2013; **59**: 300-307 [PMID: 23603669 DOI: 10.1016/j.jhep.2013.04.009]
 - 49 **Shibata T**, Iimuro Y, Yamamoto Y, Maetani Y, Ametani F, Itoh K, Konishi J. Small hepatocellular carcinoma: comparison of radiofrequency ablation and percutaneous microwave coagulation therapy. *Radiology* 2002; **223**: 331-337 [PMID: 11997534]
 - 50 **Ellestad LE**, Carre W, Muchow M, Jenkins SA, Wang X, Cogburn LA, Porter TE. Gene expression profiling during cellular differentiation in the embryonic pituitary gland using cDNA microarrays. *Physiol Genomics* 2006; **25**: 414-425 [PMID: 16493019]
 - 51 **Goldberg SN**, Grassi CJ, Cardella JF, Charboneau JW, Dodd GD, Dupuy DE, Gervais D, Gillams AR, Kane RA, Lee FT, Livraghi T, McGahan J, Phillips DA, Rhim H, Silverman SG. Image-guided tumor ablation: standardization of terminology and reporting criteria. *Radiology* 2005; **235**: 728-739 [PMID: 15845798 DOI: 10.1148/radiol.2353042205]
 - 52 **Ferrari FS**, Megliola A, Scorzelli A, Stella A, Vigni F, Drudi FM, Venezia D. Treatment of small HCC through radiofrequency ablation and laser ablation. Comparison of techniques and long-term results. *Radiol Med* 2007; **112**: 377-393 [PMID: 17447018 DOI: 10.1007/s11547-007-0148-2]
 - 53 **Orlacchio A**, Bazzocchi G, Pastorelli D, Bolacchi F, Angelico M, Almerighi C, Masala S, Simonetti G. Percutaneous cryoablation of small hepatocellular carcinoma with US guidance and CT monitoring: initial experience. *Cardiovasc Intervent Radiol* 2008; **31**: 587-594 [PMID: 18236104 DOI: 10.1007/s00270-008-9293-9]
 - 54 **Ei S**, Hibi T, Tanabe M, Itano O, Shinoda M, Kitago M, Abe Y, Yagi H, Okabayashi K, Sugiyama D, Wakabayashi G, Kitagawa Y. Cryoablation Provides Superior Local Control of Primary Hepatocellular Carcinomas of $\geq 2\text{ cm}$ Compared with Radiofrequency Ablation and Microwave Coagulation Therapy: An Underestimated Tool in the Toolbox. *Ann Surg Oncol* 2015; **22**: 1294-1300 [PMID: 25287439 DOI: 10.1245/s10434-014-4114-7]
 - 55 **Wang C**, Wang H, Yang W, Hu K, Xie H, Hu KQ, Bai W, Dong Z, Lu Y, Zeng Z, Lou M, Wang H, Gao X, Chang X, An L, Qu J, Li J, Yang Y. Multicenter randomized controlled trial of percutaneous cryoablation versus radiofrequency ablation in hepatocellular carcinoma. *Hepatology* 2014; Epub ahead of print [PMID: 25284802 DOI: 10.1002/hep.27548]
 - 56 **Lo CM**, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164-1171 [PMID: 11981766 DOI: 10.1053/jhep.2002.33156]
 - 57 **Tsochatzis EA**, Germani G, Burroughs AK. Transarterial chemoembolization, transarterial chemotherapy, and intra-arterial chemotherapy for hepatocellular carcinoma treatment. *Semin Oncol* 2010; **37**: 89-93 [PMID: 20494700 DOI: 10.1053/j.seminoncol.2010.03.007]
 - 58 **Lin S**, Hoffmann K, Schemmer P. Treatment of hepatocellular carcinoma: a systematic review. *Liver Cancer* 2012; **1**: 144-158 [PMID: 24159579 DOI: 10.1159/000343828]
 - 59 **Kudo M**, Han KH, Kokudo N, Cheng AL, Choi BI, Furuse J, Izumi N, Park JW, Poon RT, Sakamoto M. Liver Cancer Working Group report. *Jpn J Clin Oncol* 2010; **40** Suppl 1: i19-i27 [PMID: 20870915 DOI: 10.1093/jco/hyq123]
 - 60 **Zhang ZM**, Guo JX, Zhang ZC, Jiang N, Zhang ZY, Pan LJ. Therapeutic options for intermediate-advanced hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 1685-1689 [PMID: 21483627 DOI: 10.3748/wjg.v17.i13.1685]
 - 61 **Forner A**, Llovet JM, Bruix J. Chemoembolization for intermediate HCC: is there proof of survival benefit? *J Hepatol* 2012; **56**: 984-986 [PMID: 22008737 DOI: 10.1016/j.jhep.2011.08.017]
 - 62 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
 - 63 **Oliveri RS**, Wetterslev J, Gluud C. Transarterial (chemo)-embolisation for unresectable hepatocellular carcinoma. *Cochrane Database Syst Rev* 2011; **16**: CD004787 [PMID: 21412886 DOI: 10.1002/14651858.CD004787.pub2]
 - 64 **Cabibbo G**, Genco C, Di Marco V, Barbara M, Enea M, Parisi P, Brancatelli G, Romano P, Craxi A, Cammà C. Predicting survival in patients with hepatocellular carcinoma treated by transarterial chemoembolisation. *Aliment Pharmacol Ther* 2011; **34**: 196-204 [PMID: 21564144 DOI: 10.1111/j.1365-2036.2011.04694.x]
 - 65 **Dhanasekaran R**, Kooby DA, Staley CA, Kauh JS, Khanna V, Kim HS. Prognostic factors for survival in patients with unresectable hepatocellular carcinoma undergoing chemoembolization with doxorubicin drug-eluting beads: a preliminary study. *HPB (Oxford)* 2010; **12**: 174-180 [PMID: 20590884 DOI: 10.1111/j.1477-2574.2009.00138.x]
 - 66 **Sawhney S**, Montano-Loza AJ, Salat P, McCarthy M, Kneteman N, Meza-Junco J, Owen R. Transarterial chemoembolization in patients with hepatocellular carcinoma: predictors of survival. *Can J Gastroenterol* 2011; **25**: 426-432 [PMID: 21912767]
 - 67 **Bolondi L**, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, Piscaglia F, Raoul JL, Sangro B. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis* 2012; **32**: 348-359 [PMID: 23397536 DOI: 10.1055/s-0032-1329906]
 - 68 **Varela M**, Real MI, Burrell M, Forner A, Sala M, Brunet M, Ayuso C, Castells L, Montañá X, Llovet JM, Bruix J. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007; **46**: 474-481 [PMID: 17239480 DOI: 10.1016/j.jhep.2006.10.020]
 - 69 **Lammer J**, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, Pitton M, Sergeant G, Pfammatter T, Terraz S, Benhamou Y, Avajon Y, Gruenberger T, Pomoni M, Langenberger H, Schuchmann M, Dumortier J, Mueller C, Chevallier P, Lencioni R. Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Intervent Radiol* 2010; **33**: 41-52 [PMID: 19908093 DOI: 10.1007/s00270-009-9711-7]
 - 70 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]
 - 71 **Raoul JL**, Sangro B, Forner A, Mazzaferro V, Piscaglia F, Bolondi L, Lencioni R. Evolving strategies for the management of intermediate-stage hepatocellular carcinoma: available evidence and expert opinion on the use of transarterial chemoembolization. *Cancer Treat Rev* 2011; **37**: 212-220 [PMID: 20724077 DOI: 10.1016/j.ctrv.2010.07.006]
 - 72 **Piscaglia F**, Bolondi L. The intermediate hepatocellular carcinoma stage: Should treatment be expanded? *Dig Liver Dis* 2010; **42** Suppl 3: S258-S263 [PMID: 20547312 DOI: 10.1016/S1590-8658(10)60514-2]
 - 73 **Sieghart W**, Huckle F, Pinter M, Graziadei I, Vogel W, Müller C, Heinzl H, Trauner M, Peck-Radosavljevic M. The ART of

- decision making: retreatment with transarterial chemoembolization in patients with hepatocellular carcinoma. *Hepatology* 2013; **57**: 2261-2273 [PMID: 23316013 DOI: 10.1002/hep.26256]
- 74 **Hucke F**, Sieghart W, Pinter M, Graziadei I, Vogel W, Müller C, Heinzl H, Waneck F, Trauner M, Peck-Radosavljevic M. The ART-strategy: sequential assessment of the ART score predicts outcome of patients with hepatocellular carcinoma re-treated with TACE. *J Hepatol* 2014; **60**: 118-126 [PMID: 24012941 DOI: 10.1016/j.jhep.2013.08.022]
 - 75 **Salem R**, Mazzaferro V, Sangro B. Yttrium 90 radioembolization for the treatment of hepatocellular carcinoma: biological lessons, current challenges, and clinical perspectives. *Hepatology* 2013; **58**: 2188-2197 [PMID: 23512791 DOI: 10.1002/hep.26382]
 - 76 **Sangro B**, Iñarrairaegui M, Bilbao JJ. Radioembolization for hepatocellular carcinoma. *J Hepatol* 2012; **56**: 464-473 [PMID: 21816126 DOI: 10.1016/j.jhep.2011.07.012]
 - 77 **Kim YH**, Kim do Y. Yttrium-90 radioembolization for hepatocellular carcinoma: what we know and what we need to know. *Oncology* 2013; **84** Suppl 1: 34-39 [PMID: 23428856 DOI: 10.1159/000345887]
 - 78 **Salem R**, Lewandowski RJ, Mulcahy MF, Riaz A, Ryu RK, Ibrahim S, Atassi B, Baker T, Gates V, Miller FH, Sato KT, Wang E, Gupta R, Benson AB, Newman SB, Omary RA, Abecassis M, Kulik L. Radioembolization for hepatocellular carcinoma using Yttrium-90 microspheres: a comprehensive report of long-term outcomes. *Gastroenterology* 2010; **138**: 52-64 [PMID: 19766639 DOI: 10.1053/j.gastro.2009.09.006]
 - 79 **Sangro B**, Carpanese L, Cianni R, Golfieri R, Gasparini D, Ezziddin S, Paprottka PM, Fiore F, Van Buskirk M, Bilbao JJ, Ettorre GM, Salvatori R, Giampalma E, Geatti O, Wilhelm K, Hoffmann RT, Izzo F, Iñarrairaegui M, Maini CL, Urigo C, Cappelli A, Vit A, Ahmadzadehfar H, Jakobs TF, Lastoria S. Survival after yttrium-90 resin microsphere radioembolization of hepatocellular carcinoma across Barcelona clinic liver cancer stages: a European evaluation. *Hepatology* 2011; **54**: 868-878 [PMID: 21618574 DOI: 10.1002/hep.24451]
 - 80 **Salem R**, Lewandowski RJ, Kulik L, Wang E, Riaz A, Ryu RK, Sato KT, Gupta R, Nikolaidis P, Miller FH, Yaghamai V, Ibrahim SM, Senthilnathan S, Baker T, Gates VL, Atassi B, Newman S, Memon K, Chen R, Vogelzang RL, Nemcek AA, Resnick SA, Chrisman HB, Carr J, Omary RA, Abecassis M, Benson AB, Mulcahy MF. Radioembolization results in longer time-to-progression and reduced toxicity compared with chemoembolization in patients with hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 497-507.e2 [PMID: 21044630 DOI: 10.1053/j.gastro.2010.10.049]
 - 81 **Louis C**, Dewas S, Mirabel X, Lacomere T, Adenis A, Bonodeau F, Lartigau E. Stereotactic radiotherapy of hepatocellular carcinoma: preliminary results. *Technol Cancer Res Treat* 2010; **9**: 479-487 [PMID: 20815419 DOI: 10.1177/153303461000900506]
 - 82 **Blomgren H**, Lax I, Näslund I, Svanström R. Stereotactic high dose fraction radiation therapy of extracranial tumors using an accelerator. Clinical experience of the first thirty-one patients. *Acta Oncol* 1995; **34**: 861-870 [PMID: 7576756 DOI: 10.3109/02841869509127197]
 - 83 **Herfarth KK**, Debus J, Lohr F, Bahner ML, Rhein B, Fritz P, Höss A, Schlegel W, Wannenmacher MF. Stereotactic single-dose radiation therapy of liver tumors: results of a phase I/II trial. *J Clin Oncol* 2001; **19**: 164-170 [PMID: 11134209]
 - 84 **Wulf J**, Guckenberger M, Haedinger U, Oppitz U, Mueller G, Baier K, Flentje M. Stereotactic radiotherapy of primary liver cancer and hepatic metastases. *Acta Oncol* 2006; **45**: 838-847 [PMID: 16982548 DOI: 10.1080/02841860600904821]
 - 85 **Lai CL**, Wu PC, Chan GC, Lok AS, Lin HJ. Doxorubicin versus no antitumor therapy in inoperable hepatocellular carcinoma. A prospective randomized trial. *Cancer* 1988; **62**: 479-483 [PMID: 2839280]
 - 86 **Fuchs CS**, Clark JW, Ryan DP, Kulke MH, Kim H, Earle CC, Vincitore M, Mayer RJ, Stuart KE. A phase II trial of gemcitabine in patients with advanced hepatocellular carcinoma. *Cancer* 2002; **94**: 3186-3191 [PMID: 12115351 DOI: 10.1002/cncr.10607]
 - 87 **Yeo W**, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ. A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005; **97**: 1532-1538 [PMID: 16234567 DOI: 10.1093/jnci/dji315]
 - 88 **Nowak AK**, Stockler MR, Chow PK, Findlay M. Use of tamoxifen in advanced-stage hepatocellular carcinoma. A systematic review. *Cancer* 2005; **103**: 1408-1414 [PMID: 15744746 DOI: 10.1002/cncr.20963]
 - 89 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]
 - 90 **Barbare JC**, Bouché O, Bonnetain F, Raoul JL, Rougier P, Abergel A, Boige V, Denis B, Blanchi A, Pariente A, Milan C, Bedenne L. Randomized controlled trial of tamoxifen in advanced hepatocellular carcinoma. *J Clin Oncol* 2005; **23**: 4338-4346 [PMID: 15994145 DOI: 10.1200/JCO.2005.05.470]
 - 91 **Chow PK**, Tai BC, Tan CK, Machin D, Win KM, Johnson PJ, Soo KC. High-dose tamoxifen in the treatment of inoperable hepatocellular carcinoma: A multicenter randomized controlled trial. *Hepatology* 2002; **36**: 1221-1226 [PMID: 12395333 DOI: 10.1053/jhep.2002.36824]
 - 92 **Leung TW**, Patt YZ, Lau WY, Ho SK, Yu SC, Chan AT, Mok TS, Yeo W, Liew CT, Leung NW, Tang AM, Johnson PJ. Complete pathological remission is possible with systemic combination chemotherapy for inoperable hepatocellular carcinoma. *Clin Cancer Res* 1999; **5**: 1676-1681 [PMID: 10430068]
 - 93 **Cervello M**, McCubrey JA, Cusimano A, Lampiasi N, Azzolina A, Montalto G. Targeted therapy for hepatocellular carcinoma: novel agents on the horizon. *Oncotarget* 2012; **3**: 236-260 [PMID: 22470194]
 - 94 **Chang YS**, Adnane J, Trail PA, Levy J, Henderson A, Xue D, Bortolon E, Ichetovkin M, Chen C, McNabola A, Wilkie D, Carter CA, Taylor IC, Lynch M, Wilhelm S. Sorafenib (BAY 43-9006) inhibits tumor growth and vascularization and induces tumor apoptosis and hypoxia in RCC xenograft models. *Cancer Chemother Pharmacol* 2007; **59**: 561-574 [PMID: 17160391 DOI: 10.1007/s00280-006-0393-4]
 - 95 **Nojiri K**, Sugimoto K, Shiraki K, Tameda M, Inagaki Y, Ogura S, Kasai C, Kusagawa S, Yoneda M, Yamamoto N, Takei Y, Nobori T, Ito M. Sorafenib and TRAIL have synergistic effect on hepatocellular carcinoma. *Int J Oncol* 2013; **42**: 101-108 [PMID: 23123700 DOI: 10.3892/ijo.2012.1676]
 - 96 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
 - 97 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]
 - 98 **Kudo M**, Tateishi R, Yamashita T, Ikeda M, Furuse J, Ikeda K, Kokudo N, Izumi N, Matsui O. Current status of hepatocellular carcinoma treatment in Japan: case study and discussion-voting system. *Clin Drug Investig* 2012; **32** Suppl 2: 37-51 [PMID: 22873626 DOI: 10.2165/1163024-S0-000000000-00000]
 - 99 **Kudo M**, Ueshima K, Arizumi T. Real-life clinical practice with sorafenib in advanced hepatocellular carcinoma: a single-center experience. *Dig Dis* 2012; **30**: 609-616 [PMID: 23258103 DOI: 10.1159/000343091]
 - 100 **Cammà C**, Cabibbo G, Petta S, Enea M, Iavarone M, Grieco A, Gasbarrini A, Villa E, Zavaglia C, Bruno R, Colombo M, Craxi

- A. Cost-effectiveness of sorafenib treatment in field practice for patients with hepatocellular carcinoma. *Hepatology* 2013; **57**: 1046-1054 [PMID: 23299720 DOI: 10.1002/hep.26221]
- 101 **Dufour JF**, Hoppe H, Heim MH, Helbling B, Maurhofer O, Szucs-Farkas Z, Kickuth R, Borner M, Candinas D, Saar B. Continuous administration of sorafenib in combination with transarterial chemoembolization in patients with hepatocellular carcinoma: results of a phase I study. *Oncologist* 2010; **15**: 1198-1204 [PMID: 21036880 DOI: 10.1634/theoncologist.2010-0180]
 - 102 **Pinter M**, Sieghart W, Graziadei I, Vogel W, Maieron A, Königsberg R, Weissmann A, Kornek G, Plank C, Peck-Radosavljevic M. Sorafenib in unresectable hepatocellular carcinoma from mild to advanced stage liver cirrhosis. *Oncologist* 2009; **14**: 70-76 [PMID: 19144684 DOI: 10.1634/theoncologist.2008-0191]
 - 103 **Lencioni R**, Kudo M, Ye SL, Bronowicki JP, Chen XP, Dagher L, Furuse J, Geschwind JF, Ladrón de Guevara L, Papandreou C, Sanyal AJ, Takayama T, Yoon SK, Nakajima K, Cihon F, Heldner S, Marrero JA. First interim analysis of the GIDEON (Global Investigation of therapeutic decisions in hepatocellular carcinoma and of its treatment with sorafenib) non-interventional study. *Int J Clin Pract* 2012; **66**: 675-683 [PMID: 22698419 DOI: 10.1111/j.1742-1241.2012.02940.x]
 - 104 **Lencioni R**, Kudo M, Ye SL, Bronowicki JP, Chen XP, Dagher L, Furuse J, Geschwind JF, de Guevara LL, Papandreou C, Takayama T, Yoon SK, Nakajima K, Lehr R, Heldner S, Sanyal AJ. GIDEON (Global Investigation of therapeutic DECisions in hepatocellular carcinoma and Of its treatment with sorafenib): second interim analysis. *Int J Clin Pract* 2014; **68**: 609-617 [PMID: 24283303 DOI: 10.1111/ijcp.12352]
 - 105 **Cheng AL**, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J Clin Oncol* 2013; **31**: 4067-4075 [PMID: 24081937 DOI: 10.1200/JCO.2012.45.8372]
 - 106 **Cainap C**, Qin S, Huang WT, Chung JJ, Pan H, Cheng Y, Kudo M, Kang YK, Chen PJ, Toh HC, Gorbunova V, Eskens F, Qian J, McKee MD, Ricker JL, Carlson DM, El Nowiem S. Phase III trial of linifanib versus sorafenib in patients with advanced hepatocellular carcinoma (HCC). *J Clin Oncol* 2013; **31** Suppl 4: abstr 249
 - 107 **Johnson PJ**, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Aviña J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, Decaens T, Tak WY, Jeng LB, Liu D, Ezzeddine R, Walters I, Cheng AL. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J Clin Oncol* 2013; **31**: 3517-3524 [PMID: 23980084 DOI: 10.1200/JCO.2012.48.4410]
 - 108 **Llovet JM**, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, Kang YK, Assenat E, Lim HY, Boige V, Mathurin P, Fartoux L, Lin DY, Bruix J, Poon RT, Sherman M, Blanc JF, Finn RS, Tak WY, Chao Y, Ezzeddine R, Liu D, Walters I, Park JW. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. *J Clin Oncol* 2013; **31**: 3509-3516 [PMID: 23980090 DOI: 10.1200/JCO.2012.47.3009]
 - 109 **Zhu AX**, Rosmorduc O, Evans TR, Ross PJ, Santoro A, Carrilho FJ, Bruix J, Qin S, Thuluvath PJ, Llovet JM, Leberre MA, Jensen M, Meinhardt G, Kang YK. SEARCH: A Phase III, Randomized, Double-Blind, Placebo-Controlled Trial of Sorafenib Plus Erlotinib in Patients With Advanced Hepatocellular Carcinoma. *J Clin Oncol* 2015; **33**: 559-566 [PMID: 25547503 DOI: 10.1200/JCO.2013.53.7746]
 - 110 **Zhu AX**, Kudo M, Assenat E, Cattani S, Kang YK, Lim HY, Poon RT, Blanc JF, Vogel A, Chen CL, Dorval E, Peck-Radosavljevic M, Santoro A, Daniele B, Furuse J, Jappe A, Perraud K, Anak O, Sellami DB, Chen LT. Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. *JAMA* 2014; **312**: 57-67 [PMID: 25058218 DOI: 10.1001/jama.2014.7189]
 - 111 **O'Reilly KE**, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006; **66**: 1500-1508 [PMID: 16452206 DOI: 10.1158/0008-5472.CAN-05-2925]
 - 112 **Lim HY**, Yen CJ, Tak WY, Heo J, Choi HJ, Lin CY, Yoon JH, Hsu C, Rau KM, Poon RTP, Yeo W, Park JW, Tay MH, Hsieh WS, Kappeler C, Rajagopalan P, Krissel H. A phase II trial of MEK inhibitor BAY 86-9766 in combination with sorafenib as first-line systemic treatment for patients with unresectable hepatocellular carcinoma (HCC). *J Clin Oncol* 2012; **30** Suppl 15: abstr4103
 - 113 **Choo SP**, Ng QS, Chen WJJ, CK, Yong WP, Wang LZ, Koh TS, Goh BC, Thng CH, Huynh HT, Zee YK, Low L, Toh HC. A phase I/II study of AZD6244 in combination with sorafenib in advanced hepatocellular carcinoma. *J Clin Oncol* 2012; **30** Suppl 15: abstr4100
 - 114 **Finn RS**, Poon RT, Yau T, Klumpen HJ, Chen LT, Kang YK, Kim TY, Gomez-Martin C, Rodriguez-Lopez C, Kunz T, Paquet T, Brandt U, Sellami D, Bruix J. Phase I study investigating everolimus combined with sorafenib in patients with advanced hepatocellular carcinoma. *J Hepatol* 2013; **59**: 1271-1277 [PMID: 23928403 DOI: 10.1016/j.jhep.2013.07.029]
 - 115 **Faivre SJ**, Fartoux L, Bouattour M, Bumsel F, Dreyer C, Raymond E, Rosmorduc O. A phase I study of AVE1642, a human monoclonal antibody-blocking insulin-like growth factor-1 receptor (IGF-1R), given as a single agent and in combination with sorafenib as first-line therapy in patients with advanced hepatocellular carcinoma (HCC). *J Clin Oncol* 2011; **29** Suppl 4: abstr270
 - 116 **Ishikawa T**. Secondary prevention of recurrence by interferon therapy after ablation therapy for hepatocellular carcinoma in chronic hepatitis C patients. *World J Gastroenterol* 2008; **14**: 6140-6144 [PMID: 18985803 DOI: 10.3748/wjg.14.6140]
 - 117 **Sakaguchi Y**, Kudo M, Fukunaga T, Minami Y, Chung H, Kawasaki T. Low-dose, long-term, intermittent interferon-alpha-2b therapy after radical treatment by radiofrequency ablation delays clinical recurrence in patients with hepatitis C virus-related hepatocellular carcinoma. *Intervirology* 2005; **48**: 64-70 [PMID: 15785092 DOI: 10.1159/000082097]
 - 118 **Shen YC**, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. *J Hepatol* 2010; **52**: 889-894 [PMID: 20395009 DOI: 10.1016/j.jhep.2009.12.041]
 - 119 **Miyake Y**, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *J Viral Hepat* 2010; **17**: 287-292 [PMID: 19732321 DOI: 10.1111/j.1365-2893.2009.01181]
 - 120 **Singal AG**, Waljee AK, Shiffman M, Bacon BR, Schoenfeld PS. Meta-analysis: re-treatment of genotype 1 hepatitis C nonresponders and relapsers after failing interferon and ribavirin combination therapy. *Aliment Pharmacol Ther* 2010; **32**: 969-983 [PMID: 20937042 DOI: 10.1111/j.1365-2036.2010.04427]
 - 121 **Hsu YC**, Ho HJ, Wu MS, Lin JT, Wu CY. Postoperative peg-interferon plus ribavirin is associated with reduced recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatology* 2013; **58**: 150-157 [PMID: 23389758 DOI: 10.1002/hep.26300]
 - 122 **Tan YJ**. Hepatitis B virus infection and the risk of hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 4853-4857 [PMID: 22171125 DOI: 10.3748/wjg.v17.i44.4853]
 - 123 **Hann HW**, Coben R, Brown D, Needleman L, Rosato E, Min A, Hann RS, Park KB, Dunn S, DiMarino AJ. A long-term study of the effects of antiviral therapy on survival of patients with HBV-associated hepatocellular carcinoma (HCC) following local tumor ablation. *Cancer Med* 2014; **3**: 390-396 [PMID: 24519810 DOI: 10.1002/cam4.197]
 - 124 **Yamasaki S**, Hasegawa H, Kinoshita H, Furukawa M, Imaoka S, Takasaki K, Kakumoto Y, Saito H, Yamada R, Oosaki Y, Arai S, Okamoto E, Monden M, Ryu M, Kusano S, Kanematsu T, Ikeda

- K, Yamamoto M, Saoshiro T, Tsuzuki T. A prospective randomized trial of the preventive effect of pre-operative transcatheter arterial embolization against recurrence of hepatocellular carcinoma. *Jpn J Cancer Res* 1996; **87**: 206-211 [PMID: 8609071 DOI: 10.1111/j.1349-7006.1996.tb03160.x]
- 125 **Lau WY**, Leung TW, Ho SK, Chan M, Machin D, Lau J, Chan AT, Yeo W, Mok TS, Yu SC, Leung NW, Johnson PJ. Adjuvant intra-arterial iodine-131-labelled lipiodol for resectable hepatocellular carcinoma: a prospective randomised trial. *Lancet* 1999; **353**: 797-801 [PMID: 10459961 DOI: 10.1016/S0140-6736(98)06475-7]
 - 126 **Boucher E**, Corbinais S, Rolland Y, Bourguet P, Guyader D, Boudjema K, Meunier B, Raoul JL. Adjuvant intra-arterial injection of iodine-131-labeled lipiodol after resection of hepatocellular carcinoma. *Hepatology* 2003; **38**: 1237-1241 [PMID: 14578862 DOI: 10.1053/jhep.2003.50473]
 - 127 **Takayama T**, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, Shimada K, Sakamoto M, Hirohashi S, Ohashi Y, Kakizoe T. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. *Lancet* 2000; **356**: 802-807 [PMID: 11022927 DOI: 10.1016/S0140-6736(00)02654-4]
 - 128 **Muto Y**, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, Tanaka T, Tsurumi K, Okuno M, Tomita E, Nakamura T, Kojima T. Prevention of second primary tumors by an acyclic retinoid, polyphenolic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N Engl J Med* 1996; **334**: 1561-1567 [PMID: 8628336]
 - 129 **Liu CJ**, Lee PH, Lin DY, Wu CC, Jeng LB, Lin PW, Mok KT, Lee WC, Yeh HZ, Ho MC, Yang SS, Lee CC, Yu MC, Hu RH, Peng CY, Lai KL, Chang SS, Chen PJ. Heparanase inhibitor PI-88 as adjuvant therapy for hepatocellular carcinoma after curative resection: a randomized phase II trial for safety and optimal dosage. *J Hepatol* 2009; **50**: 958-968 [PMID: 19303160 DOI: 10.1016/j.jhep.2008.12.023]
 - 130 **Barone C**, Koeberle D, Metselaer H, Parisi G, Sansonno D, Spinzi G. Multidisciplinary approach for HCC patients: hepatology for the oncologists. *Ann Oncol* 2013; **24** Suppl 2: ii15-ii23 [PMID: 23715939 DOI: 10.1093/annonc/mdt053]
 - 131 **Cucchetti A**, Cescon M, Bigonzi E, Piscaglia F, Golfieri R, Ercolani G, Cristina Morelli M, Ravaioli M, Daniele Pinna A. Priority of candidates with hepatocellular carcinoma awaiting liver transplantation can be reduced after successful bridge therapy. *Liver Transpl* 2011; **17**: 1344-1354 [PMID: 21837731 DOI: 10.1002/lt.22397]
 - 132 **Yao FY**, Kerlan RK, Hirose R, Davern TJ, Bass NM, Feng S, Peters M, Terrault N, Freise CE, Ascher NL, Roberts JP. Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. *Hepatology* 2008; **48**: 819-827 [PMID: 18688876 DOI: 10.1002/hep.22412]
 - 133 **Belghiti J**, Carr BI, Greig PD, Lencioni R, Poon RT. Treatment before liver transplantation for HCC. *Ann Surg Oncol* 2008; **15**: 993-1000 [PMID: 18236111 DOI: 10.1245/s10434-007-9787-8]
 - 134 **Schwartz M**, Roayaie S, Uva P. Treatment of HCC in patients awaiting liver transplantation. *Am J Transplant* 2007; **7**: 1875-1881 [PMID: 17532747]
 - 135 **Llovet JM**, Mas X, Aponte JJ, Fuster J, Navasa M, Christensen E, Rodés J, Bruix J. Cost effectiveness of adjuvant therapy for hepatocellular carcinoma during the waiting list for liver transplantation. *Gut* 2002; **50**: 123-128 [PMID: 11772979]
 - 136 **Bouchard-Fortier A**, Lapointe R, Perreault P, Bouchard L, Pomier-Layrargues G. Transcatheter arterial chemoembolization of hepatocellular carcinoma as a bridge to liver transplantation: a retrospective study. *Int J Hepatol* 2011; **2011**: 974514 [PMID: 21994880 DOI: 10.4061/2011/974514]
 - 137 **Lee KT**, Wang SR. The impact of sorafenib on early recurrence of HCC after hepatic surgery. ILCA 2012: The International Liver Cancer Association's 6th Annual Conference; 2012 Sep 14-16; Berlin, Germany. Berlin: International Liver Cancer Association, 2012: abstr 197
 - 138 **Lubienski A**, Bitsch RG, Schemmer P, Grenacher L, Dux M, Kauffmann GW. [Long-term results of interventional treatment of large unresectable hepatocellular carcinoma (HCC): significant survival benefit from combined transcatheter arterial chemoembolization (TACE) and percutaneous ethanol injection (PEI) compared to TACE monotherapy]. *Rofo* 2004; **176**: 1794-1802 [PMID: 15573291]
 - 139 **Morimoto M**, Numata K, Kondou M, Nozaki A, Morita S, Tanaka K. Midterm outcomes in patients with intermediate-sized hepatocellular carcinoma: a randomized controlled trial for determining the efficacy of radiofrequency ablation combined with transcatheter arterial chemoembolization. *Cancer* 2010; **116**: 5452-5460 [PMID: 20672352]
 - 140 **Liapi E**, Geschwind JF. Combination of local transcatheter arterial chemoembolization and systemic anti-angiogenic therapy for unresectable hepatocellular carcinoma. *Liver Cancer* 2012; **1**: 201-215 [PMID: 24159585 DOI: 10.1159/000343835]
 - 141 **Weintraub JL**, Salem R. Treatment of hepatocellular carcinoma combining sorafenib and transarterial locoregional therapy: state of the science. *J Vasc Interv Radiol* 2013; **24**: 1123-1134 [PMID: 23562168 DOI: 10.1016/j.jvir.2013.01.494]
 - 142 **Kim HY**, Park JW. Clinical trials of combined molecular targeted therapy and locoregional therapy in hepatocellular carcinoma: past, present, and future. *Liver Cancer* 2014; **3**: 9-17 [PMID: 24804173 DOI: 10.1159/000343854]
 - 143 **Printz C**. Clinical trials of note. Sorafenib as adjuvant treatment in the prevention of disease recurrence in patients with hepatocellular carcinoma (HCC) (STORM). *Cancer* 2009; **115**: 4646 [PMID: 19806596 DOI: 10.1002/cncr.24673]
 - 144 **Li X**, Feng GS, Zheng CS, Zhuo CK, Liu X. Expression of plasma vascular endothelial growth factor in patients with hepatocellular carcinoma and effect of transcatheter arterial chemoembolization therapy on plasma vascular endothelial growth factor level. *World J Gastroenterol* 2004; **10**: 2878-2882 [PMID: 15334691]
 - 145 **Wang B**, Xu H, Gao ZQ, Ning HF, Sun YQ, Cao GW. Increased expression of vascular endothelial growth factor in hepatocellular carcinoma after transcatheter arterial chemoembolization. *Acta Radiol* 2008; **49**: 523-529 [PMID: 18568538 DOI: 10.1080/02841850801958890]
 - 146 **Shim JH**, Park JW, Kim JH, An M, Kong SY, Nam BH, Choi JI, Kim HB, Lee WJ, Kim CM. Association between increment of serum VEGF level and prognosis after transcatheter arterial chemoembolization in hepatocellular carcinoma patients. *Cancer Sci* 2008; **99**: 2037-2044 [PMID: 19016764 DOI: 10.1111/j.1349-7006.2008.00909.x]
 - 147 **Sergio A**, Cristofori C, Cardin R, Pivetta G, Ragazzi R, Baldan A, Girardi L, Cillo U, Burra P, Giacomini A, Farinati F. Transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC): the role of angiogenesis and invasiveness. *Am J Gastroenterol* 2008; **103**: 914-921 [PMID: 18177453 DOI: 10.1111/j.1572-0241.2007.01712.x]
 - 148 **Pawlik TM**, Reyes DK, Cosgrove D, Kamel IR, Bhagat N, Geschwind JF. Phase II trial of sorafenib combined with concurrent transarterial chemoembolization with drug-eluting beads for hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 3960-3967 [PMID: 21911714 DOI: 10.1200/JCO.2011.37.1021]
 - 149 **Park JW**, Koh YH, Kim HB, Kim HY, An S, Choi JI, Woo SM, Nam BH. Phase II study of concurrent transarterial chemoembolization and sorafenib in patients with unresectable hepatocellular carcinoma. *J Hepatol* 2012; **56**: 1336-1342 [PMID: 22314421 DOI: 10.1016/j.jhep.2012.01.006]
 - 150 **Chung YH**, Han G, Yoon JH, Yang J, Wang J, Shao GL, Kim BI, Lee TY, Chao Y. Interim analysis of START: Study in Asia of the combination of TACE (transcatheter arterial chemoembolization) with sorafenib in patients with hepatocellular carcinoma trial. *Int J Cancer* 2013; **132**: 2448-2458 [PMID: 23129123 DOI: 10.1002/ijc.27925]
 - 151 **Lencioni R**, Llovet JM, Han G, Tak WY, Yang J, Leberre M, Niu W, Nicholson K, Meinhardt G, Bruix J. Sorafenib or placebo in combination with transarterial chemoembolization (TACE) with doxorubicin-eluting beads (DEBDOX) for intermediate stage hepatocellular carcinoma (HCC): phase II, randomized, double-blind SPACE trial. *J Clin Oncol* 2012; **30** Suppl 4: LBA154

- 152 **Qu XD**, Chen CS, Wang JH, Yan ZP, Chen JM, Gong GQ, Liu QX, Luo JJ, Liu LX, Liu R, Qian S. The efficacy of TACE combined sorafenib in advanced stages hepatocellular carcinoma. *BMC Cancer* 2012; **12**: 263 [PMID: 22721173 DOI: 10.1186/1471-2407-12-263]
- 153 **Cabrera R**, Pannu DS, Caridi J, Firpi RJ, Soldevila-Pico C, Morelli G, Clark V, Suman A, George TJ, Nelson DR. The combination of sorafenib with transarterial chemoembolisation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2011; **34**: 205-213 [PMID: 21605146 DOI: 10.1111/j.1365-2036.2011.04697.x]
- 154 **Sansonno D**, Lauletta G, Russi S, Contedua V, Sansonno L, Dammacco F. Transarterial chemoembolization plus sorafenib: a sequential therapeutic scheme for HCV-related intermediate-stage hepatocellular carcinoma: a randomized clinical trial. *Oncologist* 2012; **17**: 359-366 [PMID: 22334456 DOI: 10.1634/theoncologist.2011-0313]
- 155 **Kudo M**, Imanaka K, Chida N, Nakachi K, Tak WY, Takayama T, Yoon JH, Hori T, Kumada H, Hayashi N, Kaneko S, Tsubouchi H, Suh DJ, Furuse J, Okusaka T, Tanaka K, Matsui O, Wada M, Yamaguchi I, Ohya T, Meinhardt G, Okita K. Phase III study of sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma. *Eur J Cancer* 2011; **47**: 2117-2127 [PMID: 21664811 DOI: 10.1016/j.ejca.2011.05.007]
- 156 **Han G**, Yang J, Shao G, Teng G, Wang M, Yang J, Liu Z, Feng G, Yang R, Lu L, Chao Y, Wang J. Sorafenib in combination with transarterial chemoembolization in Chinese patients with hepatocellular carcinoma: a subgroup interim analysis of the START trial. *Future Oncol* 2013; **9**: 403-410 [PMID: 23469975 DOI: 10.2217/fon.13.11]
- 157 **Hsu C**, Po-Ching-Liang S, Hu FC, Cheng AL. Perspectives on the design of clinical trials combining transarterial chemoembolization and molecular targeted therapy. *Liver Cancer* 2012; **1**: 168-176 [PMID: 24159581 DOI: 10.1159/000343830]
- 158 **Martin RC**, Keck G, Robbins K, Strnad B, Dubel G, Longares J, Padr R, Narayanan G. Evaluation of sorafenib in combination with doxorubicin-loaded DC bead as a combination treatment option for HCC. Gastrointestinal Cancers Symposium, 2010: abstr 216
- 159 **Gadani S**, Mahvash A, Avritscher R, Chasen B, Kaseb A, Murthy R. Yttrium-90 resin microspheres as an adjunct to sorafenib in patients with unresectable HCC: A retrospective study for evaluation of survival benefit and adverse events. *J Vasc Interv Radiol* 2013; **24**: S35 [DOI: 10.1016/j.jvir.2013.01.075]
- 160 **Abou-Alfa GK**, Johnson P, Knox JJ, Capanu M, Davidenko I, Lacava J, Leung T, Gansukh B, Saltz LB. Doxorubicin plus sorafenib vs doxorubicin alone in patients with advanced hepatocellular carcinoma: a randomized trial. *JAMA* 2010; **304**: 2154-2160 [PMID: 21081728 DOI: 10.1001/jama.2010.1672]
- 161 **Prete SD**, Montella L, Caraglia M, Maiorino L, Cennamo G, Montesarchio V, Piai G, Febbraro A, Tarantino L, Capasso E, Palmieri G, Guarrasi R, Bianco M, Mamone R, Savastano C, Pisano A, Vincenzi B, Sabia A, D'Agostino A, Faiola V, Addeo R. Sorafenib plus octreotide is an effective and safe treatment in advanced hepatocellular carcinoma: multicenter phase II So.LAR. study. *Cancer Chemother Pharmacol* 2010; **66**: 837-844 [PMID: 20041325 DOI: 10.1007/s00280-009-1226-z]
- 162 **Yau T**, Chan P, Cheung FY, Lee AS, Yau TK, Choo SP, Lau J, Wong JS, Fan ST, Poon RT. Phase II trial of sorafenib with locally advanced or metastatic hepatocellular carcinoma. *European Journal of Cancer Supplements* 2009; **7**: 20-21 [DOI: 10.1016/S1359-6349(09)72082-8]
- 163 **Pettrini I**, Lencioni M, Ricasoli M, Iannopollo M, Orlandini C, Oliveri F, Bartolozzi C, Ricci S. Phase II trial of sorafenib in combination with 5-fluorouracil infusion in advanced hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2012; **69**: 773-780 [PMID: 22033636 DOI: 10.1007/s00280-011-1753-2]
- 164 **Lee SJ**, Lee J, Park SH, Park JO, Park YS, Kang WK, Lee J, Yim DS, Lim HY. Phase I trial of S-1 in combination with sorafenib for patients with advanced hepatocellular carcinoma. *Invest New Drugs* 2012; **30**: 1540-1547 [PMID: 21695438 DOI: 10.1007/s10637-011-9706-5]
- 165 **Abou-Alfa GK**, Chan SL, Lin CC, Chiorean EG, Holcombe RF, Mulcahy MF, Carter WD, Patel K, Wilson WR, Melink TJ, Gutheil JC, Tsao CJ. PR-104 plus sorafenib in patients with advanced hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2011; **68**: 539-545 [PMID: 21594722 DOI: 10.1007/s00280-011-1671-3]
- 166 **Hsu CH**, Shen YC, Lin ZZ, Chen PJ, Shao YY, Ding YH, Hsu C, Cheng AL. Phase II study of combining sorafenib with metronomic tegafur/uracil for advanced hepatocellular carcinoma. *J Hepatol* 2010; **53**: 126-131 [PMID: 20416968 DOI: 10.1016/j.jhep.2010.01.035]
- 167 **Giuliana FAR**, Addeo R, Febbraio A, Rizzi D, Macello E, del Prete S, Pisconti S, Fico M, Colucci G. Sorafenib plus cisplatin and gemcitabine in the treatment of advanced hepatocellular carcinoma: a phase II study by the Gruppo Oncologico Dell'Italia Meridionale (PROT. GOIM 2705). *Cancer Treat Rev* 2010; **36** Suppl 4: S96
- 168 Abstracts of the American Association for the Study of Liver Diseases 61st Annual Meeting and Postgraduate Course. October 29-November 2, 2010. Boston, Massachusetts, USA. *Hepatology* 2010; **52** Suppl: 320A-1291A [PMID: 20949695]
- 169 **National Cancer Institute (NCI)**. Sorafenib Tosylate With or Without Gemcitabine Hydrochloride and Oxaliplatin in Treating Patients With Locally Advanced, Unresectable, or Metastatic Liver Cancer. 2009. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [Cited 2014 October]. Available from: URL: <http://clinicaltrials.gov/ct2/show/NCT00941967> NLM Identifier: NCT00941967
- 170 **Zhu AX**. Sorafenib mFOLFOX for Hepatocellular Carcinoma. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2013. [Cited 2014 October]. Available from: URL: <http://clinicaltrials.gov/ct2/show/NCT01775501> NLM Identifier: NCT01775501
- 171 **The University of Hong Kong**. Sorafenib With Capecitabine and Oxaliplatin for Advanced or Metastatic Hepatocellular Carcinoma (SECOX). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2008. [Cited 2014 October]. Available from: URL: <http://clinicaltrials.gov/ct2/show/NCT00752063> NLM Identifier: NCT00752063
- 172 **Dollinger M**. Sorafenib Plus Doxorubicin Versus Sorafenib Alone for the Treatment of Advanced Hepatocellular Carcinoma: a Randomized Phase II Trial (SoraDox). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2011. [Cited 2014 October]. Available from: URL: <http://clinicaltrials.gov/ct2/show/NCT01272557> NLM Identifier: NCT01272557
- 173 **Abdel-Rahman O**, Fouad M. Sorafenib-based combination as a first line treatment for advanced hepatocellular carcinoma: a systematic review of the literature. *Crit Rev Oncol Hematol* 2014; **91**: 1-8 [PMID: 24457121 DOI: 10.1016/j.critrevonc.2013.12.013]
- 174 **Xie B**, Wang DH, Spechler SJ. Sorafenib for treatment of hepatocellular carcinoma: a systematic review. *Dig Dis Sci* 2012; **57**: 1122-1129 [PMID: 22451120 DOI: 10.1007/s10620-012-2136-1]

P- Reviewer: Hann HW, Sun XY **S- Editor:** Tian YL
L- Editor: A **E- Editor:** Liu SQ



Cirrhosis and portal hypertension: The importance of risk stratification, the role of hepatic venous pressure gradient measurement

Vincenzo La Mura, Antonio Nicolini, Giulia Tosetti, Massimo Primignani

Vincenzo La Mura, Antonio Nicolini, Giulia Tosetti, Massimo Primignani, Fondazione IRCCS, Ca' Granda, Ospedale Maggiore Policlinico, 20100 Milano, Italy

Author contributions: La Mura V contributed to review concept and design; La Mura V and Tosetti G contributed to drafting of the manuscript; Nicolini A and Primignani M contributed to critical revision of the manuscript for important intellectual content.

Conflict-of-interest: There are no conflict of interest to declare related with the manuscript.

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

Correspondence to: Vincenzo La Mura, MD, PhD, Fondazione IRCCS, Ca' Granda, Ospedale Maggiore Policlinico, via F. Sforza, 20100 Milano, Italy. vin.lamura@gmail.com

Telephone: +39-02-5035432

Fax: +39-02-50320410

Received: August 29, 2014

Peer-review started: August 29, 2014

First decision: October 14, 2014

Revised: November 11, 2014

Accepted: January 9, 2015

Article in press: January 12, 2015

Published online: April 8, 2015

for any stage of risk. Experts recommend to move toward a pathophysiological classification of cirrhosis that considers both structural and functional changes. The hepatic venous pressure gradient HVPG, is the reference gold standard to estimate the severity of portal hypertension in cirrhosis. It correlates with both structural and functional changes that occur in cirrhosis and carries valuable prognostic information to stratify the mortality risk. This article provides a general overview of the pathophysiology and natural course of cirrhosis and portal hypertension. We propose a simplified classification of cirrhosis based on low, intermediate and high mortality stage. The prognostic information provided by HVPG is presented according to each stage. A comparison with prognostic models based on clinical and endoscopic variables is discussed in order to evidence the additional contribute given by HVPG on top of other clinical and instrumental variables widely used in clinical practice.

Key words: Cirrhosis; Portal hypertension; Hepatic venous pressure gradient; Variceal bleeding; Prognosis

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Recently a pathophysiological classification of cirrhosis has been strongly encouraged. Hepatic venous pressure gradient (HVPG) measurement is the most reliable tool to estimate the severity of portal hypertension in cirrhosis but several methodological concerns have limited its use in clinical practice. The article summarizes the results published about the prognostic value of HVPG and originally offers a critical revision of the information provided by this hemodynamic parameter on top of the most widely used models based on non-hemodynamic parameters.

Abstract

Portal hypertension is the main prognostic factor in cirrhosis. The recent emergence of potent antiviral drugs and new algorithm of treatment for the management of complications due to portal hypertension have sensibly changed our perception of cirrhosis that can be now considered as a multistage liver disease whose mortality risk can be reduced by a tailored approach

La Mura V, Nicolini A, Tosetti G, Primignani M. Cirrhosis and

portal hypertension: The importance of risk stratification, the role of hepatic venous pressure gradient measurement. *World J Hepatol* 2015; 7(4): 688-695 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/688.htm> DOI: <http://dx.doi.org/10.4254/wjhl.v7.i4.688>

INTRODUCTION

Advanced chronic hepatitis, whatever the etiology, accounts for the development of regenerative nodules surrounded by fibrotic septa that are the histological hallmark of liver cirrhosis^[1]. These architectural changes are associated with a relevant increase of intra-hepatic resistance to portal blood flow and, as a consequence, an increase of portal pressure^[2]. In particular, a portal pressure gradient (the difference between the portal pressure and the inferior cava vein pressure) greater than 5 mmHg defines the condition of portal hypertension^[3]. The hepatic venous pressure gradient (HVPG) (the difference between wedged and free hepatic venous pressure measurement) accurately reflects the portal pressure gradient in the most common causes of cirrhosis^[4,5].

No specific symptoms are associated with chronic hepatitis, even in the early phases of cirrhosis, up to the development of clinical significant portal hypertension (CSPH, HVPG ≥ 10 mmHg)^[6]. Ascites and gastroesophageal varices are the most frequent manifestations of CSPH. Other complications as variceal bleeding (VB), spontaneous bacterial peritonitis (SBP), and infections other than SBP, hepato-renal syndrome (HRS) and hepatic encephalopathy (HE) parallel the severity of portal hypertension and substantially worsen the prognosis. As a consequence, cirrhosis should no longer be considered "*per se*" an end-stage liver disease, but a multi-stage disease with different outcomes according to the mortality risk^[7]. By contrast hepatocellular carcinoma (HCC) can occur at any stage of liver disease although the severity of portal hypertension influences the therapeutic options^[8].

In the last years, the development of very effective antiviral drugs and new therapeutic strategies to prevent or manage acute complications of portal hypertension have changed our general perception of cirrhosis^[9]. At present, cirrhosis should be regarded as a disease whose mortality risk can be significantly reduced by a specific tailored approach for any stage of risk^[7].

This article is focused on the clinical implications of cirrhosis as a multistage liver disease with low, intermediate and high mortality risk (Table 1) and discusses the use of HVPG measurement as a surrogate indicator of clinical outcome for any stage.

THE PATHOPHYSIOLOGY OF CIRRHOSIS (SHORT OVERVIEW)

Portal hypertension is the main driving factor in the

natural history of cirrhosis^[10]. In the earliest stages of the disease, portal pressure is determined by the increase of liver resistance to portal blood flow caused by histological changes of the liver, like fibrosis and nodules^[6-11]. On top of these histological features, there is an over-activation of contractile elements at the portal and sinusoidal level (e.g., hepatic stellate cells, hepatic myofibroblasts, vascular smooth muscle cells) that weakens the ability of the liver to accommodate the vascular tone to portal blood flow^[12]. Along with the progression of the disease, extrahepatic vascular changes occur^[13]. Indeed, arterial vasodilation and neo-angiogenesis at the splanchnic circulation creates a condition of "effective hypovolemia" featured by a discrepancy between the relatively low blood volume flowing into the great central vessels and the increased vascular filling capacity. Effective hypovolemia is the trigger of a hyperkinetic syndrome that causes portal venous blood flow increase^[2]. Hallmarks of the hyperkinetic syndrome are a high cardiac output and a low peripheral vascular resistance. Along with these changes, an activation of neuro-humoral vasoactive systems occurs that, finally, causes hydro-electrolyte retention by the kidney^[14]. The final consequence is a major increase of blood volume flowing through the splanchnic circulation. The inability of the liver to accommodate sinusoidal resistance to this high blood flow fosters a further increase of portal pressure that determines fluid escape into the peritoneal cavity (ascites) and induces the development of collateral vessels (varices). In the latest stages of cirrhosis, the extreme consequences of vasodilation and hydro-electrolyte imbalance are type-2 hepato-renal syndrome (HSR-2) and/or hyponatremia^[15,16]. Recently, a reduction of cardiac output, and the consequent reversal of the hyperdynamic syndrome into a (relative) hypodynamic circulatory state has been shown to trigger type-1 HRS^[17]. Given the importance of splanchnic vasodilation in the genesis of effective hypovolemia and the hyperdynamic circulation, and the consequent further increase of portal pressure, any superimposed condition able to further reduce the vascular tone and/or the cardiac function is, potentially, life-threatening, as it could irreversibly impair organ perfusion. This may occur either in the presence of SBP (or, potentially, other infections), or in the case of large volume paracentesis without adequate volume expansion with albumin (post-paracentesis circulatory dysfunction)^[18-22]. All in all, along with the development of severe portal hypertension, the control of vascular homeostasis and organ perfusion becomes frail and this is among the main pathogenic mechanisms underlying the high mortality rate of acute on chronic liver failure^[23]. The occurrence of hepato-cellular insufficiency is another important pathogenic mechanism clinically manifested in advanced stages of cirrhosis. We know that portal pressure reduction can ameliorate liver function^[24]. Further emergent factors such as cytokine release^[25], the prothrombotic imbalance described in cirrhosis^[26],

Table 1 Natural history of cirrhosis

Stages of cirrhosis Mortality risk at 1 yr			
	Low ≤ 1%	Intermediate 1%-20%	High > 20%
Clinical features	Asymptomatic no varices	Varices/ascites or both	Bleeding/re-bleeding SBP Refractory ascites HRS/AKI Infection other than SBP
HVPG of risk	10 mmHg		
Main outcome to prevent	Decompensation and/or HCC and/or varices	Decompensation and/or HCC mortality	HCC and/or mortality
Main pathophysiologic factor	Intrahepatic structural and hemodynamic changes Portal pressure	Extrahepatic hemodynamic changes Portal pressure	Hepatocellular dysfunction Portal pressure Cytokine release Peripheral perfusion Coagulopathy? Other?

Each category of risk is presented with the clinical features, the hepatic venous pressure gradient value, the main outcome to prevent, the main pathophysiologic factor related with that category of risk. SBP: Spontaneous bacterial peritonitis; HRS: Hepato-renal syndrome; AKI: Acute kidney injury; HCC: Hepatocellular carcinoma; ACLF: Acute on chronic liver failure; HVPG: Hepatic venous pressure gradient.

the increased levels of von Willebrand factor^[27,28] and the intra- and extrahepatic changes induced by bacterial derived byproducts^[29-31] could, in our opinion, play a relevant role in determining the outcome of advanced cirrhosis.

PROGNOSTIC INDICATORS IN CLINICAL PRACTICE: ADVANTAGES AND LIMITATIONS

The appearance of any among ascites, variceal bleeding, hepatic encephalopathy, hepato-renal syndrome, spontaneous bacterial infection or jaundice heralds the passage from compensated to decompensated cirrhosis^[32,33]. This distinction is commonly used to establish two conditions with different prognosis, however it has both advantages and drawbacks. It is easy to use in clinical practice and appears rationale, as several studies have consistently demonstrated that such events are associated with a relevant mortality rate and worsen the prognosis. However, major drawbacks rely on considering at similar risk episodes of decompensation of diverse severity (e.g., the first appearance of ascites responsive to diuretics or spontaneous bacterial peritonitis, that is an infective complication of ascites), or including in the same prognostic group complications strictly related with the degree of portal hypertension and complications due to hepato-cellular insufficiency (such as jaundice).

Some of these limitations were overcome by the Child-Pugh classification that distinguishes between compensated (Child-Pugh class A) and decompensated patients and subgroups the latter category of patients in intermediate (Child-Pugh class B) or high degree of liver dysfunction (Child-Pugh class C). However, some of the parameters of Child-Pugh classification are subjective and influenced by therapy, thus decreasing

the accuracy of the classification^[34]. Moreover, the categorization of continuous laboratory parameters, characteristic of this classification system, reduces the discriminative power of the model. A further, important inadequacy of the Child-Pugh classification is that it does not take into account the renal function, whose impairment is associated with a high mortality rate in patients with liver cirrhosis. The more recently introduced MELD score, firstly devised to predict the outcome of patients undergoing transjugular intra-hepatic porto-systemic shunt (TIPS)^[35], but currently used in daily practice to prioritize patients for liver transplantation, is certainly more objective and reproducible^[34].

All these systems are widely used in clinical practice. They simplify the work-up of physicians to define the degree of liver dysfunction and prognosis of patients by using the most common clinical and laboratory findings and are, for that, very easy-to-use.

However, these models ignore the problems related with the presence or not of endoscopic signs of portal hypertension, whose detection indicate the use of pharmacologic and/or endoscopic therapy to manage the bleeding risk. Indeed, according to international guidelines, all patients diagnosed with cirrhosis should be screened for the presence of gastro-esophageal varices every 2-3 years^[10]. The presence of varices and a history of previous bleeding define different clinical scenarios, each associated with a specific mortality risk: prevention of varices development and first bleeding episode (pre-primary and primary prophylaxis) in compensated patients; treatment of acute variceal bleeding and prevention of recurrent bleeding (secondary prophylaxis) in decompensated patients. In this respect, a further discrimination of patients at high risk of bleeding and bleeding related mortality has been encouraged since a good pre-selection of patients may determine better results on survival by using more

aggressive strategies of treatment^[36].

In recent years, the availability of non-invasive tools to diagnose cirrhosis, together with the development of potent antiviral drugs able to reduce fibrosis, has significantly increased the proportion of patients with chronic liver disease diagnosed in the asymptomatic stage of cirrhosis with the ultimate result that the surveillance schedule for the presence of varices should be revised in order to reduce the number of unnecessary endoscopies performed in daily clinical practice^[37]. Another clinical outcome that sensibly influences the outcome of patients with cirrhosis is the possibility of predicting the response to pharmacologic therapy to prevent variceal bleeding/rebleeding. Unfortunately, the low intra- and inter-observer reproducibility of the endoscopic features does not allow recommending the use of this technique systematically^[38].

In summary, the natural history of cirrhosis is strongly dependent on portal hypertension related complications. The use of several clinical and/or instrumental signs, in particular, upper gastro-intestinal endoscopy, characterizes the work-up of physicians to predict and manage the risk of portal hypertension related complications in several stages of risk mainly based on varices, ascites and bleeding^[39]. However, recently, a pathophysiological classification of cirrhosis has been evoked to promote a system that better reflects the dynamic state of cirrhosis and provides a useful tool to predict outcomes and individualize therapy^[7].

THE ADDITIONAL PROGNOSTIC INFORMATION PROVIDED BY HVPG

Given the importance of portal hypertension in the natural history of patients with cirrhosis, it would be expected that HVPG measurement hold prognostic information in this setting. Theoretically, the use of HVPG as a prognostic tool has several advantages: (1) it is an objective and continuous variable; (2) it changes in presence of specific therapeutic interventions and/or an improvement of liver function^[40-42]; and (3) it has been widely studied in cross sectional-, longitudinal-studies, randomized controlled trials and metanalysis to consider it as one of the most reliable surrogate markers of clinical outcome in Hepatology^[43]. However, there are several disadvantages limiting its use^[3,44,45]: (1) the costs associated with the methodology; (2) the need of trained physicians to get a reliable measurement; and (3) the relatively invasiveness of the methodology. Since its introduction, evidence has been accumulated that HVPG correlates with the degree of liver dysfunction^[46-49]. Due for that, it has been recommended to test the prognostic value of the HVPG in the context of multivariable analysis in order to quantify the additional prognostic information provided on top of that achieving by common clinical and instrumental parameters.

Low risk cirrhosis

With the term of low risk cirrhosis we defined the condition of asymptomatic cirrhosis without neither gastroesophageal varices nor other gastro-intestinal signs of portal hypertension with a mortality rate at one year less than 1%. In this context the main clinical outcome to be predicted is the appearance of the first decompensation, that, often, is the appearance of ascites, but another clinical outcome to predict is the development of varices at risk of bleeding. At this stage, HVPG significantly reflects structural and functional changes occurring in the liver^[6,11]. However, above the threshold of 10 mmHg, the high correlation of HVPG with intrahepatic changes is blunted^[50] and the degree of portal pressure is determined by the concomitant activation of extrahepatic hemodynamic and humoral changes that foster a state of hyperdynamic circulation^[13]. A large longitudinal study including patients with compensated cirrhosis without varices demonstrated that patients with a HVPG < 10 mmHg carry a negligible risk of developing varices and a very low risk of decompensation where a HVPG \geq 10 mmHg is associated with 28% rate of varices development and 20% of first decompensation at two years^[51,52]. This demonstrates that patients with asymptomatic cirrhosis, classically defined as compensated and/or Child-Pugh class A, are a heterogeneous population at different risk of becoming symptomatic. However, opportunely designed studies are warranted to translate this prognostic information into the decision making process. Interesting, from the same cohort of patients, it was also demonstrated that HVPG \geq 10 mmHg is associated with a 6-fold increase of HCC risk^[53]. In this context, 10 mmHg was, again, the threshold able to identify the candidates to liver resection at risk of decompensation. Such valuable prognostic information allows considering HVPG as the strongest surrogate marker of clinical outcome to guide the decision making in this setting.

Intermediate risk cirrhosis

The diagnosis of varices, with or without ascites, or ascites alone is the hallmark of CSPH. The need at this step is to predict the risk of portal hypertension related complications (*e.g.*, bleeding, SBP, refractory ascites, *etc.*), and the hemodynamic response to non-selective beta-blockers (NSBBs), the mainstay of pharmacologic treatment to reduce portal pressure. We know that esophageal varices, whatever the diameter, are at risk of bleeding for HVPG \geq 12 mmHg^[54,55]. Moreover a second hemodynamic study that demonstrates the achievement of a HVPG below this value or a reduction \geq 20% of the basal value is highly specific of a good clinical response to NSBBs treatment^[3]. At this stage, a reduction of HVPG also correlates with a reduced risk of SBP or bacteremia^[42]. Importantly, the prognostic contribution provided by HVPG appears to be independent by the degree of liver insufficiency and the

bleeding risk assessed by endoscopy markers of risk such as variceal diameter and the presence of red wale markers of risk (North Italian Endoscopic Club)^[56], thus suggesting that the degree of portal hypertension is the main determinant of prognosis, and its reduction should be the principal aim at this stage of the disease^[41,42]. One limitation of monitoring response to NSBBs treatment by HVPG is that two measurements are needed. This doubles the costs, but also the invasive procedures for the patients. Moreover, not all patients classified as non-responders to NSBBs will bleed in the follow-up, while some patients can bleed before the second HVPG measurement^[57]. To overcome these limitations, a single HVPG measurement by testing the acute hemodynamic response to intra-venous propranolol has been proposed and proved to correlate with the chronic response to NSBBs. Moreover, such single measurement seemed to be able to predict the prognosis of candidates to NSBBs treatment more accurately than the HVPG response to NSBBs requiring two hemodynamic sessions^[58,59]. Further clinical studies to confirm the role of acute HVPG response to propranolol in the decision making are waited.

High risk cirrhosis

This stage includes all patients experiencing a first or further episode of decompensation at high mortality risk such as VB, refractory ascites, SBP, HRS, hyponatremia and HE. Recently, a metanalysis^[60] showed that any kind of bacterial infection is dramatically associated with a 67% mortality rate at one year, even though patients overcome the infection. The role of a prognostic marker, at this stage, is to help in identifying, among patients with decompensation, those at the highest mortality risk in order to implement more aggressive therapeutic strategies.

In several studies HVPG disclosed an independent predictive value on mortality in decompensated patients^[39]. Ripoll *et al.*^[61] in a series of 393 patients, mostly with previous decompensation, showed that the HVPG, independently by MELD, had an overall effect of 3% increase of mortality for each 1 mmHg of HVPG increase. Unfortunately, the addition of the HVPG to the MELD score augmented very little the discriminative power of the MELD score alone to rank the mortality risk of decompensated patients. Then, although portal hypertension can further increase at this advanced stage of cirrhosis, hepato-cellular dysfunction, and, probably, other factors related to acute on chronic liver failure, like inflammatory cytokine release or the impairment of cardiac function and peripheral organ perfusion, probably play a major prognostic role in this clinical setting.

Another important aspect is that the relationship between HVPG and the risk of death could be non-linear. This can be evidenced by considering dichotomization of the HVPG value. In this respect, several studies found an increased mortality risk in patients with HVPG beyond the threshold of 16 mmHg^[48,62-64]. The biological rationale behind this finding is intriguing but still unclear. The

threshold of 16 mmHg would allow an early detection of patients at high mortality risk, and is a promising threshold to be tested in the clinical decision process.

Acute variceal bleeding is the complication of advanced cirrhosis with the highest mortality reduction achieved in the last decades, from 40%-50% to 10%-20%^[65]. In acute variceal bleeding, an HVPG ≥ 20 mmHg identifies patients at high risk of early rebleeding and bleeding related mortality^[66]. Recently, two randomized trials have shown that the reduction of portal pressure achieved by an early TIPS (within 72 h from bleeding) significantly decreases the mortality rate of "high risk patients"^[67,68] (those with a HVPG ≥ 20 mmHg or patient in Child-Pugh class C or in class B who have persistent bleeding at endoscopy). The positive effect on survival achieved by TIPS in patients at high risk of mortality during acute variceal bleeding confirms the importance of the degree of portal pressure in this clinical setting.

Beyond HVPG measurement: Liver/spleen stiffness

Several clinical studies have tried to demonstrate that portal pressure can be indirectly estimated by also non-invasive techniques, in particular, measurement of liver stiffness and, more recently, splenic stiffness by transient elastography^[69,70]. Moreover, several attempts have been made in order to test the ability of these parameters (alone or in combination with other clinical variables) to predict the presence of varices, the risk of decompensation, the presence of CSPH in order to facilitate the sub-classification of cirrhosis by a non-invasive methodology^[71,72].

Liver stiffness is mainly determined by fibrosis that plays a determinant role in the pathogenesis of portal hypertension in the earliest stages of cirrhosis. Due for that, it shows a good correlation with HVPG only before extrahepatic hemodynamic changes determine the increase of portal pressure over the threshold of CSPH. The accuracy of this parameter to detect CSPH is high, good for the detection of varices, however the cut-offs proposed to predict these clinical conditions differs from one study to another^[69]. In our opinion this sensibly reduces the possibility of introducing liver stiffness measurement in the clinical decision process neither allows to detecting the response to pharmacological reduction of portal pressure. Theoretically, splenic stiffness would be a better candidate for the non-invasive assessment of portal pressure since spleen congestion and fibrosis are both effects related with portal pressure increase^[73]. At now the most interesting results have been achieved in hepatitis c virus positive cirrhotic patients. In this clinical subset splenic stiffness has been showed promising results for the non-invasive assessment of portal hypertension, the detection of varices, the prediction of the first decompensation^[74,75].

CONCLUSION

The introduction of potent antiviral drugs and the definition of new algorithms of treatments to prevent

and manage complications due to portal hypertension has revealed that cirrhosis is a multi-stage disease featured by different survival risks. The HVPG measurement is, at present, the most reliable tool for clinicians to predict the clinical outcome and dictate the decision making in several complications. HVPG is particularly promising in low risk cirrhosis for any complication related to portal hypertension and for HCC screening and management. HVPG is a useful tool to predict the response to NSBBs in primary and secondary prevention of variceal bleeding, respectively, in intermediate and high risk cirrhosis. The occurrence of critical complications of portal hypertension and the increasing hepato-cellular insufficiency in this stage of high mortality risk apparently reduce the prognostic contribution of HVPG measurement. Probably, at this clinical stage, other clinical variables may substitute HVPG with a good level of accuracy.

Liver/spleen stiffness are new non-invasive emerging tools to, potentially, estimate the degree of portal hypertension. They might resemble in several aspects the HVPG measurement with the additional advantage of a higher tolerability and reduced costs. However, further studies are needed before a non-invasive "portal sphygmomanometer" can substitute the prognostic information provided by HVPG in cirrhosis.

REFERENCES

- 1 **Tsochatzis EA**, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014; **383**: 1749-1761 [PMID: 24480518 DOI: 10.1016/S0140-6736(14)60121-5]
- 2 **Bosch J**, Abraldes JG, Fernández M, García-Pagán JC. Hepatic endothelial dysfunction and abnormal angiogenesis: new targets in the treatment of portal hypertension. *J Hepatol* 2010; **53**: 558-567 [PMID: 20561700 DOI: 10.1016/j.jhep.2010.03.021]
- 3 **Bosch J**, Abraldes JG, Berzigotti A, García-Pagán JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 573-582 [PMID: 19724251 DOI: 10.1038/nrgastro.2009.149]
- 4 **Boyer TD**, Triger DR, Horisawa M, Redeker AG, Reynolds TB. Direct transhepatic measurement of portal vein pressure using a thin needle. Comparison with wedged hepatic vein pressure. *Gastroenterology* 1977; **72**: 584-589 [PMID: 838210 DOI: 10.1016/S0016-5085(77)80136-4]
- 5 **Perelló A**, Escorsell A, Bru C, Gilibert R, Moitinho E, García-Pagán JC, Bosch J. Wedged hepatic venous pressure adequately reflects portal pressure in hepatitis C virus-related cirrhosis. *Hepatology* 1999; **30**: 1393-1397 [PMID: 10573517 DOI: 10.1002/hep.510300628]
- 6 **Nagula S**, Jain D, Groszmann RJ, Garcia-Tsao G. Histological-hemodynamic correlation in cirrhosis-a histological classification of the severity of cirrhosis. *J Hepatol* 2006; **44**: 111-117 [PMID: 16274836 DOI: 10.1016/j.jhep.2005.07.036]
- 7 **Garcia-Tsao G**, Friedman S, Iredale J, Pinzani M. Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis. *Hepatology* 2010; **51**: 1445-1449 [PMID: 20077563 DOI: 10.1002/hep.23478]
- 8 **European Association For The Study Of The Liver**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- 9 **Albillos A**, Garcia-Tsao G. Classification of cirrhosis: the clinical use of HVPG measurements. *Dis Markers* 2011; **31**: 121-128 [PMID: 22045397 DOI: 10.3233/DMA-2011-0834]
- 10 **de Franchis R**. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; **53**: 762-768 [PMID: 20638742 DOI: 10.1016/j.jhep.2010.06.004]
- 11 **Sethasine S**, Jain D, Groszmann RJ, Garcia-Tsao G. Quantitative histological-hemodynamic correlations in cirrhosis. *Hepatology* 2012; **55**: 1146-1153 [PMID: 22109744 DOI: 10.1002/hep.24805]
- 12 **Iwakiri Y**, Groszmann RJ. Vascular endothelial dysfunction in cirrhosis. *J Hepatol* 2007; **46**: 927-934 [PMID: 17391799 DOI: 10.1016/j.jhep.2007.02.006]
- 13 **Iwakiri Y**, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. *Hepatology* 2006; **43**: S121-S131 [PMID: 16447289 DOI: 10.1002/hep.20993]
- 14 **Salerno F**, Guevara M, Bernardi M, Moreau R, Wong F, Angeli P, Garcia-Tsao G, Lee SS. Refractory ascites: pathogenesis, definition and therapy of a severe complication in patients with cirrhosis. *Liver Int* 2010; **30**: 937-947 [PMID: 20492521 DOI: 10.1111/j.1478-3231.2010.02272.x]
- 15 **Schrier RW**, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodés J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988; **8**: 1151-1157 [PMID: 2971015 DOI: 10.1002/hep.1840080532]
- 16 **Porcel A**, Díaz F, Rendón P, Macías M, Martín-Herrera L, Girón-González JA. Dilutional hyponatremia in patients with cirrhosis and ascites. *Arch Intern Med* 2002; **162**: 323-328 [PMID: 11822925 DOI: 10.1001/archinte.162.3.323]
- 17 **Ruiz-del-Arbol L**, Urman J, Fernández J, González M, Navasa M, Monescillo A, Albillos A, Jiménez W, Arroyo V. Systemic, renal, and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 2003; **38**: 1210-1218 [PMID: 14578859 DOI: 10.1053/jhep.2003.50447]
- 18 **Ginès P**, Arroyo V. Paracentesis in the management of cirrhotic ascites. *J Hepatol* 1993; **17** Suppl 2: S14-S18 [PMID: 8491965 DOI: 10.1016/S0168-8278(05)80449-0]
- 19 **Ginès A**, Fernández-Esparrach G, Monescillo A, Vila C, Domènech E, Abecasis R, Angeli P, Ruiz-Del-Arbol L, Planas R, Solà R, Ginès P, Terg R, Inglada L, Vaqué P, Salerno F, Vargas V, Clemente G, Quer JC, Jiménez W, Arroyo V, Rodés J. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. *Gastroenterology* 1996; **111**: 1002-1010 [PMID: 8831595 DOI: 10.1016/S0016-5085(96)70068-9]
- 20 **Sort P**, Navasa M, Arroyo V, Aldegue X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409 [PMID: 10432325 DOI: 10.1056/NEJM199908053410603]
- 21 **Fernández J**, Monteagudo J, Bargallo X, Jiménez W, Bosch J, Arroyo V, Navasa M. A randomized unblinded pilot study comparing albumin versus hydroxyethyl starch in spontaneous bacterial peritonitis. *Hepatology* 2005; **42**: 627-634 [PMID: 16108036 DOI: 10.1002/hep.20829]
- 22 **Guevara M**, Terra C, Nazar A, Solà E, Fernández J, Pavesi M, Arroyo V, Ginès P. Albumin for bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. A randomized, controlled study. *J Hepatol* 2012; **57**: 759-765 [PMID: 22732511 DOI: 10.1016/j.jhep.2012.06.013]
- 23 **Moreau R**, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426-1437, 1437.e1-9 [PMID: 23474284 DOI: 10.1053/j.gastro.2013.02.042]
- 24 **Abraldes JG**, Albillos A, Bañares R, Turnes J, González R, García-Pagán JC, Bosch J. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized

- controlled trial. *Gastroenterology* 2009; **136**: 1651-1658 [PMID: 19208350 DOI: 10.1053/j.gastro.2009.01.043]
- 25 **Cazzaniga M**, Dionigi E, Gobbo G, Fioretti A, Monti V, Salerno F. The systemic inflammatory response syndrome in cirrhotic patients: relationship with their in-hospital outcome. *J Hepatol* 2009; **51**: 475-482 [PMID: 19560225 DOI: 10.1016/j.jhep.2009.04.017]
- 26 **Tripodi A**, Mannucci PM. The coagulopathy of chronic liver disease. *N Engl J Med* 2011; **365**: 147-156 [PMID: 21751907 DOI: 10.1056/NEJMra1011170]
- 27 **La Mura V**, Reverter JC, Flores-Arroyo A, Raffa S, Reverter E, Seijo S, Abraldes JG, Bosch J, García-Pagán JC. Von Willebrand factor levels predict clinical outcome in patients with cirrhosis and portal hypertension. *Gut* 2011; **60**: 1133-1138 [PMID: 21427197 DOI: 10.1136/gut.2010.235689]
- 28 **Ferlitsch M**, Reiberger T, Hoke M, Salzl P, Schwengerer B, Ulbrich G, Payer BA, Trauner M, Peck-Radosavljevic M, Ferlitsch A. von Willebrand factor as new noninvasive predictor of portal hypertension, decompensation and mortality in patients with liver cirrhosis. *Hepatology* 2012; **56**: 1439-1447 [PMID: 22532296 DOI: 10.1002/hep.25806]
- 29 **Tandon P**, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis* 2008; **28**: 26-42 [PMID: 18293275 DOI: 10.1055/s-2008-1040319]
- 30 **Bellet P**, García-Pagán JC, Francés R, Abraldes JG, Navasa M, Pérez-Mateo M, Such J, Bosch J. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology* 2010; **52**: 2044-2052 [PMID: 20979050 DOI: 10.1002/hep.23918]
- 31 **La Mura V**, Pasarin M, Meireles CZ, Miquel R, Rodríguez-Vilarrupla A, Hide D, Gracia-Sancho J, García-Pagán JC, Bosch J, Abraldes JG. Effects of simvastatin administration on rodents with lipopolysaccharide-induced liver microvascular dysfunction. *Hepatology* 2013; **57**: 1172-1181 [PMID: 23184571 DOI: 10.1002/hep.26127]
- 32 **Saunders JB**, Walters JR, Davies AP, Paton A. A 20-year prospective study of cirrhosis. *Br Med J (Clin Res Ed)* 1981; **282**: 263-266 [PMID: 6779978 DOI: 10.1136/bmj.282.6260.263]
- 33 **Ginés P**, Quintero E, Arroyo V, Terés J, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128 [PMID: 3804191 DOI: 10.1002/hep.1840070124]
- 34 **Pagliaro L**. MELD: the end of Child-Pugh classification? *J Hepatol* 2002; **36**: 141-142 [PMID: 11804679 DOI: 10.1016/S0168-8278(01)00302-6]
- 35 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]
- 36 **García-Tsao G**, Bosch J. Management of varices and variceal hemorrhage in cirrhosis. *N Engl J Med* 2010; **362**: 823-832 [PMID: 20200386 DOI: 10.1056/NEJMra0901512]
- 37 **Rudler M**, Benosman H, Lebray P, Nghiem D, Ngo Y, Munteanu M. Screening for esophageal varices in patients newly diagnosed with cirrhosis in 2011: 84% of upper gastrointestinal endoscopies are futile. *Hepatology* 2011; **54** (Suppl): 935A [DOI: 10.1002/hep.24666]
- 38 **Winkfield B**, Aubé C, Burtin P, Calès P. Inter-observer and intra-observer variability in hepatology. *Eur J Gastroenterol Hepatol* 2003; **15**: 959-966 [PMID: 12923367 DOI: 10.1097/00042737-200309000-00004]
- 39 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]
- 40 **Vorobioff J**, Groszmann RJ, Picabea E, Gamen M, Villavicencio R, Bordato J, Morel I, Audano M, Tanno H, Lerner E, Passamonti M. Prognostic value of hepatic venous pressure gradient measurements in alcoholic cirrhosis: a 10-year prospective study. *Gastroenterology* 1996; **111**: 701-709 [PMID: 8780575 DOI: 10.1053/gast.1996.v111.pm8780575]
- 41 **Abraldes JG**, Tarantino I, Turnes J, García-Pagán JC, Rodés J, Bosch J. Hemodynamic response to pharmacological treatment of portal hypertension and long-term prognosis of cirrhosis. *Hepatology* 2003; **37**: 902-908 [PMID: 12668985 DOI: 10.1053/jhep.2003.50133]
- 42 **Turnes J**, García-Pagán JC, Abraldes JG, Hernández-Guerra M, Dell'Era A, Bosch J. Pharmacological reduction of portal pressure and long-term risk of first variceal bleeding in patients with cirrhosis. *Am J Gastroenterol* 2006; **101**: 506-512 [PMID: 16542287]
- 43 **Gluud C**, Brok J, Gong Y, Koretz RL. Hepatology may have problems with putative surrogate outcome measures. *J Hepatol* 2007; **46**: 734-742 [PMID: 17316871]
- 44 **Groszmann RJ**, Wongcharatrawee S. The hepatic venous pressure gradient: anything worth doing should be done right. *Hepatology* 2004; **39**: 280-282 [PMID: 14767976 DOI: 10.1002/hep.20062]
- 45 **La Mura V**, Abraldes JG, Berzigotti A, Erice E, Flores-Arroyo A, García-Pagán JC, Bosch J. Right atrial pressure is not adequate to calculate portal pressure gradient in cirrhosis: a clinical-hemodynamic correlation study. *Hepatology* 2010; **51**: 2108-2116 [PMID: 20512998 DOI: 10.1002/hep.23612]
- 46 **Brailion A**, Cales P, Valla D, Gaudy D, Geoffroy P, Lebrec D. Influence of the degree of liver failure on systemic and splanchnic haemodynamics and on response to propranolol in patients with cirrhosis. *Gut* 1986; **27**: 1204-1209 [PMID: 3781335 DOI: 10.1136/gut.27.10.1204]
- 47 **Gluud C**, Henriksen JH, Nielsen G. Prognostic indicators in alcoholic cirrhotic men. *Hepatology* 1988; **8**: 222-227 [PMID: 3258578 DOI: 10.1002/hep.1840080205]
- 48 **Stanley AJ**, Robinson I, Forrest EH, Jones AL, Hayes PC. Haemodynamic parameters predicting variceal haemorrhage and survival in alcoholic cirrhosis. *QJM* 1998; **91**: 19-25 [PMID: 9519209]
- 49 **Le Moine O**, Hadengue A, Moreau R, Sogni P, Soupison T, Yang S, Hartleb M, Lebrec D. Relationship between portal pressure, esophageal varices, and variceal bleeding on the basis of the stage and cause of cirrhosis. *Scand J Gastroenterol* 1997; **32**: 731-735 [PMID: 9246716 DOI: 10.3109/00365529708996526]
- 50 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971 DOI: 10.1002/hep.21665]
- 51 **Groszmann RJ**, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, García-Pagán JC, Patch D, Matloff DS, Gao H, Makuch R. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med* 2005; **353**: 2254-2261 [PMID: 16306522 DOI: 10.1056/NEJMoa044456]
- 52 **Ripoll C**, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, Escorsell A, García-Pagán JC, Makuch R, Patch D, Matloff DS, Bosch J. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; **133**: 481-488 [PMID: 17681169]
- 53 **Ripoll C**, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, Burroughs A, Planas R, Escorsell A, García-Pagán JC, Makuch R, Patch D, Matloff DS. Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. *J Hepatol* 2009; **50**: 923-928 [PMID: 19303163 DOI: 10.1016/j.jhep.2009.01.014]
- 54 **García-Tsao G**, Groszmann RJ, Fisher RL, Conn HO, Atterbury CE, Glickman M. Portal pressure, presence of gastroesophageal varices and variceal bleeding. *Hepatology* 1985; **5**: 419-424 [PMID: 3873388]
- 55 **Casado M**, Bosch J, García-Pagán JC, Bru C, Bañares R, Bandi JC, Escorsell A, Rodríguez-Láiz JM, Gilabert R, Feu F, Schorlemer C, Echenagusia A, Rodés J. Clinical events after transjugular intrahepatic portosystemic shunt: correlation with hemodynamic findings. *Gastroenterology* 1998; **114**: 1296-1303 [PMID: 9609767 DOI: 10.1016/S0016-5085(98)70436-6]
- 56 **North Italian Endoscopic Club for the Study and Treatment of Esophageal Varices**. Prediction of the first variceal hemorrhage in patients with cirrhosis of the liver and esophageal varices. A

- prospective multicenter study. *N Engl J Med* 1988; **319**: 983-989 [PMID: 3262200 DOI: 10.1056/NEJM198810133191505]
- 57 **Thalheimer U**, Bosch J, Burroughs AK. How to prevent varices from bleeding: shades of grey--the case for nonselective beta blockers. *Gastroenterology* 2007; **133**: 2029-2036 [PMID: 18054573]
 - 58 **Villanueva C**, Aracil C, Colomo A, Hernández-Gea V, López-Balaguer JM, Alvarez-Urturi C, Torras X, Balanzó J, Guarner C. Acute hemodynamic response to beta-blockers and prediction of long-term outcome in primary prophylaxis of variceal bleeding. *Gastroenterology* 2009; **137**: 119-128 [PMID: 19344721 DOI: 10.1053/j.gastro.2009.03.048]
 - 59 **La Mura V**, Abraldes JG, Raffà S, Retto O, Berzigotti A, García-Pagán JC, Bosch J. Prognostic value of acute hemodynamic response to i.v. propranolol in patients with cirrhosis and portal hypertension. *J Hepatol* 2009; **51**: 279-287 [PMID: 19501930 DOI: 10.1016/j.jhep.2009.04.015]
 - 60 **Arvaniti V**, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010; **139**: 1246-1256, 1256.e1-5 [PMID: 20558165 DOI: 10.1053/j.gastro.2010.06.019]
 - 61 **Ripoll C**, Bañares R, Rincón D, Catalina MV, Lo Iacono O, Salcedo M, Clemente G, Núñez O, Matilla A, Molinero LM. Influence of hepatic venous pressure gradient on the prediction of survival of patients with cirrhosis in the MELD Era. *Hepatology* 2005; **42**: 793-801 [PMID: 16175621 DOI: 10.1002/hep.20871]
 - 62 **Merkel C**, Bolognesi M, Sacerdoti D, Bombonato G, Bellini B, Bighin R, Gatta A. The hemodynamic response to medical treatment of portal hypertension as a predictor of clinical effectiveness in the primary prophylaxis of variceal bleeding in cirrhosis. *Hepatology* 2000; **32**: 930-934 [PMID: 11050041]
 - 63 **Merkel C**, Bolognesi M, Bellon S, Zuin R, Noventa F, Finucci G, Sacerdoti D, Angeli P, Gatta A. Prognostic usefulness of hepatic vein catheterization in patients with cirrhosis and esophageal varices. *Gastroenterology* 1992; **102**: 973-979 [PMID: 1537533]
 - 64 **Berzigotti A**, Rossi V, Tiani C, Pierpaoli L, Zappoli P, Riili A, Serra C, Andreone P, Morelli MC, Golfieri R, Rossi C, Magalotti D, Zoli M. Prognostic value of a single HVPG measurement and Doppler-ultrasound evaluation in patients with cirrhosis and portal hypertension. *J Gastroenterol* 2011; **46**: 687-695 [PMID: 21213113 DOI: 10.1007/s00535-010-0360-z]
 - 65 **Chalasani N**, Kahi C, Francois F, Pinto A, Marathe A, Bini EJ, Pandya P, Sitaraman S, Shen J. Improved patient survival after acute variceal bleeding: a multicenter, cohort study. *Am J Gastroenterol* 2003; **98**: 653-659 [PMID: 12650802 DOI: 10.1016/S0002-9270(02)06016-1]
 - 66 **Moitinho E**, Escorsell A, Bandi JC, Salmerón JM, García-Pagán JC, Rodés J, Bosch J. Prognostic value of early measurements of portal pressure in acute variceal bleeding. *Gastroenterology* 1999; **117**: 626-631 [PMID: 10464138 DOI: 10.1016/S0016-5085(99)70455-5]
 - 67 **Monescillo A**, Martínez-Lagares F, Ruiz-del-Arbol L, Sierra A, Guevara C, Jiménez E, Marrero JM, Buceta E, Sánchez J, Castellot A, Peñate M, Cruz A, Peña E. Influence of portal hypertension and its early decompression by TIPS placement on the outcome of variceal bleeding. *Hepatology* 2004; **40**: 793-801 [PMID: 15382120 DOI: 10.1002/hep.20386]
 - 68 **García-Pagán JC**, Caca K, Bureau C, Laleman W, Appenrodt B, Luca A, Abraldes JG, Nevens F, Vinel JP, Mössner J, Bosch J. Early use of TIPS in patients with cirrhosis and variceal bleeding. *N Engl J Med* 2010; **362**: 2370-2379 [PMID: 20573925 DOI: 10.1056/NEJMoa0910102]
 - 69 **Castera L**, Pinzani M, Bosch J. Non invasive evaluation of portal hypertension using transient elastography. *J Hepatol* 2012; **56**: 696-703 [PMID: 21767510 DOI: 10.1016/j.jhep.2011.07.005]
 - 70 **Singh S**, Eaton JE, Murad MH, Tanaka H, Iijima H, Talwalkar JA. Accuracy of spleen stiffness measurement in detection of esophageal varices in patients with chronic liver disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2014; **12**: 935-45.e4 [PMID: 24055985 DOI: 10.1016/j.cgh.2013.09.013]
 - 71 **Berzigotti A**, Seijo S, Arena U, Abraldes JG, Vizzutti F, García-Pagán JC, Pinzani M, Bosch J. Elastography, spleen size, and platelet count identify portal hypertension in patients with compensated cirrhosis. *Gastroenterology* 2013; **144**: 102-111.e1 [PMID: 23058320 DOI: 10.1053/j.gastro.2012.10.001]
 - 72 **Augustin S**, Millán L, González A, Martell M, Gelabert A, Segarra A, Serres X, Esteban R, Genescà J. Detection of early portal hypertension with routine data and liver stiffness in patients with asymptomatic liver disease: a prospective study. *J Hepatol* 2014; **60**: 561-569 [PMID: 24211744 DOI: 10.1016/j.jhep.2013.10.027]
 - 73 **Abrales JG**, Reverter E, Berzigotti A. Spleen stiffness: toward a noninvasive portal sphygmomanometer? *Hepatology* 2013; **57**: 1278-1280 [PMID: 23339063 DOI: 10.1002/hep.26239]
 - 74 **Colecchia A**, Montrone L, Scafoli E, Bacchi-Reggiani ML, Colli A, Casazza G, Schiumerini R, Turco L, Di Biase AR, Mazzella G, Marzi L, Arena U, Pinzani M, Festi D. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. *Gastroenterology* 2012; **143**: 646-654 [PMID: 22643348 DOI: 10.1053/j.gastro.2012.05.035]
 - 75 **Colecchia A**, Colli A, Casazza G, Mandolesi D, Schiumerini R, Reggiani LB, Marasco G, Taddia M, Lisotti A, Mazzella G, Di Biase AR, Golfieri R, Pinzani M, Festi D. Spleen stiffness measurement can predict clinical complications in compensated HCV-related cirrhosis: a prospective study. *J Hepatol* 2014; **60**: 1158-1164 [PMID: 24607624 DOI: 10.1016/j.jhep.2014.02.024]

P- Reviewer: Qin JM, Zhu X **S- Editor:** Song XX
L- Editor: A **E- Editor:** Liu SQ



Host cellular microRNA involvement in the control of hepatitis B virus gene expression and replication

Yoshiaki Mizuguchi, Toshihiro Takizawa, Eiji Uchida

Yoshiaki Mizuguchi, Eiji Uchida, Department of Surgery, Nippon Medical School Hospital, Bunkyo-Ku, Tokyo 113-8603, Japan

Toshihiro Takizawa, Department of Anatomy, Nippon Medical School, Bunkyo-Ku, Tokyo 113-8603, Japan

Author contributions: Takizawa T and Uchida E contributed equally to this work for generating the figures and revising the manuscript; Mizuguchi Y contributed to the writing of the manuscript.

Conflict-of-interest: The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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

Correspondence to: Yoshiaki Mizuguchi, MD, PhD, Department of Surgery, Nippon Medical School Hospital, 1-1-5 Sendagi, Bunkyo-Ku, Tokyo 113-8603, Japan. yoshi1224@gmail.com

Telephone: +81-3-38222131

Fax: +81-3-58146135

Received: September 17, 2014

Peer-review started: September 20, 2014

First decision: October 28, 2014

Revised: November 28, 2014

Accepted: January 15, 2015

Article in press: January 19, 2015

Published online: April 8, 2015

(miRNAs), their growth factors and their downstream agents is required for the initiation and completion of pathogenesis in the liver. miRNAs are thought to exert a profound effect on almost every aspect of liver biology and pathology. Accumulating evidence indicates that several miRNAs are involved in the hepatitis B virus (HBV) life cycle and infectivity, in addition to HBV-associated liver diseases including fibrosis, cirrhosis and hepatocellular carcinoma (HCC). In turn, HBV can modulate the expression of several cellular miRNAs, thus promoting a favorable environment for its replication and survival. In this review, we focused on the involvement of host cellular miRNAs that are directly and indirectly associated with HBV RNA or HBV associated transcription factors. Exploring different facets of the interactions among miRNA, HBV and HCV infections, and the carcinogenesis and progress of HCC, could facilitate the development of novel and effective treatment approaches for liver disease.

Key words: Hepatitis B virus; Gene expression; Gene replication; Transcription; MicroRNA

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: A large number of studies have demonstrated that the synergistic collaboration of a number of microRNAs (miRNAs), their growth factors and their downstream agents is required for the initiation and completion of pathogenesis in the liver. miRNAs are thought to exert a profound effect on almost every aspect of biology and pathology. In this review, we focused on the miRNAs that play an important role in hepatitis B virus replication and gene expression, and summarized the involvement of host cellular miRNAs that are directly and indirectly associated with hepatitis B virus (HBV) RNA or HBV associated transcription factors.

Abstract

A large number of studies have demonstrated that the synergistic collaboration of a number of microRNAs

Mizuguchi Y, Takizawa T, Uchida E. Host cellular microRNA

involvement in the control of hepatitis B virus gene expression and replication. *World J Hepatol* 2015; 7(4): 696-702 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/696.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i4.696>

INTRODUCTION

MicroRNAs (miRNAs) participate in crucial biological processes, including development, differentiation, apoptosis, and proliferation^[1,2], by either inhibiting target mRNA translation or inducing its degradation by pairing with complementary sequences within the 3'-untranslated regions (3'-UTRs) of targeted transcripts at the post-transcriptional and/or translational level^[3]. There are more than 2500 homo sapiens miRNAs (1881 precursors, 2588 mature; data taken from miRbase 21; <http://www.mirbase.org/>), each of which can influence hundreds of gene transcripts^[4]. Several miRNAs can regulate a specific mRNA, engendering substantial complexity with respect to their capacity to modulate fundamental biological processes.

MiRNAs are crucial in normal liver development^[5] and biological processes, including liver differentiation, hepatocyte development, and many metabolic functions.

Recent studies have demonstrated that the profiling of miRNAs facilitates the classification of various tumors (whereas mRNA profiles are relatively inaccurate), which suggests that miRNAs possess a high potential for facilitating cancer diagnosis^[6]. Another study using sequencing demonstrated that a set of miRNAs account for the majority of the differences in miRNA profiles among cell lineages and tissues^[7]. A large number of miRNAs are expected to be involved in critical aspects of liver physiology, because the liver serves as an endocrine and exocrine organ with numerous functions, including carbohydrate, lipid and amino-acid metabolism, urea synthesis, detoxification of drugs and toxic endogenous compounds, bile production and plasma protein secretion^[8]. The dysregulation of tissue and serum miRNA expression has also been observed in the context of specific liver pathologies, including hepatitis, cirrhosis, and liver cancers, as well as chronic cholestasis^[9].

MiRNAs are typically transcribed from genes in the nucleus by RNA polymerase II into initial transcripts of either monocistronic or polycistronic primary-miRNAs (pri-miRNAs; Figure 1). These pri-miRNAs are further processed *via* the canonical pathway (by Drosha-DGCR8)^[10] into hairpin-shaped precursor miRNAs (pre-miRNAs). These pre-miRNAs are exported *via* Exportin-5/RanGTP^[11] from the nucleus into the cytoplasm, where they undergo cleavage by the RNase III enzyme known as Dicer, as well as TBRP^[12], which produces an imperfect miRNA duplex. This duplex then splits, thereby generating a single-stranded mature miRNA that is loaded onto the RNA-induced silencing complex^[12], which is then guided to the target mRNA through interactions with members of the Argonaute family. Due to

complementarity with its target gene sequence, mature miRNA principally induces either translational repression or mRNA degradation^[13]. However, several reports have indicated that miRNAs and their associated protein complexes (micro-ribonucleoproteins or microRNPs) can also post-transcriptionally stimulate gene expression *via* direct and indirect mechanisms^[14,15]. miRNAs primarily regulate mRNAs by interacting with their 5' end (5p) and 3'-UTR, although it has recently been suggested that the miRNA target sites may be located in the 5'-UTR or even at simultaneous 5'-UTR and 3'-UTR interaction sites^[16].

In the following section, we review recent advances in the understanding of the involvement of miRNA in the control of hepatitis B virus (HBV) gene expression and replication.

HBV INFECTION

The estimated number of new hepatocellular carcinoma (HCC) cases rose to 564300, with 548600 of these patients dying (*i.e.*, 97.2% of those receiving a diagnosis of HCC). The World Health Organization estimates that 2 billion people worldwide have been infected with the HBV, which represents the most common cause of HCC and, furthermore, that 350 million people have chronic-type HBV infection^[9]. Because the current therapeutic options for HCC patients are limited, there is an urgent need to analyze the molecular oncogenic mechanisms of HCC, to determine novel targets for specific systemic therapy and to detect novel biomarkers for early diagnosis. The biology and lifecycle of the HBV remains to be elucidated, but it is presumed to involve the following processes^[17]: (1) entry into hepatocytes *via* the fusion of viral and cellular membranes, such that the viral capsid is transported into the nucleus; (2) the HBV-relaxed circular genome is released into the nucleus and converted to 1-50 covalently closed circular DNA (cccDNA); (3) 3.2 kb cccDNA contains four major open reading frames of the pre-core/core gene, the polymerase gene, the *preS1/L-*, *preS2/M-*, and *Surface/S-* gene, and the *X* gene. The genes are transcribed into subgenomic RNA (sgRNA) and pregenomic RNA (pgRNA), HBV surface antigen, HBV core protein, viral reverse DNA polymerase, and X protein, as well as pgRNA; (4) following nuclear export, the pgRNA is translated into the core protein and viral polymerase. The sgRNA is translated into regulatory X-protein and three envelope proteins; and (5) complex formation and reverse transcription of pgRNA produces an RNA-containing nucleocapsid. RNA-containing nucleocapsids mature into DNA-containing nucleocapsids within the cytoplasm.

Accumulating evidence indicates that several miRNAs are involved in the HBV life cycle and infectivity, in addition to HBV-associated liver diseases including fibrosis, cirrhosis and HCC. In turn, HBV can modulate the expression of several cellular miRNAs, thus promoting a favorable environment for its replication and

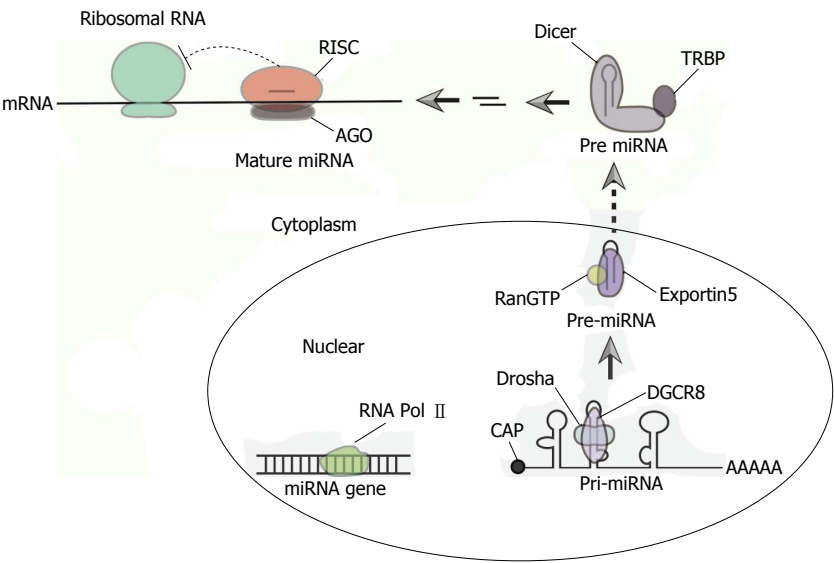


Figure 1 Schematic illustration of microRNA biogenesis. mRNA: Messenger RNA; RISC: RNA-induced silencing complex; AGO: Argonaute; Pre-miRNA: Premature-microRNA; Pri-miRNA: Primary-microRNA; RNA pol II: RNA polymerase II.

Table 1 Direct interactions of microRNAs with hepatitis C virus and hepatitis B virus RNA

Host miRNA	Binding location	Function	Expression	Ref.
miR-20a	X and polymerase	Inhibits HBV replication	Up	[23]
miR-92a-1	X and polymerase	Inhibits HBV replication	Up	[23]
miR-122	Core and DNA polymerase	Inhibits HBV replication	Down	[20]
miR-125a-5p	HBsAg	Inhibits HBV replication	Up	[22]
miR-199a- 3p	HBsAg	Inhibits HBV replication	Up	[21]
miR-205	HBx	Suppresses HBx production	Down	[25]
miR-210	PreS1	Inhibits HBV replication	Up	[21]
miR-1231	HBx/HB core	Inhibits HBV replication	Up	[26]

HBsAg: Hepatitis B surface antigens; HBV: Hepatitis B virus; HBx: HBV X.

survival. Several studies exploring the involvement of HBV in hepatocytes utilized HepG2.2.15 cells, derived from HepG2 and containing a stable transfected full-length *HBV* genome (ayw subtype), hepatitis B surface antigens (HBsAg) and hepatitis B e antigens (HBeAg), thereby supporting full HBV replication^[18].

DIRECT INTERACTION BETWEEN HBV TRANSCRIPTS AND HOST miRNAs

Although HBV is a DNA virus, its transcripts may be targeted and regulated by several cellular miRNAs similar to those of HCV (Table 1). The direct interaction between HCV RNA and miR-122, which results in a stable heterotrimeric structure, enhances HCV translation and protects against HCV RNA degradation^[19]. Conversely, all of the interactions reported thus far between host cellular miRNAs, including miR-122, and HBV RNA transcripts inhibit HBV genome replication. Chen *et al.*^[20] demonstrated that miR-122, a liver-specific miRNA, down-regulates *HBV* gene expression and replication, as determined using HBsAg and HBeAg. MiR-122 can inhibit *HBV* gene expression by interacting with the target sequence coding for nucleotides 2738-2760 and

by targeting sequences located at the coding region of the mRNA for viral polymerase and the 3'-UTR region for the core protein of the HBV genome, *via* base-pairing interactions. Chen *et al.*^[20] also demonstrated an inverse linear relationship *in vivo* between miR-122 levels and viral load in the peripheral blood mononuclear cells of HBV-positive patients. Zhang *et al.*^[21] demonstrated that miR-199a-3p and miR-210 effectively reduced HBsAg expression in HepG2 2.2.15 cells containing an integrated *HBV* genome. Bioinformatics analysis indicated a putative binding site for miR-199a-3p in the HBsAg coding region and a putative binding site for miR-210 in the HBV pre-S1 region^[21]. Comparison of the expression levels of miR-199a-3p and miR-210 between HepG2 2.2.15 cells and the parent cell line (*i.e.*, HepG2 cells) revealed a 9-fold increase in miR-199a-3p and miR- 210 in HepG2 2.2.15 cells compared with HepG2 cells^[21]. Potenza *et al.*^[22] reported that miR-125a-5p can interact with the HBV surface antigen and interfere with its expression, thus reducing the amount of HBsAg secreted. A recent study by Jung *et al.*^[23] demonstrated that HBV infection transactivates c-Myc, after which it up-regulates the miR-17-92 cluster. Conversely, miR-20a and miR-92a can down-regulate HBV pregenomic

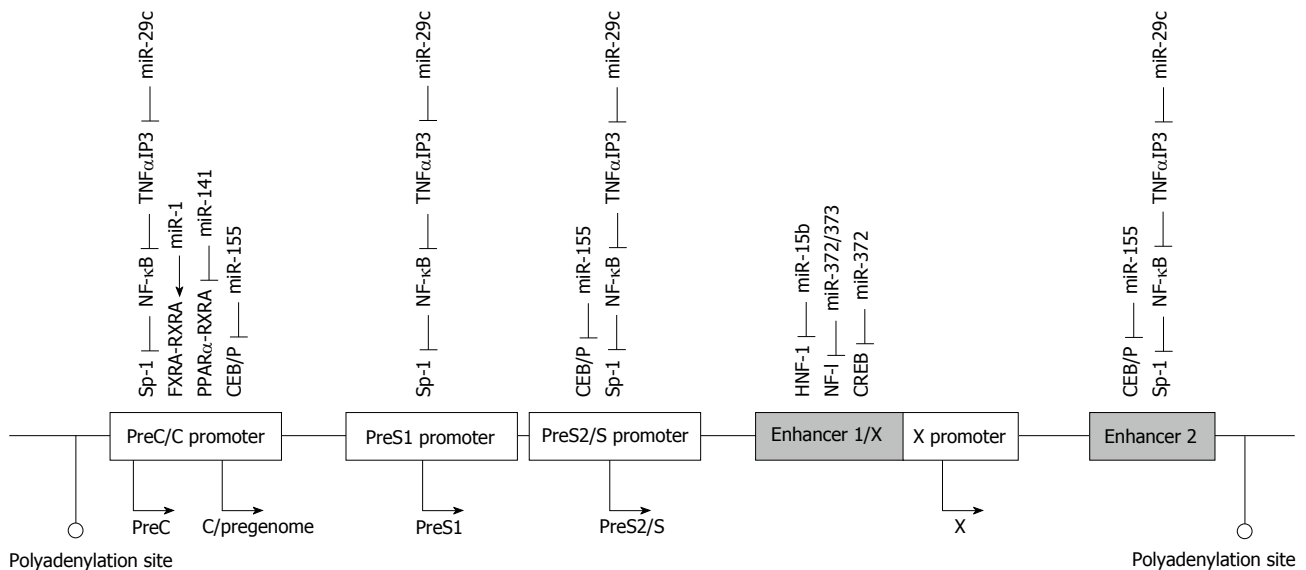


Figure 2 Summary of the effect of miRNAs on hepatitis B virus transcription. Binding sites of ubiquitous and hepatocyte-enriched transcription factors within *HBV* promoter and enhancer regions as well as miRNAs that can modulate target transcription factors are indicated. The various *HBV* promoter and enhancer sites are schematically depicted as boxes. *HBV*: Hepatitis B virus.

RNA by directly targeting its *X* and polymerase gene, indicating that these miRNAs suppress *HBV* replication by creating a negative feedback loop. *HBV X* protein (*HBx*) plays a crucial role in the development of HCC by inducing epigenetic changes within host genetic and epigenetic architecture, including aberrations in DNA methylation, histone modifications, and miRNA expression^[24]. Zhang *et al.*^[25] demonstrated that *HBx* can down-regulate miR-205, otherwise known as down-regulated miRNA in HCC, by inducing hypermethylation of the miR-205 promoter within cells. In turn, miR-205 suppresses *HBx* expression by directly targeting *HBx* mRNA. Kohno *et al.*^[26] recently demonstrated that in *HBV*-transfected HepG2 cells, the overexpression of hsa-miR-1231 is associated with the suppression of *HBV* replication and *HBV* core reduction. The mechanism by which these interactions between host cellular miRNAs and *HBV* RNA transcripts affect infectivity could involve the maintenance of a suitable reduction in virus antigen level and virion production, thereby contributing to a persistent, chronic *HBV* infection or latent *HBV* state.

CELLULAR miRNAs CONTROL *HBV* TRANSCRIPTION BY TARGETING TRANSCRIPTION FACTORS

In addition to direct interactions, several miRNAs regulate *HBV* replication by targeting *HBV*-associated genes, including transcription factors (Table 2 and Figure 2).

HBV contains a 3.2-kb partially double-stranded DNA genome with four promoters (*i.e.*, core, pre-S1, pre-S2/S, and *X* promoters) and two enhancer regions (ENI and ENII), which are involved in viral transcription regulation and thus play a central role in the control of

HBV replication^[27]. The transcription of *HBV* cccDNA is tightly regulated according to epigenetic mechanisms, such as DNA methylation, acetylation, and histone modifications^[28]. *HBV* utilizes a number of ubiquitous and liver-enriched transcription factors, in addition to nuclear receptors in hepatocytes, to cause the efficient transcription of the *HBV* gene by binding to *HBV* promoter/enhancer elements^[27]. The control of *HBV* during transcription thus influences both *HBV* gene expression and replication. Zhang *et al.*^[29] demonstrated that miR-1 enhanced *HBV* core promoter transcription activity by augmenting the expression of farnesoid *X* receptor alpha (*FXRα*), a liver-enriched transcription factor activated by bile acids. *FXRα* can function as a transcription factor by binding to the *HBV* enhancer II and core promoter in heterodimers with retinoid *X* receptor α (*RXRα*). Zhang *et al.*^[29] also demonstrated that miR-1 targets deacetylase 4 and E2F transcription factor 5, thus suppressing *HBV* replication. Liver-specific miR-122 is part of a complicated signaling network for *HBV* infectivity, through which it can down-regulate *HBV* replication as discussed above. For instance, the inhibition of miR-122 causes an increase in cellular heme oxygenase-1, which can decrease *HBV* cccDNA levels^[30]. Cyclin G₁ is one target of miR-122 and is involved in the regulation of *HBV* replication. Wang *et al.*^[31] demonstrated that interactions between cyclin G₁ and p53 block the specific binding of p53 to *HBV* enhancer elements and simultaneously abrogate p53-mediated inhibition of *HBV* transcription. Fan *et al.*^[32] reported an inverse correlation between the expression levels of miR-122 and *NDRG3* (a member of the *N-myc* downstream-regulated gene) in *HBV*-related HCC specimens, which might accelerate *HBeAg* and *HBsAg* secretion and *HBV* DNA replication. miR-372/373 represses *PRKACB* and *NFIB* by targeting its 3'-UTR,

Table 2 MicroRNAs that control hepatitis B virus gene replication and expression through cellular targets

miRNAs	Cellular targets	Effects on HBV biology or pathogenesis	Expression	Ref.
miR-1	HDAC4/E2F5	Increases HBV replication by augmenting FXR activity	Down	[29]
miR-15b	HNF1 α	Promotes HBV replication and expression of HBV antigens, including HBx protein	Down	[39]
miR-29c	TNFAIP3	Suppresses HBV DNA replication and cell proliferation and inhibits apoptosis of HCC	Down (HCC)	[41]
miR-122	Cyclin G1	Suppresses HBV replication	Down	[31]
	(HO-1)	Promotes HBV expression by inhibiting HO-1 expression		[30]
	NDRG3	Inhibits viral replication and HBV HCC proliferation		[32]
miR-125b	SCNN1A	Inhibits HBV expression	Down	[42]
miR-141	PPAR α	Suppresses HBV replication by inhibiting PPAR α -HBV promoter interaction		[44]
miR-155	C/EBP	Elevates HCC levels/promotes HCC cell growth	Up	[46]
miR-372/373	NFIB	Promotes HBV replication	Up	[33]
upmiR-372	CREB	Promotes HBV replication		[34]
miR-501	HBIP (HBx inhibitor)	Promotes HBV replication	Up	[45]

HBV: Hepatitis B virus; HBx: HBV X; CREB: cAMP-response element binding protein; C/EBP: CCAAT/enhancer binding protein beta; HCC: Hepatocellular carcinoma; HNF1 α : Human hepatocyte nuclear factor 1 α .

resulting in reduced expression of HBV^[33,34]. PPKACB induces the phosphorylation of cAMP-response element binding protein (CREB) and dissociates CREB from its promoter. CREB is required for the expression of all HBV transcription units in the binding of viral enhancer I^[35]. NFIB is significantly down-regulated in HBV-associated liver cirrhosis (LC), compared with non-LC tissues, whereas pre-miR-372 is increased significantly in liver cirrhosis. Together, these independent findings confirm that *HBV* genomic nuclear factor I sites play important and complex roles in the regulation of HBV expression^[36-38]. Conversely, a recent study has reported that peroxisome proliferator-activated receptor (PPAR)- γ and the HBx down-regulate miR-122 transcription. Dai X reported that miR-15b promotes HBV replication by augmenting HBV enhancer I activity *via* direct targeting of human hepatocyte nuclear factor 1 α , while HBV replication and antigen expression, particularly of the HBx protein, repress the expression of miR-15b^[39].

The miR-29 family members miR-29a, miR-29b, and miR-29c exert a suppressive action on tumors and are downregulated in several types of cancer, in which miR-29 directly targets DNA methyltransferase 3A and -3B, two key enzymes involved in DNA methylation, in addition to methylation-silencing tumor suppressor genes, such as fragile histidine triad and WW domain containing oxidoreductase^[40]. Wang *et al*^[41] reported that miR-29c functions as a tumor-suppressive gene by targeting TNFAIP3, a key regulator in inflammation and immunity. Furthermore, this interaction results in the suppression of HBV replication, as indexed by HBsAg/HBeAg secretion and HBV DNA replication.

Zhang *et al*^[42] used a miRNA microarray to assess miRNA expression during HBV infection *in vitro*. miR-125b expression was decreased in both HepG2-HBV1.3 (a HepG2 cell line transiently transfected with an HBV expression plasmid) and HepG2.2.15 cells. Furthermore, the ectopic expression of miR-125b inhibited HBV DNA intermediates and the secretion of HBsAg and HBeAg. miR-125b also reduced SCNN1A mRNA and protein levels. Using a dual luciferase assay,

SCNN1A was shown to be one of the targets of miR-125b, indicating that miR-125b inhibits HBV expression by targeting the *SCNN1A* gene. These results suggest a potential role of miRNA in HBV infection.

The core promoters pre-S1 promoter, X promoter, ENI and ENII all contain a PPAR α binding site; these regions are transactivated in the presence of RXR α and PPAR α ^[27], suggesting that PPAR α likely plays a critical role in HBV biogenesis. miRNA 141, a member of the miR-200 family, plays a central role in epithelial mesenchymal transition^[43]. Hu *et al*^[44] demonstrated that miR-141 can repress HBV replication effectively, and further that miR-141 inhibitor transfection precipitates a marked increase in HBsAg/HBeAg expression, which had no significant effect on HBV DNA replication, through direct targeting of the PPAR α mRNA 3'-UTR.

miR-501 expression was significantly up-regulated in hepatocellular carcinoma tissues, in which the level of HBV replication remained high^[45]. Down-regulating miR-501 significantly inhibited HBV replication but did not influence the growth of HepG2.2.15 cells^[45]. Luciferase reporter and western blot assays revealed that HBx-interacting protein, an HBV replication inhibitor, is a potential target of miR-501^[45].

Although the association between miR-155 and HBV replication remains to be demonstrated, it has been reported that CCAAT/enhancer binding protein beta, which can bind to HBV promoters and enhancers, is one target of miR-155^[46].

CONCLUSION

In this review, we focused on the role of miRNAs in *HBV* gene expression and replication. The available evidence suggests that several miRNAs mediate HBV RNA accumulation. miRNAs might have other functions specific to individual miRNAs, cell types or tissue environments and may also play a suppressive role in multi-target gene expression, thereby suggesting that miRNAs may serve as novel targets for therapeutic interventions. Despite progress in drug discovery and

development, as evidenced by novel protease inhibitors and polymerase inhibitors, the clinical use of direct-acting antivirals is limited due to poor compliance and rapid-onset drug resistance. The use of imatinib mesylate, a platelet-derived growth factor receptor, and other tyrosine kinase inhibitors, as molecular targets against HCC progression, has been proposed^[47,48]; however, clinical trials have indicated a lack of efficacy using this approach^[49]. The development of more effective, cost efficient, and better-tolerated novel treatments is crucial to control the development of hepatitis, cirrhosis, and hepatocellular carcinoma. Exploring different facets of the interactions among miRNA, HBV and HCV infections, and the carcinogenesis and progression of HCC could facilitate the development of novel and effective treatment approaches for liver disease.

Although relatively poorly understood in the context of human cancers, evidence continues to accumulate indicating that long non-coding RNAs play a crucial role in regulating numerous developmental and biological genomic pathways^[50].

REFERENCES

- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438 DOI: 10.1016/S0092-8674(04)00045-5]
- Harfe BD. MicroRNAs in vertebrate development. *Curr Opin Genet Dev* 2005; **15**: 410-415 [PMID: 15979303 DOI: 10.1016/j.gde.2005.06.012]
- Rajewsky N. microRNA target predictions in animals. *Nat Genet* 2006; **38** Suppl: S8-13 [PMID: 16736023 DOI: 10.1038/ng1798]
- Lim LP, Lau NC, Garrett-Engle P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005; **433**: 769-773 [PMID: 15685193 DOI: 10.1038/nature03315]
- Si-Tayeb K, Lemaigre FP, Duncan SA. Organogenesis and development of the liver. *Dev Cell* 2010; **18**: 175-189 [PMID: 20159590 DOI: 10.1016/j.devcel.2010.01.011]
- Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 2005; **435**: 834-838 [PMID: 15944708 DOI: 10.1038/nature03702]
- Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foà R, Schliwka J, Fuchs U, Novosel A, Müller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M, Tuschl T. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* 2007; **129**: 1401-1414 [PMID: 17604727 DOI: 10.1016/j.cell.2007.04.040]
- Chen Y, Verfaillie CM. MicroRNAs: the fine modulators of liver development and function. *Liver Int* 2014; **34**: 976-990 [PMID: 24517588 DOI: 10.1111/liv.12496]
- Mizuguchi Y, Mishima T, Yokomuro S, Arima Y, Kawahigashi Y, Shigehara K, Kanda T, Yoshida H, Uchida E, Tajiri T, Takizawa T. Sequencing and bioinformatics-based analyses of the microRNA transcriptome in hepatitis B-related hepatocellular carcinoma. *PLoS One* 2011; **6**: e15304 [PMID: 21283620 DOI: 10.1371/journal.pone.0015304]
- Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 2001; **294**: 858-862 [PMID: 11679671 DOI: 10.1126/science.1065062]
- Lund E, Güttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. *Science* 2004; **303**: 95-98 [PMID: 14631048 DOI: 10.1126/science.1090599]
- Hutvagner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. *Science* 2002; **297**: 2056-2060 [PMID: 12154197 DOI: 10.1126/science.1073827]
- He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 2004; **5**: 522-531 [PMID: 15211354 DOI: 10.1038/nrg1379]
- Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science* 2007; **318**: 1931-1934 [PMID: 18048652 DOI: 10.1126/science.1149460]
- Vasudevan S. Posttranscriptional upregulation by microRNAs. *Wiley Interdiscip Rev RNA* 2012; **3**: 311-330 [PMID: 22072587 DOI: 10.1002/wrna.121]
- Lee I, Ajay SS, Yook JI, Kim HS, Hong SH, Kim NH, Dhanasekaran SM, Chinnaiyan AM, Athey BD. New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. *Genome Res* 2009; **19**: 1175-1183 [PMID: 19336450 DOI: 10.1101/gr.089367.108]
- Urban S, Schulze A, Dandri M, Petersen J. The replication cycle of hepatitis B virus. *J Hepatol* 2010; **52**: 282-284 [PMID: 20056291 DOI: 10.1016/j.jhep.2009.10.031]
- Sells MA, Chen ML, Acs G. Production of hepatitis B virus particles in Hep G2 cells transfected with cloned hepatitis B virus DNA. *Proc Natl Acad Sci USA* 1987; **84**: 1005-1009 [PMID: 3029758 DOI: 10.1073/pnas.84.4.1005]
- Li X, Yang W, Ye W, Jin L, He J, Lou L. microRNAs: novel players in hepatitis C virus infection. *Clin Res Hepatol Gastroenterol* 2014; **38**: 664-675 [PMID: 24875730 DOI: 10.1016/j.clinre.2014.04.008]
- Chen Y, Shen A, Rider PJ, Yu Y, Wu K, Mu Y, Hao Q, Liu Y, Gong H, Zhu Y, Liu F, Wu J. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. *FASEB J* 2011; **25**: 4511-4521 [PMID: 21903935 DOI: 10.1096/fj.11-187781]
- Zhang GL, Li YX, Zheng SQ, Liu M, Li X, Tang H. Suppression of hepatitis B virus replication by microRNA-199a-3p and microRNA-210. *Antiviral Res* 2010; **88**: 169-175 [PMID: 20728471 DOI: 10.1016/j.antiviral.2010.08.008]
- Potenza N, Papa U, Mosca N, Zerbini F, Nobile V, Russo A. Human microRNA hsa-miR-125a-5p interferes with expression of hepatitis B virus surface antigen. *Nucleic Acids Res* 2011; **39**: 5157-5163 [PMID: 21317190 DOI: 10.1093/nar/gkr067]
- Jung YJ, Kim JW, Park SJ, Min BY, Jang ES, Kim NY, Jeong SH, Shin CM, Lee SH, Park YS, Hwang JH, Kim N, Lee DH. c-Myc-mediated overexpression of miR-17-92 suppresses replication of hepatitis B virus in human hepatoma cells. *J Med Virol* 2013; **85**: 969-978 [PMID: 23532756 DOI: 10.1002/jmv.23534]
- 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]
- Zhang T, Zhang J, Cui M, Liu F, You X, Du Y, Gao Y, Zhang S, Lu Z, Ye L, Zhang X. Hepatitis B virus X protein inhibits tumor suppressor miR-205 through inducing hypermethylation of miR-205 promoter to enhance carcinogenesis. *Neoplasia* 2013; **15**: 1282-1291 [PMID: 24339740]
- Kohno T, Tsuge M, Murakami E, Hiraga N, Abe H, Miki D, Imamura M, Ochi H, Hayes CN, Chayama K. Human microRNA hsa-miR-1231 suppresses hepatitis B virus replication by targeting core mRNA. *J Viral Hepat* 2014; **21**: e89-e97 [PMID: 24835118 DOI: 10.1111/jvh.12240]
- Quasdorff M, Protzer U. Control of hepatitis B virus at the level of transcription. *J Viral Hepat* 2010; **17**: 527-536 [PMID: 20546497 DOI: 10.1111/j.1365-2893.2010.01315.x]
- Liu WH, Yeh SH, Chen PJ. Role of microRNAs in hepatitis B virus

- replication and pathogenesis. *Biochim Biophys Acta* 2011; **1809**: 678-685 [PMID: 21565290 DOI: 10.1016/j.bbagr.2011.04.008]
- 29 **Zhang X**, Zhang E, Ma Z, Pei R, Jiang M, Schlaak JF, Roggendorf M, Lu M. Modulation of hepatitis B virus replication and hepatocyte differentiation by MicroRNA-1. *Hepatology* 2011; **53**: 1476-1485 [PMID: 21520166 DOI: 10.1002/hep.24195]
- 30 **Qiu L**, Fan H, Jin W, Zhao B, Wang Y, Ju Y, Chen L, Chen Y, Duan Z, Meng S. miR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. *Biochem Biophys Res Commun* 2010; **398**: 771-777 [PMID: 20633528 DOI: 10.1016/j.bbrc.2010.07.021]
- 31 **Wang S**, Qiu L, Yan X, Jin W, Wang Y, Chen L, Wu E, Ye X, Gao GF, Wang F, Chen Y, Duan Z, Meng S. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1) -modulated P53 activity. *Hepatology* 2012; **55**: 730-741 [PMID: 22105316 DOI: 10.1002/hep.24809]
- 32 **Fan CG**, Wang CM, Tian C, Wang Y, Li L, Sun WS, Li RF, Liu YG. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. *Oncol Rep* 2011; **26**: 1281-1286 [PMID: 21725618 DOI: 10.3892/or.2011.1375]
- 33 **Guo H**, Liu H, Mitchelson K, Rao H, Luo M, Xie L, Sun Y, Zhang L, Lu Y, Liu R, Ren A, Liu S, Zhou S, Zhu J, Zhou Y, Huang A, Wei L, Guo Y, Cheng J. MicroRNAs-372/373 promote the expression of hepatitis B virus through the targeting of nuclear factor I/B. *Hepatology* 2011; **54**: 808-819 [PMID: 21608007 DOI: 10.1002/hep.24441]
- 34 **Wang J**, Liu X, Wu H, Ni P, Gu Z, Qiao Y, Chen N, Sun F, Fan Q. CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. *Nucleic Acids Res* 2010; **38**: 5366-5383 [PMID: 20423907 DOI: 10.1093/nar/gkq285]
- 35 **Kim BK**, Lim SO, Park YG. Requirement of the cyclic adenosine monophosphate response element-binding protein for hepatitis B virus replication. *Hepatology* 2008; **48**: 361-373 [PMID: 18615500 DOI: 10.1002/hep.22359]
- 36 **Pineau P**, Volinia S, McJunkin K, Marchio A, Battiston C, Terris B, Mazzaferro V, Lowe SW, Croce CM, Dejean A. miR-221 overexpression contributes to liver tumorigenesis. *Proc Natl Acad Sci USA* 2010; **107**: 264-269 [PMID: 20018759 DOI: 10.1073/pnas.0907904107]
- 37 **Shaul Y**, Ben-Levy R, De-Medina T. High affinity binding site for nuclear factor I next to the hepatitis B virus S gene promoter. *EMBO J* 1986; **5**: 1967-1971 [PMID: 3463507]
- 38 **Ben-Levy R**, Faktor O, Berger I, Shaul Y. Cellular factors that interact with the hepatitis B virus enhancer. *Mol Cell Biol* 1989; **9**: 1804-1809 [PMID: 2725524]
- 39 **Dai X**, Zhang W, Zhang H, Sun S, Yu H, Guo Y, Kou Z, Zhao G, Du L, Jiang S, Zhang J, Li J, Zhou Y. Modulation of HBV replication by microRNA-15b through targeting hepatocyte nuclear factor 1 α . *Nucleic Acids Res* 2014; **42**: 6578-6590 [PMID: 24705650 DOI: 10.1093/nar/gku260]
- 40 **Fabbri M**, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci USA* 2007; **104**: 15805-15810 [PMID: 17890317 DOI: 10.1073/pnas.0707628104]
- 41 **Wang CM**, Wang Y, Fan CG, Xu FF, Sun WS, Liu YG, Jia JH. miR-29c targets TNFAIP3, inhibits cell proliferation and induces apoptosis in hepatitis B virus-related hepatocellular carcinoma. *Biochem Biophys Res Commun* 2011; **411**: 586-592 [PMID: 21763284 DOI: 10.1016/j.bbrc.2011.06.191]
- 42 **Zhang Z**, Chen J, He Y, Zhan X, Zhao R, Huang Y, Xu H, Zhu Z, Liu Q. miR-125b inhibits hepatitis B virus expression in vitro through targeting of the SCNN1A gene. *Arch Virol* 2014; **159**: 3335-3343 [PMID: 25173609 DOI: 10.1007/s00705-014-2208-y]
- 43 **Mizuguchi Y**, Isse K, Specht S, Lunz JG, Corbitt N, Takizawa T, Demetris AJ. Small proline rich protein 2a in benign and malignant liver disease. *Hepatology* 2014; **59**: 1130-1143 [PMID: 24123265 DOI: 10.1002/hep.26889]
- 44 **Hu W**, Wang X, Ding X, Li Y, Zhang X, Xie P, Yang J, Wang S. MicroRNA-141 represses HBV replication by targeting PPAR α . *PLoS One* 2012; **7**: e34165 [PMID: 22479552 DOI: 10.1371/annotation/cbbe9454-0b72-44b3-a972-10dca22db68]
- 45 **Jin J**, Tang S, Xia L, Du R, Xie H, Song J, Fan R, Bi Q, Chen Z, Yang G, Liu J, Shi Y, Fan D. MicroRNA-501 promotes HBV replication by targeting HBXIP. *Biochem Biophys Res Commun* 2013; **430**: 1228-1233 [PMID: 23266610 DOI: 10.1016/j.bbrc.2012.12.071]
- 46 **Wang B**, Majumder S, Nuovo G, Kutay H, Volinia S, Patel T, Schmittgen TD, Croce C, Ghoshal K, Jacob ST. Role of microRNA-155 at early stages of hepatocarcinogenesis induced by choline-deficient and amino acid-defined diet in C57BL/6 mice. *Hepatology* 2009; **50**: 1152-1161 [PMID: 19711427 DOI: 10.1002/hep.23100]
- 47 **Treiber G**, Wex T, Schleyer E, Troeger U, Hosius C, Malfertheiner P. Imatinib for hepatocellular cancer--focus on pharmacokinetic/pharmacodynamic modelling and liver function. *Cancer Lett* 2008; **260**: 146-154 [PMID: 18083304 DOI: 10.1016/j.canlet.2007.10.041]
- 48 **Höpfner M**, Schuppan D, Scherübl H. Growth factor receptors and related signalling pathways as targets for novel treatment strategies of hepatocellular cancer. *World J Gastroenterol* 2008; **14**: 1-14 [PMID: 18176955 DOI: 10.3748/wjg.14.1]
- 49 **Eckel F**, von Delius S, Mayr M, Dobritz M, Fend F, Hosius C, Schleyer E, Schulte-Frohlinde E, Schmid RM, Lersch C. Pharmacokinetic and clinical phase II trial of imatinib in patients with impaired liver function and advanced hepatocellular carcinoma. *Oncology* 2005; **69**: 363-371 [PMID: 16319507 DOI: 10.1159/000089990]
- 50 **Gupta RA**, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 2010; **464**: 1071-1076 [PMID: 20393566 DOI: 10.1038/nature08975]

P- Reviewer: De Minicis S, Sugimura H, Vespasiani-Gentilucci U

S- Editor: Tian YL **L- Editor:** A **E- Editor:** Liu SQ



Retrospective Study

Pre-treatment prediction of response to peginterferon plus ribavirin in chronic hepatitis C genotype 3

Sebastián Marciano, Silvia M Borzi, Melisa Dirchwolf, Ezequiel Ridruejo, Manuel Mendizabal, Fernando Bessone, María E Sirotinsky, Diego H Giunta, Julieta Trinks, Pablo A Olivera, Omar A Galdame, Marcelo O Silva, Hugo A Fainboim, Adrián C Gadano

Sebastián Marciano, Omar A Galdame, Adrián C Gadano, Liver Unit, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina

Silvia M Borzi, Hepatology Section, Hospital R. Rossi, La Plata, Buenos Aires, Argentina

Melisa Dirchwolf, Hugo A Fainboim, Liver Infectious Disease Unit, Hospital F.J. Muñiz, Uspallata 2272, Buenos Aires, Argentina
Ezequiel Ridruejo, Hepatology Section, Department of Medicine, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno "CEMIC", Avenida Galván 4102, Buenos Aires, Argentina
Ezequiel Ridruejo, Manuel Mendizabal, Marcelo O Silva, Hepatology and Liver Transplant Unit, Hospital Universitario Austral, Buenos Aires, Argentina

Fernando Bessone, Hepatology Unit, Sanatorio del Parque, Rosario, Argentina

María E Sirotinsky, HEPATOSUR group, Comodoro Rivadavia, Chubut, Argentina

Diego H Giunta, Internal Medicine Research Unit, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina

Julieta Trinks, Basic Science and Experimental Medicine Institute, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina

Pablo A Olivera, Internal Medicine, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno "CEMIC", Buenos Aires, Argentina

Author contributions: Marciano S studied design, data acquisition, analyze the data, wrote the manuscript; Borzi SM, Dirchwolf M, Ridruejo E, Mendizabal M, Bessone F, Sirotinsky ME, Olivera PA, Galdame OA, Silva MO, Fainboim HA and Gadano AC contributed equally in data acquisition, concept design and reviewing of the manuscript; Giunta DH contributed to statistical analyses, concept design and reviewing of the manuscript; Trinks J processed *INFL3* polymorphisms, conceived design and reviewed of the manuscript.

Ethics approval: The study was reviewed and approved by the Hospital Italiano de Buenos Aires Institutional Review Board.

Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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

Data sharing: None.

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

Correspondence to: Sebastián Marciano, MD, Liver Unit, Hospital Italiano de Buenos Aires, Juan D. Peron 4190, C1181ACH Capital Federal, Buenos Aires, Argentina. sebastian.marciano@hospitalitaliano.org.ar

Telephone: +54-11-49590200-5370

Fax: +54-11-49590346

Received: August 29, 2014

Peer-review started: August 30, 2014

First decision: November 1, 2014

Revised: November 5, 2014

Accepted: January 18, 2015

Article in press: January 20, 2015

Published online: April 8, 2015

Abstract

AIM: To evaluate pre-treatment factors associated with sustained virological response (SVR) in patients with hepatitis C virus (HCV) genotype 3 treated with peginterferon and ribavirin (RBV).

METHODS: We retrospectively analyzed treatment naive, mono-infected HCV genotype 3 patients treated with peginterferon and RBV. Exclusion criteria included presence of other liver disease, alcohol consumption and African American or Asian ethnicity. The variables collected and compared between patients who achieved an SVR and patients who did not were as follows: gender, age, fibrosis stage, diabetes, body mass index,

steatosis, *INFL3* polymorphism, pre-treatment HCV-RNA, type of peginterferon, RBV dose and adherence.

RESULTS: A total of 107 patients treated between June, 2004 and March, 2013 were included. Mean treatment duration was 25.1 (\pm 1.8) wk. Overall, 58% (62/107) of the patients achieved an SVR and 42% (45/107) did not. In the multivariate logistic regression analysis, pre-treatment HCV-RNA \geq 600000 UI/mL (OR = 0.375, 95%CI: 0.153-0.919, P = 0.032) and advanced fibrosis (OR = 0.278, 95%CI: 0.113-0.684, P = 0.005) were significantly associated with low SVR rates. In patients with pre-treatment HCV-RNA \geq 600000 UI/mL and advanced fibrosis, the probability of achieving an SVR was 29% (95%CI: 13.1-45.2). In patients with pre-treatment HCV-RNA < 600000 UI/mL and mild to moderate fibrosis, the probability of achieving an SVR was 81% (95%CI: 68.8-93.4).

CONCLUSION: In patients with HCV genotype 3 infections the presence of advance fibrosis and high pre-treatment viral load might be associated with poor response to peginterferon plus RBV. These patients could benefit the most from new direct antiviral agents-based regimes.

Key words: Sustained virological response; Direct antiviral agents; Sofosbuvir; Cirrhosis; Viral load

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Our study evaluates pre-treatment factors associated with sustained virological response in patients with hepatitis C virus genotype 3 treated with peginterferon plus ribavirin. We identified a sub-group of patients with high pre-treatment viral load and advanced fibrosis whose chance of achieving a sustained virological response is as low as 29%. We believe these patients should be prioritized to access new treatment strategies.

Marciano S, Borzi SM, Dirchwolf M, Ridruejo E, Mendizabal M, Bessone F, Sirotinsky ME, Giunta DH, Trinks J, Olivera PA, Galdame OA, Silva MO, Fainboim HA, Gadano AC. Pre-treatment prediction of response to peginterferon plus ribavirin in chronic hepatitis C genotype 3. *World J Hepatol* 2015; 7(4): 703-709 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/703.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i4.703>

INTRODUCTION

Hepatitis C virus (HCV) is a major health problem affecting more than 180 million people worldwide^[1]. It is estimated that at least 350000 HCV infected people die annually due to liver-related causes^[2].

Six genotypes of HCV have been identified. In Latin America, genotype 1 is the most prevalent, followed by genotypes 2 and 3^[3,4]. In some areas of this region

the prevalence of HCV genotype 3 (HCV-3) is as high as 30%^[3].

Traditionally, HCV-2 and HCV-3 have been grouped together as "easy to treat" genotypes. However we now know that sustained virological response (SVR) rates of HCV-3 patients treated with peginterferon plus ribavirin (RBV) are sub-optimal. The global SVR rates for HCV-3 patients treated with peginterferon plus RBV are around 65%-70%^[5,6]. Since these data mainly arise from randomized trials, SVR rates are expected to be lower in real-life patients with adverse factors^[7].

Several host and viral factors have an impact on the SVR rate of patients infected with HCV-3 treated with peginterferon plus RBV. Pre-treatment factors that have been proposed to have a negative impact on SVR are advanced fibrosis or cirrhosis, male gender, non-Caucasian race, high body weight, diabetes mellitus, and high pre-treatment HCV-RNA^[7-12]. More recently, *INFL3* (formerly IL28B) polymorphisms were evaluated, but a clear association between the favorable *INFL3* genotypes and SVR could not be established^[13-15].

A major limitation of the studies that assessed predictors of SVR in HCV-3 patients lies in the fact that they evaluated HCV-2 and HCV-3 together, and difficulties arise when trying to draw conclusions for HCV-3 individually.

By the end of 2013, sofosbuvir was approved for the treatment of chronic HCV-3, being the standard of care in a minority of countries at the moment this manuscript was submitted. However, in regions like Latin America and Middle East/Africa, payer-related barriers were reported^[16], which might hamper adequate treatment delivery.

When new treatments become globally available, a careful selection of candidates will be mandatory, particularly for cost-related reasons. Therefore, it will be necessary to identify patients at higher risk of fibrosis progression and treatment failure to current therapies.

Thus, we decided to conduct this study that evaluates pre-treatment variables associated with SVR in patients with chronic HCV-3 treated with peginterferon plus RBV.

MATERIALS AND METHODS

This is a retrospective multicenter study performed in 7 Liver Units from Argentina.

The centers involved in the study were, Hospital Italiano from Buenos Aires, Hospital R. Rossi from La Plata, Hospital F. J. Muñiz, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Hospital Universitario Austral, Sanatorio Parque from Rosario, and the HEPATOSUR Group representing the Patagonia.

The survey was discussed by all the participating centers. The local institutional review board of each center approved the study. The study was conducted

according to the principles of the Declaration of Helsinki.

Patients included in this study were aged ≥ 18 years and received their first treatment with peginterferon plus RBV for chronic hepatitis C genotype 3. Patients received weekly peginterferon alfa-2a 180 μg or peginterferon alfa-2b 1.5 $\mu\text{g}/\text{kg}$ of body weight. Ribavirin dose could be either fixed (800 mg/d) or adjusted to weight (1000 mg/d in patients ≤ 75 kg; 1200 mg/d in patients > 75 kg).

Exclusion criteria included alcohol intake greater than 20 g/d, history of organ transplantation, creatinine clearance < 50 mL/min, co-infection with hepatitis B virus or human immunodeficiency virus and African American or Asian ethnicity. Patients were also excluded if they presented evidence of other liver disease, such as autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease or alfa-1-antitrypsin deficiency.

Each center provided detailed information of patients included in the study. Patient management and treatment candidacy were determined in each center. All data records were checked for missing values and inconsistencies, queries were sent to all participating institutions, and corrections were made at the data coordinating center, namely Hospital Italiano.

Efficacy assessment

Pre-treatment and follow-up blood samples were collected for virologic testing in each participating center. Serum HCV-RNA was either qualitatively or quantitatively evaluated.

The primary end-point was SVR, defined as an undetectable serum HCV-RNA at 24 wk after the end of treatment. Rapid virological response was defined as an undetectable HCV-RNA by treatment week four. Virologic relapse was defined as a detectable HCV-RNA during follow-up in patients who had had undetectable HCV-RNA at the end of treatment. Those patients who never achieved a negative on-treatment HCV-RNA were classified as non-responders.

Pre-treatment characteristics

The variables that were collected and compared between patients who achieved an SVR and patients who did not were as follows: gender, age, fibrosis stage, diabetes, body mass index (BMI), steatosis, *INFL3* polymorphism, pre-treatment HCV-RNA, type of peginterferon, RBV dose and adherence.

For the purpose of this study, high HCV-RNA was defined as ≥ 600000 UI/mL.

Fibrosis grade was staged either by biopsy or Transient Elastography (Fibroscan®). In patients without fibrosis evaluation, the aspartate aminotransferase to Platelet Ration Index (APRI) was calculated^[17]. Patients were divided into two groups: patients with mild to moderate fibrosis, including patients without fibrosis and patients with up to METAVIR F2 fibrosis; and patients with advanced fibrosis, including patients with METAFIR F3 and cirrhosis^[18]. Patients with clinical

or endoscopic findings of cirrhosis were included in the advanced fibrosis group.

The presence of steatosis was evaluated either by ultrasound or histology.

Since most patients did not have data regarding *INFL3* polymorphism, they were contacted and invited to participate in this study in order to determine the genotype. Patients signed an informed consent before *INFL3* genotyping. Genomic DNA was extracted from oral swabs using QIAamp DNA Blood Mini Kit (QIAGEN, GmbH, Hilden, Germany) following the manufacturer's protocol.

SNP rs12979860 (*INFL3*) was PCR-amplified from isolated genomic DNA with standard Taq polymerase (Inbio-Highway, Tandil, Argentina)^[19]. The PCR-amplified fragments were bi-directionally sequenced using Big-Dye Termination chemistry system (Applied Biosystems, Life Technologies Corp., Foster City, CA, United States). The sequencing chromatogram was analyzed by using the BioEdit Sequence Alignment Editor version 7.1.3.0 in order to discriminate between homozygotes and heterozygotes. Patients were grouped as *INFL3* CC and *INFL3* non-CC (including patients with *INFL3* TT and CT).

Sample size calculation

In order to generate a predictive model including four variables, at least 40 patients without SVR had to be included. Assuming an SVR rate of 65%-70% in patients with HCV-3 treated with peginterferon plus RBV, between 100 and 110 patients had to be included^[5,6].

Statistical analysis

We presented data as percentages or medians and interquartile ranges. We evaluated the association of pre-treatment characteristics with SVR using the Mann-Whitney test for continuous variables and the χ^2 test for categorical variables.

In order to identify independent predictors of SVR we used a logistic regression model for the variables that showed a level of significance lower than 0.1 in the univariate analysis. We compared different models by estimating the area under the receiver operating characteristic (ROC) curve as a measure of predictive accuracy. We chose the model with the greatest area under the ROC and we presented the estimated probabilities predicted by the regression model with their 95%CI. Tests were two-sided and significance was accepted at $P < 0.05$. Statistical analysis was performed using software SPSS, version 17.0 (Chicago, IL).

RESULTS

Table 1 provides an overview of the patients' characteristics. A total of 122 HCV-3 patients treated with peginterferon plus RBV patients were identified. Fifteen were excluded because of missing data, whereas

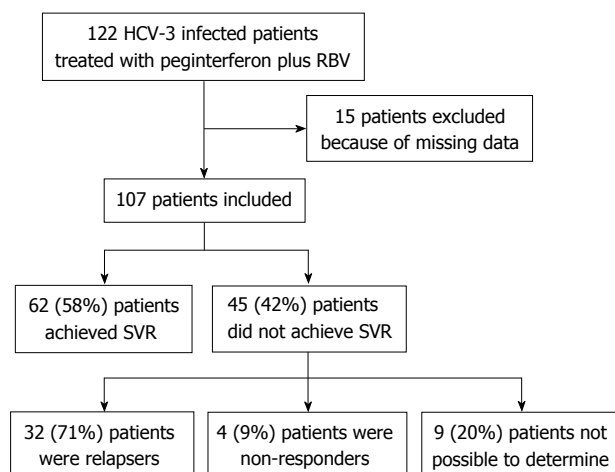


Figure 1 Inclusion, exclusion and outcome of patients. HCV: Hepatitis C virus; RBV: Ribavirin; SVR: Sustained virological response.

107 were included (Figure 1). Patients were treated between June/2004 and March/2013. The mean age at treatment was 47.5 (\pm 8.8) years, and 68% (73/107) of the patients were male. In 93% (103/107) of the patients, a pre-treatment serum sample was collected. Of those patients, 55% (57/103) had high HCV-RNA. Pre-treatment fibrosis could be determined in 97% (104/107) of the patients, either by biopsy (62%), Fibroscan® (26%) or by a combination of clinical or endoscopic features and the APRI score (12%). Of these patients, 40% (41/104) had advanced fibrosis. Steatosis and a BMI > 27 were reported in 48% (51/107) and 54% (58/107) of the patients, respectively. Data regarding INFL3 polymorphism was available in 60% (64/107) of the patients, of whom 28% (18/64) were CC.

Mean treatment durations was 25.1 (\pm 1.8) wk. Fix dose RBV was prescribed in 64% (68/107) patients, while the remaining ones received a weight-adjusted approach. More than 80% of the peginterferon and RBV planned doses were received by 94% (101/107) of the patients.

Virologic response

Overall, 58% (62/107) of the patients achieved an SVR. Of the 45 patients who did not achieve an SVR, 71% (32/45) were relapsers and 9% (4/45) were non-responders. In 20% (9/45) of the cases, the type of treatment failure was not possible to characterize due to lack of precise viral kinetic information (Figure 1).

In the univariate analysis, SVR rate was significantly lower in patients with high baseline HCV-RNA (OR = 0.330, 95%CI: 0.145-0.755, P = 0.009), advanced fibrosis (OR = 0.274, 95%CI: 0.145-0.755, P = 0.001) and BMI > 27 (OR = 0.422, 95%CI: 0.149-0.892, P = 0.033) (Table 2).

These variables were included in the multivariate logistic regression analysis and only high HCV-RNA (OR = 0.375, 95%CI: 0.153-0.919, P = 0.032) and

Table 1 Characteristics of the study population n (%)

Characteristic	Patients (n = 107)
Age, yr (mean \pm SD) ¹	47.5 (\pm 8.8)
Gender	
Male	68.2 (73)
Female	31.8 (34)
INFL3 polymorphism ²	
CT	56.2 (36)
CC	28.1 (18)
TT	15.7 (10)
Pre treatment HCV-RNA ³	
< 600000 UI/mL	44.7 (46)
\geq 600000 UI/mL	55.3 (57)
Pre treatment elevated ALT ⁴	88.5 (92)
Fibrosis ⁵	
Mild to moderate ⁶	59.8 (61)
Advanced ⁷	40.2 (41)
Steatosis	47.6 (51)
BMI (kg/m ²) > 27	54.2 (58)
Diabetes	5.6 (6)
Type of peginterferon	
alfa 2a	75.7 (81)
alfa 2b	24.3 (26)
RBV dose	
Fix ⁸	63.6 (68)
Weight-Based ⁹	36.4 (39)
80/80/80 adherence ¹⁰	94.4 (101)

¹Available for 105 patients; ²Available for 64 patients; ³Available for 103 patients; ⁴Available for 104 patients; ⁵Available for 102 patients; ⁶Mild to moderate fibrosis includes patients without fibrosis and patients with up to METAVIR F2 fibrosis; ⁷Advanced fibrosis includes patients with METAVIR F3 and cirrhosis; ⁸800 mg/d; ⁹1000 mg/d in patients \leq 75 kg weight, 1200 mg/d in patients > 75 kg weight; ¹⁰Patients received at least 80% of the peginterferon and RBV doses, and completed at least 80% of the expected treatment duration. ALT: Alanine aminotransferase; HCV: Hepatitis C virus; RBV: Ribavirin; BMI: Body mass index.

advanced fibrosis (OR = 0.278, 95%CI: 0.113-0.684, P = 0.005) had a statistically significant negative association with SVR.

The SVR rate was estimated according to the fibrosis grade and to the pre-treatment HCV-RNA. In patients with low pre-treatment HCV-RNA and mild to moderate fibrosis, the probability of achieving an SVR was 81% (95%CI: 68.8-93.4). In patients with high baseline HCV-RNA and advanced fibrosis, the probability of achieving an SVR was 29% (95%CI: 13.1-45.2) (Table 3).

DISCUSSION

In the present study, we assessed pre-treatment predictors of SVR in patients infected with HCV-3 who were treated with peginterferon and RBV. We identified high HCV-RNA and advanced fibrosis to be independently associated with treatment failure.

Advanced fibrosis or cirrhosis have consistently been associated with lower rates of SVR in patients with HCV-3 treated with peginterferon plus RBV^[9-11,20]. The chances of achieving an SVR after a 24-wk treatment for these patients are 49%-57%^[21-23]. Besides, it is

Table 2 Association between pre-treatment characteristics and sustained virological response - univariate analyses *n* (%)

	SVR (<i>n</i> = 62)	No-SVR (<i>n</i> = 45)	OR	95%CI	<i>P</i>
Age, yr (mean ± SD) ¹	47.8 (± 9.1)	47.1 (± 8.3)	1	0.965-1.055	0.696
Male	61.3 (38)	77.8 (35)	0.425	0.190-1.079	0.093
<i>INFL3</i> polymorphism CC ²	31.4 (11)	24.1 (7)	1.44	0.475-4.372	0.585
³ HCV-RNA ≥ 600000 UI/mL	44.1 (26)	70.5 (31)	0.33	0.145-0.755	0.009
Pre treatment elevated ALT ⁴	86.7 (52)	90.9 (40)	0.65	0.183-2.312	0.553
Advanced fibrosis ^{5,6}	26.7 (16)	59.5 (25)	0.247	0.107-0.573	0.001
Steatosis	46.8 (29)	48.9 (22)	0.919	0.426-1.981	0.847
BMI (kg/m ²) > 27	46.8 (29)	64.4 (29)	0.422	0.149-0.892	0.033
Diabetes	2 (1)	11 (5)	0.131	0.015-1.165	0.08
Peginterferon alfa 2a	75.8 (47)	75.6 (34)	0.986	0.403-2.413	1
RBV fix-dose ⁷	64.5 (40)	62.2 (28)	1.104	0.498-2.447	0.841
80/80/80 adherence ⁸	95.2 (59)	93.3 (42)	0.75	0.113-2.552	0.919

¹Available for 105 patients; ²Available for 64 patients; ³Available for 103 patients; ⁴Available for 104 patients; ⁵Advanced fibrosis includes patients with METAFIR F3 and cirrhosis; ⁶Available for 102 patients; ⁷800 mg/d; ⁸Patients received at least 80% of the peginterferon and RBV doses, and completed at least 80% of the expected treatment duration. ALT: Alanine aminotransferase; HCV: Hepatitis C virus; RBV: Ribavirin; BMI: Body mass index; SVR: Sustained virological response.

Table 3 Probability of sustained virological response according to fibrosis grade and pre-treatment hepatitis C virus-RNA

HCV-RNA ≥ 600000 UI/mL	Advanced fibrosis ¹	Probability (%)	95%CI
No	No	81.1	68.8-93.4
Yes	No	62.2	46.6-77.8
No	Yes	51.8	31.3-72.3
Yes	Yes	29.2	13.1-45.2

¹Advanced fibrosis includes patients with METAFIR F3 and cirrhosis. HCV: Hepatitis C virus.

estimated that the likelihood of achieving an SVR is reduced by 59% in comparison with patients with lower fibrosis grades^[20]. The impact of the pre-treatment HCV-RNA on SVR is more difficult to determine, since different cut-off points were selected in prior studies (400.000-800.000 IU/mL). Even though it has not been consistently associated with lower SVR rates, HCV-3 patients with high pre-treatment viral load do have lower rapid virological response rates^[24].

In our study, we found that by combining baseline HCV-RNA and fibrosis stage, SVR rates could be accurately predicted. In patients with low HCV-RNA, and without advanced fibrosis, the SVR rate was 81%. On the contrary, in patients with high baseline HCV-RNA and advanced fibrosis, the SVR rate was 29%.

Other variables that were assessed in our study included age, gender, *INFL3* polymorphism, steatosis, adherence, RBV dose, type of peginterferon, diabetes and BMI. None of these were associated with SVR.

Steatosis is known to be more frequent in patients infected with HCV-3 than in other genotypes^[25,26]. In our series, 48% of the patients presented steatosis, which was diagnosed either by biopsy or by ultrasonography. Even though HCV-3 is known to cause steatosis through specific viral mechanisms, in our study, 58% of the patients had a BMI > 27, reflecting an overweight

population. In line with our results, most studies reported no impact of steatosis on SVR for patients with HCV-3^[27,28]. Although the BMI was previously reported to have an impact on SVR^[8], prior studies proposed different cut-off points and results were discordant. We selected a cut-off point of 27, and no association with SVR was found. Even though diabetes is associated with higher relapse rates in HCV G3 patients^[20], we did not find this association, probably due to the low number of patients with diabetes that were included in the study.

INFL3 polymorphisms were more recently evaluated in patients with HCV-3 treated with peginterferon plus RBV. Putting together all the available information, it seems that *INFL3*-CC is not globally associated with SVR in HCV-3 patients. Nevertheless, it does predict SVR in the sub-group of patients who do not achieve a rapid virological response^[13,15]. We did not find an association between *INFL3*-CC and SVR.

No differences in the SVR rates were found in patients who received fixed-dose or weight-adjusted RBV. This was an expected finding, since mean treatment duration was 25.7 (± 1.8) wk, and RBV dose does have an impact on SVR when treatment duration is reduced, but not for the 24-wk regimen^[21].

In order to evaluate treatment adherence we used the 80/80/80 rule. Overall, 94% of the patients were adherent. No differences were found in SVR rates between patients who were adherent and patients who were not. This was most likely due to the low proportion of patients who were non-compliant.

Until the end of 2013, the only treatment for HCV-3 was peginterferon plus RBV. At that time, sofosbuvir-based regimens were approved and released. With this new approach, more than 90% of the HCV-3 treatment-naïve patients achieve an SVR, regardless of the fibrosis stage^[29,30]. When this manuscript was submitted for its publication, sofosbuvir was only approved and available in a minority of countries. Numerous barriers related to patient, provider, government and payers are known

to affect HCV treatment accessibility^[31]. In developing countries, treatment-related costs constitute a major concern. In fact, in some Latin American countries, after more than three years of the release of Boceprevir and Telaprevir for the treatments of HCV genotype 1, accessibility is still limited.

In our study, we identified a sub-group of HCV-3 infected patients with very low chances of achieving an SVR after treatment peginterferon plus RBV. These patients with high baseline HCV-RNA and advanced fibrosis are in urgent need for new therapeutic approaches and should be prioritized when sofosbuvir or other antivirals become available. A similar approach was recently proposed to identify a sub-group of patients infected with HCV genotype 1 who might benefit from peginterferon plus RBV therapy^[32].

Owing to its retrospective nature, our study has several limitations. First, since patients were not consecutive, selection bias is possible, and therefore the SVR rate of our population cannot be extrapolated to the general population. Second, on-treatment virologic kinetics was not evaluated. Rapid virological response is probably the most important predictor of SVR across all genotypes^[24]. A major limitation of rapid virological response to predict SVR, lies in the fact that being an on-treatment variable, it is not useful to determine treatment candidacy. Third, even though the number of patients included is small, it adequately represents the sample size estimated to provide the specific power. Finally, although differences regarding treatment protocols between the participating centers might have existed, treatment duration and adherence were globally homogeneous.

In conclusion, our study identified a sub-group of patients with chronic HCV-3 with high baseline viral load and advanced fibrosis whose chances of achieving an SVR with peginterferon plus RBV were poor. These patients are in urgent need for direct antiviral agent-based regimes.

COMMENTS

Background

Hepatitis C virus (HCV) is a major health problem affecting more than 180 million people worldwide. It is estimated that at least 350000 HCV infected people die annually due to liver-related causes.

Research frontiers

Several host and viral factors have an impact on the sustained virological response (SVR) rate of patients infected with HCV genotype 3 (HCV-3) treated with peginterferon plus ribavirin (RBV). Pre-treatment factors that have been proposed to have a negative impact on SVR are advanced fibrosis or cirrhosis, male gender, non-Caucasian race, high body weight, diabetes mellitus, and high pre-treatment HCV-RNA. More recently, *INFL3* (formerly IL28B) polymorphisms were evaluated, but a clear association between the favorable *INFL3* genotypes and SVR could not be established. A major limitation of the studies that assessed predictors of SVR in HCV-3 patients lies in the fact that they evaluated HCV-2 and HCV-3 together, and difficulties arise when trying to draw conclusions for HCV-3 individually.

Innovations and breakthroughs

This study was designed to evaluate pre-treatment variables associated with SVR particularly for patients with chronic HCV-3 treated with peginterferon plus

RBV.

Applications

In the authors' study, they identified a sub-group of HCV-3 infected patients with very low chances of achieving an SVR after treatment peginterferon plus RBV. In patients with both high baseline HCV-RNA and advanced fibrosis, the probability of achieving an SVR was 29%. They believe that these patients are in urgent need for new therapeutic approaches and should be prioritized when sofosbuvir or other antivirals become available.

Terminology

Genotype refers to the genetic relatedness of the different HCV species. Six genotypes of HCV have been well characterized. Fibrosis is a process in which scarring occurs in the liver, ultimately leading to cirrhosis, which is the greatest degree of fibrosis. Sustained virological response means viral eradication or cure.

Peer-review

This study was intended to find some factors associated with SVR in patients with HCV-3 treated with peginterferon and ribavirin. Both high viral load and advanced fibrosis were concluded to be associated with low SVR rates. The authors think this manuscript is well written.

REFERENCES

- 1 **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]
- 2 **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]
- 3 **Kershenobich D**, Razavi HA, Sánchez-Avila JF, Bessone F, Coelho HS, Dagher L, Gonçalves FL, Quiroz JF, Rodríguez-Pérez F, Rosado B, Wallace C, Negro F, Silva M. Trends and projections of hepatitis C virus epidemiology in Latin America. *Liver Int* 2011; **31** Suppl 2: 18-29 [PMID: 21651701 DOI: 10.1111/j.1478-3231.2011.02538.x]
- 4 **Szabo SM**, Bibby M, Yuan Y, Donato BM, Jiménez-Mendez R, Castañeda-Hernández G, Rodríguez-Torres M, Levy AR. The epidemiologic burden of hepatitis C virus infection in Latin America. *Ann Hepatol* 2012; **11**: 623-635 [PMID: 22947522]
- 5 **Tapper EB**, Afdhal NH. Is 3 the new 1: perspectives on virology, natural history and treatment for hepatitis C genotype 3. *J Viral Hepat* 2013; **20**: 669-677 [PMID: 24010641]
- 6 **Andriulli A**, Mangia A, Iacobellis A, Ippolito A, Leandro G, Zeuzem S. Meta-analysis: the outcome of anti-viral therapy in HCV genotype 2 and genotype 3 infected patients with chronic hepatitis. *Aliment Pharmacol Ther* 2008; **28**: 397-404 [PMID: 18549461 DOI: 10.1111/j.1365-2036.2008.03763.x]
- 7 **Marcellin P**, Cheinquer H, Curescu M, Dusheiko GM, Ferenci P, Horban A, Jensen D, Lengyel G, Mangia A, Ouzan D, Puoti M, Rodríguez-Torres M, Shiffman ML, Schmitz M, Tatsch F, Rizzetto M. High sustained virologic response rates in rapid virologic response patients in the large real-world PROPHECY cohort confirm results from randomized clinical trials. *Hepatology* 2012; **56**: 2039-2050 [PMID: 22706730 DOI: 10.1002/hep.25892]
- 8 **Sarin SK**, Kumar CK. Treatment of patients with genotype 3 chronic hepatitis C—current and future therapies. *Liver Int* 2012; **32** Suppl 1: 141-145 [PMID: 22212585 DOI: 10.1111/j.1478-3231.2011.02715.x]
- 9 **Shiffman ML**, Suter F, Bacon BR, Nelson D, Harley H, Solá R, Shafraan SD, Barange K, Lin A, Soman A, Zeuzem S. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; **357**: 124-134 [PMID: 17625124 DOI: 10.1056/NEJMoa066403]
- 10 **Mangia A**, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M, Zechini F, Sogari F, Spirito F, Andriulli A. Peginterferon alfa-2b and ribavirin for 12 vs 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; **352**: 2609-2617 [PMID: 15972867 DOI: 10.1056/NEJMoa042608]

- 11 **Yu ML**, Dai CY, Huang JF, Hou NJ, Lee LP, Hsieh MY, Chiu CF, Lin ZY, Chen SC, Hsieh MY, Wang LY, Chang WY, Chuang WL. A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007; **56**: 553-559 [PMID: 16956917 DOI: 10.1136/gut.2006.102558]
- 12 **Shah SR**, Patel K, Marcellin P, Foster GR, Manns M, Kottitil S, Healey L, Pulkstenis E, Subramanian GM, McHutchison JG, Sulkowski MS, Zeuzem S, Nelson DR. Steatosis is an independent predictor of relapse following rapid virologic response in patients with HCV genotype 3. *Clin Gastroenterol Hepatol* 2011; **9**: 688-693 [PMID: 21640198 DOI: 10.1016/j.cgh.2011.04.029]
- 13 **Mangia A**, Thompson AJ, Santoro R, Piazzolla V, Tillmann HL, Patel K, Shianna KV, Mottola L, Petruzzellis D, Bacca D, Carretta V, Minerva N, Goldstein DB, McHutchison JG. An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology* 2010; **139**: 821-827, 827.e1 [PMID: 20621700 DOI: 10.1053/j.gastro.2010.05.079]
- 14 **Sarrazin C**, Susser S, Doehring A, Lange CM, Müller T, Schlecker C, Herrmann E, Löttsch J, Berg T. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. *J Hepatol* 2011; **54**: 415-421 [PMID: 21112657 DOI: 10.1016/j.jhep.2010.07.041]
- 15 **Moghaddam A**, Melum E, Reinton N, Ring-Larsen H, Verbaan H, Bjørø K, Dalgard O. IL28B genetic variation and treatment response in patients with hepatitis C virus genotype 3 infection. *Hepatology* 2011; **53**: 746-754 [PMID: 21374656 DOI: 10.1002/hep.24154]
- 16 **McGowan CE**, Monis A, Bacon BR, Mallolas J, Goncalves FL, Goulis I, Poordad F, Afdhal N, Zeuzem S, Piratvisuth T, Marcellin P, Fried MW. A global view of hepatitis C: physician knowledge, opinions, and perceived barriers to care. *Hepatology* 2013; **57**: 1325-1332 [PMID: 23315914 DOI: 10.1002/hep.26246]
- 17 **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]
- 18 **Bedossa P**, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293 [PMID: 8690394 DOI: 10.1002/hep.510240201]
- 19 **Ito K**, Higami K, Masaki N, Sugiyama M, Mukaide M, Saito H, Aoki Y, Sato Y, Imamura M, Murata K, Nomura H, Hige S, Adachi H, Hino K, Yatsushashi H, Orito E, Kani S, Tanaka Y, Mizokami M. The rs8099917 polymorphism, when determined by a suitable genotyping method, is a better predictor for response to pegylated alpha interferon/ribavirin therapy in Japanese patients than other single nucleotide polymorphisms associated with interleukin-28B. *J Clin Microbiol* 2011; **49**: 1853-1860 [PMID: 21389156 DOI: 10.1128/JCM.02139-10]
- 20 **Shoeb D**, Rowe IA, Freshwater D, Mutimer D, Brown A, Moreea S, Sood R, Marley R, Sabin CA, Foster GR. Response to antiviral therapy in patients with genotype 3 chronic hepatitis C: fibrosis but not race encourages relapse. *Eur J Gastroenterol Hepatol* 2011; **23**: 747-753 [PMID: 21691208 DOI: 10.1097/MEG.0b013e3283488aba]
- 21 **Zeuzem S**, Rizzetto M, Ferenci P, Shiffman ML. Management of hepatitis C virus genotype 2 or 3 infection: treatment optimization on the basis of virological response. *Antivir Ther* 2009; **14**: 143-154 [PMID: 19430089]
- 22 **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]
- 23 **Lagging M**, Langeland N, Pedersen C, Färkkilä M, Buhl MR, Mørch K, Dhillon AP, Alsiö A, Hellstrand K, Westin J, Norkrans G. Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. *Hepatology* 2008; **47**: 1837-1845 [PMID: 18454508 DOI: 10.1002/hep.22253]
- 24 **Fried MW**, Hadziyannis SJ, Shiffman ML, Messinger D, Zeuzem S. Rapid virological response is the most important predictor of sustained virological response across genotypes in patients with chronic hepatitis C virus infection. *J Hepatol* 2011; **55**: 69-75 [PMID: 21145856 DOI: 10.1016/j.jhep.2010.10.032]
- 25 **Leandro G**, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, Adinolfi LE, Asselah T, Jonsson JR, Smedile A, Terrault N, Piazienza V, Giordani MT, Giostra E, Sonzogni A, Ruggiero G, Marcellin P, Powell EE, George J, Negro F. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. *Gastroenterology* 2006; **130**: 1636-1642 [PMID: 16697727 DOI: 10.1053/j.gastro.2006.03.014]
- 26 **Rubbia-Brandt L**, Quadri R, Abid K, Giostra E, Malé PJ, Mentha G, Spahr L, Zarski JP, Borisch B, Hadengue A, Negro F. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. *J Hepatol* 2000; **33**: 106-115 [PMID: 10905593]
- 27 **Patton HM**, Patel K, Behling C, Bylund D, Blatt LM, Vallée M, Heaton S, Conrad A, Pockros PJ, McHutchison JG. The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. *J Hepatol* 2004; **40**: 484-490 [PMID: 15123364 DOI: 10.1016/j.jhep.2003.11.004]
- 28 **Rodriguez-Torres M**, Govindarajan S, Diago M, Morgan T, Anand B, Barange K, Suter F, Lin A, Hooper G, Shiffman M. Hepatic steatosis in patients with chronic hepatitis C virus genotype 2 or 3 does not affect viral response in patients treated with peginterferon alpha-2a (40KD) (PEGASYS) plus ribavirin (COPEGUS) for 16 or 24 weeks. *Liver Int* 2009; **29**: 237-241 [PMID: 18710427 DOI: 10.1111/j.1478-3231.2008.01859.x]
- 29 **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]
- 30 **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]
- 31 **McGowan CE**, Fried MW. Barriers to hepatitis C treatment. *Liver International* 2012; **57**: 151-156 [DOI: 10.1111/j.1478-3231.2011.02706.x]
- 32 **Andriulli A**, Nardi A, Di Marco V, Ippolito AM, Gavrila C, Aghemo A, Di Paolo D, Squadrito G, Grassi E, Calvaruso V, Valvano MR, Brancaccio G, Craxi A, Angelico M. An a priori prediction model of response to peginterferon plus ribavirin dual therapy in naïve patients with genotype 1 chronic hepatitis C. *Dig Liver Dis* 2014; **46**: 818-825 [PMID: 24953209 DOI: 10.1016/j.dld.2014.05.015]

P- Reviewer: Lakatos PL, Lisotti A, Luo GH **S- Editor:** Tian YL
L- Editor: A **E- Editor:** Liu SQ



Endotipsitis: A case report with a literature review on an emerging prosthetic related infection

Annalan MD Navaratnam, Matthew Grant, David B Banach

Annalan MD Navaratnam, Department of Infectious Disease Epidemiology, Imperial College London, W2 1PG London, United Kingdom

Matthew Grant, David B Banach, Department of Infectious Diseases, Yale School of Medicine, New Haven, CT 06510, United States

Author contributions: Navaratnam AMD and Grant M conceived the idea and drafted the manuscript; Navaratnam AMD carried out the literature review and data collection; Navaratnam AMD, Grant M and Banach DB analyzed the data; all authors revised, read and approved the final manuscript.

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

Data sharing: No additional data are available.

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

Correspondence to: Dr. Annalan MD Navaratnam, Department of Infectious Disease Epidemiology, Imperial College London, St Mary's Campus, Norfolk Place, W2 1PG London, United Kingdom. amnavaratnam@gmail.com
 Telephone: +44-20-759443287

Received: August 1, 2014

Peer-review started: August 2, 2014

First decision: November 14, 2014

Revised: December 15, 2014

Accepted: January 30, 2015

Article in press: February 2, 2015

Published online: April 8, 2015

out, reviewing all papers with specific words in the title or abstract, and excluding appropriately. Of 283 papers that were reviewed, 22 papers reporting 53 cases in total were included in the analyses.

RESULTS: No predominant etiology for endotipsitis was identified, but gram-positive organisms were more common among early-onset infections ($P < 0.01$). A higher mortality rate was associated with *Staphylococcus aureus* and *Candida* spp infections ($P < 0.01$). There was no trend in choice of antibiotic based on the microorganisms isolated and treatment varied from the guidelines of other vegetative prosthetic infections. In endotipsitis "high risk" organisms have been identified, emphasizing the importance of ensuring optimal antimicrobial therapy and adjunctive management strategies.

CONCLUSION: Higher mortality rate was associated with *Staphylococcus aureus* and *Candida* spp infections. A prospective multicenter trial is needed before specific treatment can be recommended.

Key words: Transjugular intrahepatic portosystemic shunts; Transjugular intrahepatic portosystemic shunt infection; Persistent bacteremia; Tipsitis; Antimicrobial therapy

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: We present a case of a rare disease entity called endotipsitis, vegetative infection of a transjugular intrahepatic portosystemic shunt (TIPS). This case has the longest latency, from insertion to infection, reported in the literature. The literature review supplementing this case report demonstrates an association between onset of infection from TIPS insertion and the etiological agent causing the disease. Furthermore, we demonstrate significantly poorer outcomes in specific infections.

Abstract

AIM: To investigate the etiology and management of a poorly understood complication of transjugular intrahepatic portosystemic shunt; "endotipsitis".

METHODS: A MEDLINE database search was carried

Navaratnam AMD, Grant M, Banach DB. Endotipsitis: A case report with a literature review on an emerging prosthetic related infection. *World J Hepatol* 2015; 7(4): 710-716 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/710.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i4.710>

INTRODUCTION

Transjugular intrahepatic portosystemic shunt (TIPS) insertion is a minimally invasive procedure that is used for the decompression of elevated portal pressure. Technical complications to this procedure include capsular perforation affecting up to 33% of cases, with 1%-2% leading to significant intraperitoneal haemorrhage as well as stent misplacement in 20% of cases^[1]. Although initially employed to treat refractory variceal bleeding and ascites, its list of indications has broadened^[2]. Increasing experience with this procedure has led to the recognition of multiple complications including hepatic failure, encephalopathy, sepsis and death. Sustained or relapsing bacteraemia secondary to endovascular vegetative infection of the prosthesis is a rare but serious complication^[3].

In 1998, Sanyal *et al.*^[3] proposed the term "endotipsitis" to describe this disease entity, along with a proposal for diagnostic criteria^[4]. They defined a "definite" infection as continuous clinically significant bacteraemia with vegetative/thrombi inside TIPS and "probable" infection sustained bacteraemia with no other source of infection in a patient with TIPS^[3]. Since this proposal, there have been several suggestions to improve the diagnostic accuracy and sensitivity of the disease.

Incidence and risk factors of endotipsitis are poorly understood due to difficulty in diagnosis and lack of a uniform disease definition^[2]. There are no extensive studies on this topic, the most recent and thorough being a single centre, retrospective study in 2010, which found the incidence of endotipsitis in patients with TIPS to be approximately 1%^[3]. Furthermore, no unified agreement on the best management strategy for these cases to prevent relapses or mortality has been reached. The authors report a case of endotipsitis supplemented with an analysis on a literature review of this disease process and antimicrobial therapy.

Case report

In July 2013 a 30-year-old male with a history of intravenous drug use, chronic liver disease secondary to congenital hepatic fibrosis and alcohol dependence presented with a one month history of fevers, weakness, confusion and rigors. He described a four day history of intermittent stabbing right-sided abdominal pain, non-productive cough, headache and constant fever - all of which had been gradually worsening. He also reported a three-week history of worsening peripheral edema treated with furosemide and spironolactone. Two days

before his admission his family noted confusion and impaired cognitive function.

His past medical history also included resection of a right hepatic lobe mesenchymal hemangioma at age 6, non-bleeding esophageal varices, TIPS insertion 38 mo prior to admission and splenic artery embolization to ameliorate hypersplenism. TIPS insertion was carried out in an elective setting to prevent esophageal variceal bleeds.

On examination, there was evidence of tender hepatomegaly and right flank tenderness. Although his abdomen was distended, there was no evidence of shifting dullness. He had track marks secondary to injections on both arms, symmetrical peripheral edema and tattoos on his back and arms. No abnormalities were found on neurological examination, other than impaired cognition.

Laboratory tests revealed a white blood cell count of $10.7 \times 10^9/L$ with 86% neutrophils, hemoglobin of 93 g/L, platelet count of $63 \times 10^9/L$, aspartate transaminase 819 U/L, alanine aminotransferase 148 U/L, alkaline phosphatase of 2210 U/L, total bilirubin of 23.4 mg/dL (19.8 direct) and a markedly elevated erythrocyte sedimentation rate of 111 mm/h. Blood cultures drawn at admission grew both *Klebsiella oxytoca*, only resistant to ampicillin, and *Escherichia coli* (*E. coli*) (resistant to ampicillin and sulfamethoxazole/trimethoprim).

He was initially treated with piperacillin-tazobactam and gentamicin. Diagnostic workup included doppler ultrasound of the liver showing a patent TIPS with markedly decreased velocity at the portal aspect (17 cm/s, decreased from 104 cm/s two years prior), and computed tomography imaging revealed a non-occlusive filling defect in the superior segment of the TIPS. A transthoracic echocardiogram showed mild mitral and tricuspid regurgitation but no vegetations were seen.

The patient slowly improved despite blood cultures remaining persistently positive for six days and fever continuing for nine days despite appropriate treatment. He subsequently completed a six-week course of combination intravenous antibiotic therapy, at which point antibiotics were changed to oral ciprofloxacin 500 mg daily. In light of the high risk and complication of relapsing bacteraemia and inability to remove the suspected infected TIPS, the relatively inexpensive and well tolerated use of suppressive oral ciprofloxacin was decided until transplant. He was followed for the next 14 mo without infection relapse. Unfortunately the patient was lost to follow up, so was not possible to conduct repeat imaging.

MATERIALS AND METHODS

We performed a MEDLINE database search, January 1956 to November 2013, using terms "infection", "vegetation", "bacteraemia", "sepsis", "tipsitis" and "endotipsitis" combined with the term transjugular intrahepatic portosystemic shunt, TIPSS or TIPS. The

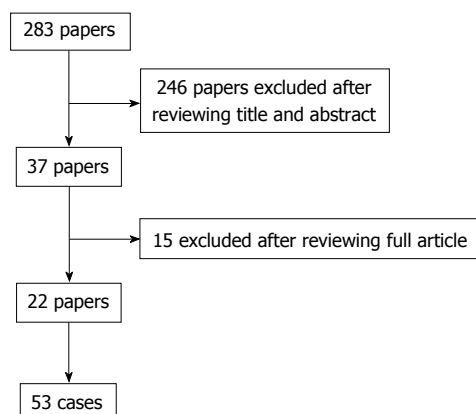


Figure 1 Number of papers excluded and include from the results of the literature review.

titles and abstracts of all papers that appeared on this search were reviewed and excluded accordingly. All data was entered in Microsoft Excel spreadsheet software. Univariate analysis was performed using a χ^2 or Fisher's exact test, as appropriate. Data was analyzed in SPSS, version 16.0^[4]. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Our literature search produced 283 papers, 246 of which were excluded on the basis of their title and abstract. Of the 37 remaining articles, another 15 were excluded after reviewing the contents of the full article. There were 22 papers that reported a sum of 53 cases of "endotipsitis"; 15 case reports and 7 case series (Figure 1). Based on the demographics of the cases, including, age, gender, co-morbidities and aetiological agent, it was possible to exclude any duplications. Analysis includes the case reported by the authors.

Patient characteristics

The mean age was 54.3 years (range 30-69 years; 14 cases unrecorded), with a predominance of males (70.4% vs 17%). The most common underlying disease process was alcoholic liver disease ($n = 32$), 6 of which were also positive for hepatitis B (HBV) ($n = 1$), hepatitis C virus (HCV) ($n = 4$) or both ($n = 1$). HCV cirrhosis ($n = 10$) was the second most common etiology of chronic liver disease, followed by cryptogenic cirrhosis ($n = 9$), HBV ($n = 1$), primary sclerosing cholangitis ($n = 1$) and one case was not recorded. There was no record of whether viral hepatitis positive cases were undergoing treatment at the time of diagnosis of endotipsitis.

Microbiology

Of the 54 cases (including our case report); there were 30 monomicrobial gram-positive infections [of which 14 were *Enterococcal* spp and 7 were *Staphylococcus aureus* (*S. aureus*)], 11 monomicrobial gram-negative

infections (of which 6 were *E. coli*), 5 polymicrobial and 6 *Candida* spp infections (4 *Candida glabrata* and 2 *Candida albicans*). The mean onset of endotipsitis from insertion of TIPS was 277 d, with bacterial being 304 d, and the longest overall being our case report 1158 d (range 6-158 d; 3 unrecorded). Among monomicrobial bacterial infections gram-positive organisms were significantly more common in early-onset infections (less than 120 d following TIPS insertion) than late-onset infections [18/19, (94.7%) vs 9/18 (50%) $P < 0.01$]. The average duration of bacteraemia was 43 d (range 3-20 d; 14 unrecorded).

Treatment

Of the 54 cases reported, 30 (56%) were treated successfully with antimicrobials, 4 (7%) underwent transplantation and 17 (31%) died (Table 1). The average duration of treatment for fungemia and bacteremia was 28 (range 6-51 wk, 3 unrecorded) and 13.5 (range 1-101 wk; 10 unrecorded) wk respectively. Of those with bacteremia, 25 cases were treated with a single antibiotic (19 cases resolved; 2 outcome not recorded), where as 14 were managed with combination antibiotic therapy (1 case patient received 6 different antibiotics; 8 cases resolved). Comparing gram-positive and gram-negative infections, there was no significant difference in mortality [9/30 (30%) vs 4/12 (33%), $P = 0.57$]. Of the 39 antibiotic therapies recorded for bacterial endotipsitis, 7 cases used an aminoglycoside (gentamicin) and 2 used rifampin. None of the staphylococcal cases were treated with gentamicin.

The organisms associated with the highest mortality rates were *S. aureus* (63% mortality) and *Candida* spp (67% mortality). Patients with infections caused by *S. aureus* and *Candida* spp had significantly higher mortality than those infections caused by all other organisms [9/14 (64.3%) vs 8/36 (22.2%) $P < 0.01$]. There was no difference in mortality rate between early and late-onset infections [8/22 (36.4%) vs 7/23 (30.4%) $P = 0.67$].

DISCUSSION

The authors have reported a case of *Klebsiella oxytoca* and *E. coli* polymicrobial endotipsitis with the longest latency of time between TIPS insertion and TIPS infection ever reported. A case of endotipsitis with similar bacteriology was reported previously but was managed quite differently (ceftriaxone monotherapy for 4 wk). Our literature review reviews the largest number of reported endotipsitis cases to date and provides a window into a rare disease, heterogeneous in both its microbiologic etiology and management approaches.

Endotipsitis is suspected in the setting of unexplained bacteremia in a patient with TIPS, supported by abnormalities in TIPS flow or the presence of vegetation

Table 1 Demographics for each case identified in the literature review as well as microorganisms isolated and duration and type of antimicrobials

Etiological agent	Outcome	Duration of bacteremia (d)	Treatment	Duration of treatment (wk)	Cirrhosis etiology	Onset (d)	Ref.
Gram positive, monomicrobial infections							
<i>Staphylococcus aureus</i>	R	6	Vancomycin	6	EtOH	110	[3]
	Tx	-	Rifampin	-	HCV	38	[17]
	D	15	Ciprofloxacin	9	HCV	8	[6]
			Penicillin				
			Vancomycin				
			Teicoplanin				
			Rifampin				
	D	-	Flucloxacillin	2	EtOH	663	[2]
	D	5	Vancomycin	1	EtOH, HBV, HCV	100	[18]
	D	-	-	-	HCV	38	[17]
<i>Staphylococcus aureus</i> (MRSA)	R	46	Vancomycin	6	Cryptogenic	300	[5]
			Linezolid				
<i>Staphylococcus epidermidis</i>	R	25	Daptomycin	6	EtOH	7	[19]
	R	30	Vancomycin	6	EtOH	120	[5]
<i>Enterococcus spp. (unspecified)</i>	R	14	Vancomycin	6	Cryptogenic	47	[5]
	R	10	Vancomycin	28	EtOH, HCV	10	[20]
			Gentamicin				
	R	3	Vancomycin	2	EtOH	60	[18]
			Gentamicin				
	R	21	Vancomycin	7	HCV	-	[20]
	D	-	Vancomycin	-	EtOH	60	[20]
	Tx	-	-	-	HCV	38	[20]
	D	-	-	-	HCV	38	[20]
	D	-	-	-	HCV	38	[20]
<i>Enterococcus faecalis</i>	R	90	Gentamicin	6	EtOH	420	[21]
			Amphotericin				
	R	100	Quinupristin	14	Cryptogenic	-	[22]
	R	10	Vancomycin	8	Cryptogenic	60	[23]
	Tx	10	Vancomycin	4	Cryptogenic	90	[16]
<i>Enterococcus faecium</i>	R	10	Vancomycin	28	EtOH	10	[20]
	-	-	Vancomycin	1	EtOH	375	[2]
<i>Streptococcus sanguis</i>	R	6	Penicillin	4	EtOH	416	[3]
<i>Gemella morbillorum</i>	R	5	Vancomycin	2	PSC	6	[18]
	R	60	Ampicillin	44	EtOH	56	[24]
<i>Streptococcus bovis</i>	R	6	Vancomycin	6	Cryptogenic	416	[3]
<i>Lactobacillus rhamnosus</i>	R	-	Netilmicin,	12	EtOH	730	[2]
			Clarithromycin				
<i>Lactobacillus acidophilus</i>	D	4	Ampicillin	4	EtOH	732	[18]
	D	3	Ampicillin	6	EtOH	146	[18]
			Gentamicin				
Gram negative, monomicrobial infections							
<i>Escherichia coli</i>	R	6	Ceftriaxone	4	EtOH	183	[3]
	R	6	Ceftriaxone	4	EtOH	532	[3]
	R	31	Ciprofloxacin	16	EtOH	1065	[6]
	D	-	Ceftriaxone	12	EtOH	282	[2]
			Gentamicin				
	D	-	Ceftriaxone	12	EtOH	1092	[2]
			Vancomycin				
			Piperacillin-				
			Tazobactam				
			Amoxicillin				
	D	-	Meropenem	3	EtOH	765	[2]
			Amikacin				
<i>Klebsiella pneumonia</i>	R	6	Ceftriaxone	4	EtOH, HCV	329	[3]
<i>Klebsiella (unspecified) MDR</i>	R	9	Tigecycline	-	EtOH	1095	[25]
<i>Serratia marcescens</i>	R	49	Imipenem	98	EtOH	21	[26]
<i>Enterobacter cloacae</i>	R	-	Ceftriaxone	12	EtOH	1065	[2]
			Gentamicin				
			Vancomycin				
			Piperacillin-				
			Tazobactam				
			Meropenem				
			Ciprofloxacin				

<i>Salmonella typhi</i>	-	-	-	-	-	-	[27]
Fungal infections							
<i>Candida glabrata</i>	R	1700	Amphotericin	51	HCV	180	[28]
	D	27	Amphotericin	27	Cryptogenic	27	[29]
	D	-	Fluconazole	-	EtOH	150	[30]
	D	60	Posaconazole	6	EtOH	-	[31]
<i>Candida albicans</i>	R	-	-	-	HBV	38	[18]
	D	-	-	-	HCV	38	[18]
Polymicrobial infections							
<i>Staphylococcus aureus</i> / <i>Pseudomonas aeruginosa</i>	D	14	Ticarcillin-clavulanic acid	-	EtOH, HBV	35	[6]
			Gentamicin				
			Amikacin				
			Ciprofloxacin				
<i>Escherichia coli</i> / <i>Klebsiella oxytoca</i>	R	6	Ceftriaxone	4	EtOH	235	[3]
<i>Escherichia coli</i> / <i>Acinetobacter</i>	R	6	Ceftriaxone	6	EtOH	285	[3]
<i>Escherichia coli</i> / <i>Klebsiella oxytoca</i>	R	6	Piperacillin- Tazobactam	6	Cryptogenic, EtOH	1158	-
			Gentamicin				
“Polymicrobial”	R	720	-	101	HCV	100	[32]
“Polymicrobial”	Tx	90	-	-	HCV	180	[15]
Unknown	-	-	-	-	-	-	[2]

D: Death; Tx: Transplant; R: Resolved; EtOH: Alcoholic liver disease; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

and remains a diagnosis of exclusion, but there have been propositions to improve the diagnostic accuracy. Bouza *et al*^[5] suggested using quantitative bacteriology, where by comparison of colony counts of peripheral venous blood and portal blood will show a substantial increase in bacteria of the latter sample. The gold standard would be to take a biopsy from the pseudo-epithelium of the TIPS, which can only be done at autopsy or after transplantation and is therefore not a viable option in clinical practice^[6].

It is evident from our review that no single micro-organism is the predominant etiological agent, but most infections were caused by gram-positive organisms^[5,6]. This trend has also been reported in studies investigating spontaneous bacterial peritonitis and nosocomial infections in cirrhotic patients and may be attributed to widespread use of fluoroquinolone prophylaxis in this population^[7,8]. Bouza *et al*^[5] suggested however that etiology of the infection could be influenced by the onset of infection post TIPS insertion, which can be categorised as early-onset (within 120 d) or late-onset (over 120 d post insertion). In their review of 36 cases, they found that all *Enterococcus* spp exclusively occurred in early infections, which was not confirmed in our review, with no significant difference between the groups in number of *Enterococcus* spp cases isolated ($P = 0.23$; 95%CI: 0.08-1.77). There was also no significant difference in mortality between early and late infections ($P = 0.76$; 95%CI: 0.35-0.44). Our data also reflect an association between early-onset infections and gram-positive organisms.

Due to the wide variety of causative pathogens and overall rarity of this disease, it is highly unlikely that prospective data will guide management decisions of this clinical entity. As there is a variation in antibiotic choice and duration of treatment on review of the literature, the evidence base is too limited to support specific antibiotic

recommendations. It may be reasonable that patients diagnosed with endotipsitis should be treated based on the principles of prosthetic valve endocarditis (PVE) management, as in many ways the two disease entities are analogous. Both are endovascular infections involving difficult to remove prosthetic material that confer a high rate of treatment failure and relapse. We found antimicrobial management strategies that were overall quite divergent from the pathogen specific treatment guidelines for PVE. For example, staphylococcal PVE is generally treated with a cell wall active agent (*e.g.*, oxacillin or vancomycin) plus rifampin and gentamicin^[9]. Of the 9 cases of staphylococcal endotipsitis reported here, 7 treatment courses were previously published with none including gentamicin and only 2 including rifampin. It is impossible to know whether addition of these agents would have improved the poor response rate we saw in this subgroup (44%). Acknowledging the reluctance of using drugs with potential hepatotoxicity and nephrotoxicity, in the face of treatment response rates below 50%, the potential benefits may outweigh the risks, when administering in a monitored setting.

S. aureus and *Candida* spp are both known to be able to resist the antimicrobial effects of antibiotics through the formation of biofilms^[10]. This propensity underlies the rationale to remove medical devices such as central venous catheters when infected with these organisms, thus reducing the attributable mortality^[11]. When comparing the treatment of monomicrobial infections in endotipsitis cases, there was a significantly lower response rate to antimicrobials alone in those with *S. aureus* or candidal infection compared to other pathogens. Therefore, when managing these patients, transplantation should be considered as a means of cure, as there is a significantly lower chance that they will respond with antibiotics and retention of the TIPS.

Of the 6 cases of fungal endotipsitis (all *Candida*

spp), only 2 responded to antifungal treatment. Similarly, *Candida* spp are the most common cause of fungal infective endocarditis (IE), with these patients more likely to have prosthetic intravascular devices^[12]. Baddley *et al*^[12] found persistently positive blood cultures were associated with this group as well as a significantly higher rate of mortality when compared to non-fungal cases of IE^[13]. With the accumulating evidence of dismal outcomes in patients with candidal endotipsitis, strong consideration for urgent transplantation needs to be contemplated in appropriate patients^[14,15].

In patients who are ineligible for transplantation aggressive medical therapy and potentially chronic suppressive antibiotics should be considered acknowledging the prognosis is poor. In general, the role and timing of liver transplantation in the setting of endotipsitis is poorly understood. In our review we identified four patients who underwent transplantation. Mizrahi *et al*^[1] did report clearance of blood cultures prior to transplant in their 2 cases^[16,17]. Jawaid *et al*^[15] described a case with repeat episodes of bacteraemia, 1 mo after TIPS insertion, with 9 different microorganisms isolated from blood cultures over the period of 11 mo. Willner *et al*^[16] reported persistent polymicrobial bacteraemia 4 d after a TIPS revision, which resulted in a transplant 3 mo later (after 3 episodes of bacteraemia). It unclear whether the patients had negative blood cultures leading up to their transplant. In both cases, a biliary-venous fistula was not identified until after the assessment of the explant. Among patients with suspected endotipsitis and persistent or relapsing bacteremia, biliary-venous fistula should be considered and in this setting transplantation would be warranted to achieve cure.

Optimal treatment of endotipsitis remains elusive given the difficulty of studying a rare disease without a broadly accepted definition and caused by a variety of microbes. Our data suggests that infections caused by *S. aureus* and *Candida* spp is associated with particularly poor outcomes. Based on the treatment of PVE, addition of gentamicin and rifampin to cell wall active antimicrobials may improve outcomes of *S. aureus* endotipsitis cases. Transplantation should be strongly considered in the setting of relapsing disease and infections caused by difficult to eradicate pathogens. More data on the epidemiology and management outcomes of endotipsitis will be needed to determine the most appropriate prevention and treatment strategies.

COMMENTS

Background

"Endotipsitis" is a poorly understood complication of transjugular intrahepatic portosystemic shunt (TIPS) procedures. Sustained or relapsing bacteraemia secondary to endovascular vegetative infection of the prosthesis, "endotipsitis", is a rare but serious complication. The authors report a case with the longest latency from insertion to infection in the literature, supplemented by a review of the literature, in particular the management of this insidious and variable disease process.

Research frontiers

Incidence and risk factors of endotipsitis are poorly understood due to difficulty

in diagnosis and lack of a uniform disease definition. Furthermore, no unified agreement on the best management strategy for these cases to prevent relapses or mortality has been reached.

Innovations and breakthroughs

The authors' data reflects an association between early-onset infections and gram-positive organisms. It also suggests that infections caused by *Staphylococcus aureus* (*S. aureus*) and *Candida* spp are associated with particularly poor outcomes. The evidence base is too limited to support specific antibiotic recommendations, but it may be reasonable to treat based on the principles of prosthetic valve endocarditis (PVE) management.

Applications

Transplantation should be strongly considered in those with infections associated with particularly poor outcomes, such as *Candida* spp. Based on the treatment of PVE, addition of gentamicin and rifampin to cell wall active antimicrobials may improve outcomes of *S. aureus* endotipsitis cases.

Terminology

TIPS insertion is a minimally invasive procedure that is used for the decompression of elevated portal pressure. Although there is no universally accepted definition of "endotipsitis", it is generally agreed that it can be defined as "definite" infection (continuous clinically significant bacteraemia with vegetative/thrombi inside TIPS) and "probable" infection (sustained bacteraemia with no other source of infection in a patient with TIPS).

Peer-review

The manuscript presents an interesting case of "endotipsitis" and reviews the literature on a rare disease. In this paper Navaratnam *et al* present a case of "endotipsitis" and performed a review of the existing literature on this field. They identified 22 papers reporting 54 patients with endotipsitis. The great majority had monomicrobial infections (gram positive agents were the majority). Infections with *S. aureus* and *Candida* spp were associated higher mortality. No homogeneous management was applied for the treatment of this condition and guidelines on antibiotics use are usually derivate from the treatment of endocarditis.

REFERENCES

- 1 Mizrahi M, Adar T, Shouval D, Bloom AI, Shibolet O. Endotipsitis-persistent infection of transjugular intrahepatic portosystemic shunt: pathogenesis, clinical features and management. *Liver Int* 2010; **30**: 175-183 [PMID: 19929905 DOI: 10.1111/j.1478-3231]
- 2 Kochar N, Tripathi D, Arestis NJ, Ireland H, Redhead DN, Hayes PC. Tipsitis: incidence and outcome-a single centre experience. *Eur J Gastroenterol Hepatol* 2010; **22**: 729-735 [PMID: 20440117 DOI: 10.1097/MEG.0b013e3282fd6917]
- 3 Sanyal AJ, Reddy KR. Vegetative infection of transjugular intrahepatic portosystemic shunts. *Gastroenterology* 1998; **115**: 110-115 [PMID: 9649465 DOI: 10.1016/S0016-5085(98)70371-3]
- 4 Fisher RA. On the interpretation of Chi-square from contingency tables and the calculation of P. *J R Stat Soc* 1922; **85**: 87-94 [DOI: 10.2307/2340521]
- 5 Bouza E, Muñoz P, Rodríguez C, Grill F, Rodríguez-Créixems M, Bañares R, Fernández J, García-Pagán JC. Endotipsitis: an emerging prosthetic-related infection in patients with portal hypertension. *Diagn Microbiol Infect Dis* 2004; **49**: 77-82 [PMID: 15183855 DOI: 10.1016/j.diagmicrobio.2004.03.006]
- 6 Armstrong PK, MacLeod C. Infection of transjugular intrahepatic portosystemic shunt devices: three cases and a review of the literature. *Clin Infect Dis* 2003; **36**: 407-412 [PMID: 12567297 DOI: 10.1086/346156]
- 7 Fernández J, Navasa M, Gómez J, Colmenero J, Vila J, Arroyo V, Rodés J. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002; **35**: 140-148 [PMID: 11786970 DOI: 10.1053/jhep.2002.30082]
- 8 Gould FK, Denning DW, Elliott TS, Foweraker J, Perry JD, Prendergast BD, Sandoe JA, Spry MJ, Watkin RW. Guidelines for the diagnosis and antibiotic treatment of endocarditis in adults: a report of the Working Party of the British Society for Antimicrobial Chemotherapy. *J Antimicrob Chemother* 2012; **67**: 269-289 [PMID: 22086858 DOI: 10.1093/jac/dkr450]
- 9 Harriott MM, Noverr MC. *Candida albicans* and *Staphylococcus*

- aureus form polymicrobial biofilms: effects on antimicrobial resistance. *Antimicrob Agents Chemother* 2009; **53**: 3914-3922 [PMID: 19564370 DOI: 10.1128/AAC.00657-09]
- 10 **Steinbach WJ**, Perfect JR, Cabell CH, Fowler VG, Corey GR, Li JS, Zaas AK, Benjamin DK. A meta-analysis of medical versus surgical therapy for *Candida* endocarditis. *J Infect* 2005; **51**: 230-247 [PMID: 16230221 DOI: 10.1016/j.jinf.2004.10.016]
 - 11 **Falcone M**, Barzaghi N, Carosi G, Grossi P, Minoli L, Ravasio V, Rizzi M, Suter F, Utili R, Viscoli C, Venditti M; Italian Study on Endocarditis. *Candida* infective endocarditis: report of 15 cases from a prospective multicenter study. *Medicine* (Baltimore) 2009; **88**: 160-168 [PMID: 19440119 DOI: 10.1097/MD.0b013e3181a693f8]
 - 12 **Baddley JW**, Benjamin DK, Patel M, Miró J, Athan E, Barsic B, Bouza E, Clara L, Elliott T, Kanafani Z, Klein J, Lerakis S, Levine D, Spelman D, Rubinstein E, Tornos P, Morris AJ, Pappas P, Fowler VG, Chu VH, Cabell C; International Collaboration on Endocarditis-Prospective Cohort Study Group (ICE-PCS). *Candida* infective endocarditis. *Eur J Clin Microbiol Infect Dis* 2008; **27**: 519-529 [PMID: 18283504 DOI: 10.1007/s10096-008-0466-x]
 - 13 **Mora-Duarte J**, Betts R, Rotstein C, Colombo AL, Thompson-Moya L, Smietana J, Lupinacci R, Sable C, Kartsonis N, Perfect J. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med* 2002; **347**: 2020-2029 [PMID: 12490683 DOI: 10.1056/NEJMoa021585]
 - 14 **Kullberg BJ**, Sobel JD, Ruhnke M, Pappas PG, Viscoli C, Rex JH, Cleary JD, Rubinstein E, Church LW, Brown JM, Schlamm HT, Oborska IT, Hilton F, Hodges MR. Voriconazole versus a regimen of amphotericin B followed by fluconazole for candidaemia in non-neutropenic patients: a randomised non-inferiority trial. *Lancet* 2005; **366**: 1435-1442 [PMID: 16243088 DOI: 10.1016/S0140-6736(05)67490-9]
 - 15 **Jawaid Q**, Saeed ZA, Di Bisceglie AM, Brunt EM, Ramrakhiani S, Varma CR, Solomon H. Biliary-venous fistula complicating transjugular intrahepatic portosystemic shunt presenting with recurrent bacteremia, jaundice, anemia and fever. *Am J Transplant* 2003; **3**: 1604-1607 [PMID: 14629294 DOI: 10.1046/j.1600-6135.2003.00267.x]
 - 16 **Willner IR**, El-Sakr R, Werkman RF, Taylor WZ, Riely CA. A fistula from the portal vein to the bile duct: an unusual complication of transjugular intrahepatic portosystemic shunt. *Am J Gastroenterol* 1998; **93**: 1952-1955 [PMID: 9772063 DOI: 10.1111/j.1572-0241.1998.00553.x]
 - 17 **Mizrahi M**, Roemi L, Shouval D, Adar T, Korem M, Moses A, Bloom A, Shibolet O. Bacteremia and "Endotipsitis" following transjugular intrahepatic portosystemic shunting. *World J Hepatol* 2011; **3**: 130-136 [PMID: 21731907 DOI: 10.4254/wjh.v3.i5.130]
 - 18 **DeSimone JA**, Beavis KG, Eschelman DJ, Henning KJ. Sustained bacteremia associated with transjugular intrahepatic portosystemic shunt (TIPS). *Clin Infect Dis* 2000; **30**: 384-386 [PMID: 10671346 DOI: 10.1086/313653]
 - 19 **Colston JM**, Scarborough M, Collier J, Bowler IC. High-dose daptomycin monotherapy cures *Staphylococcus epidermidis* 'endotipsitis' after failure of conventional therapy. *BMJ Case Rep* 2013; **2013**: pii: bcr2013009529 [PMID: 23595199 DOI: 10.1136/bcr-2013-009529]
 - 20 **Brown RS**, Brumage L, Yee HF, Lake JR, Roberts JP, Somberg KA. Enterococcal bacteremia after transjugular intrahepatic portosystemic shunts (TIPS). *Am J Gastroenterol* 1998; **93**: 636-639 [PMID: 9576462 DOI: 10.1111/j.1572-0241.1998.180_b.x]
 - 21 **Passeron A**, Mihaïla-Amrouche L, Perreira Rocha E, Wyplosz B, Capron L. [Recurrent enterococcal bacteremia associated with a transjugular intrahepatic portosystemic shunt]. *Gastroenterol Clin Biol* 2004; **28**: 1284-1286 [PMID: 15671940 DOI: 10.1016/S0399-8320(04)95222-0]
 - 22 **Zaman MM**, Recco R, Tejwani U, Scuto TJ, Ahmed S, Hypolite A, Jayaraman G. Case of vancomycin-resistant *Enterococcus faecium* infection associated with a transjugular intrahepatic portosystemic shunt that was treated with quinupristin/dalfopristin after bacteremia persisted with alatrofloxacin therapy. *Clin Infect Dis* 1999; **29**: 954-955 [PMID: 10589932 DOI: 10.1086/520480]
 - 23 **Eversman D**, Chalasani N. A case of infective endotipsitis. *Gastroenterology* 1999; **117**: 514 [PMID: 10465639 DOI: 10.1053/gast.1999.0029900514a]
 - 24 **Medina-Gens L**, López J, Manzanedo B, Pintado V. [Endotipsitis due to *Gemella morbillorum*]. *Enferm Infecc Microbiol Clin* 2007; **25**: 419-420 [PMID: 17583662 DOI: 10.1157/13106974]
 - 25 **Aggarwal S**, Park J. Endotipsitis: a diagnostic challenge. *J Postgrad Med* 2011; **57**: 134 [PMID: 21654138 DOI: 10.4103/0022-3859.81875]
 - 26 **Marques N**, Sá R, Coelho F, da Cunha S, Meliço-Silvestre A. Spondylodiscitis associated with recurrent *Serratia* bacteremia due to a transjugular intrahepatic portosystemic shunt (TIPS): a case report. *Braz J Infect Dis* 2007; **11**: 525-527 [PMID: 17962882 DOI: 10.1590/S1413-86702007000500016]
 - 27 **Barrio J**, Castiella A, Von Wichman MA, Cosme A, López P, Arenas JL. [Spontaneous bacteremia due to *Salmonella* hadar in liver cirrhosis with transjugular intrahepatic portosystemic shunt]. *Gastroenterol Hepatol* 1999; **22**: 79-81 [PMID: 10193091]
 - 28 **Brickey TW**, Trotter JF, Johnson SP. *Torulopsis glabrata* fungemia from infected transjugular intrahepatic portosystemic shunt stent. *J Vasc Interv Radiol* 2005; **16**: 751-752 [PMID: 15872333 DOI: 10.1097/01.RVI.0000153587.52276.06]
 - 29 **Darwin P**, Mergner W, Thuluvath P. *Torulopsis glabrata* fungemia as a complication of a clotted transjugular intrahepatic portosystemic shunt. *Liver Transpl Surg* 1998; **4**: 89-90 [PMID: 9457972 DOI: 10.1002/lt.500040112]
 - 30 **Schiano TD**, Atillasoy E, Fiel MI, Wolf DC, Jaffe D, Cooper JM, Jonas ME, Bodenheimer HC, Min AD. Fatal fungemia resulting from an infected transjugular intrahepatic portosystemic shunt stent. *Am J Gastroenterol* 1997; **92**: 709-710 [PMID: 9128335]
 - 31 **Anstead GM**, Martinez M, Graybill JR. Control of a *Candida glabrata* prosthetic endovascular infection with posaconazole. *Med Mycol* 2006; **44**: 273-277 [PMID: 16702108 DOI: 10.1080/13693780500049152]
 - 32 **Suhocki PV**, Smith AD, Tendler DA, Sexton DJ. Treatment of TIPS/biliary fistula-related endotipsitis with a covered stent. *J Vasc Interv Radiol* 2008; **19**: 937-939 [PMID: 18503911 DOI: 10.1016/j.jvir.2008.01.026]

P-Reviewer: De Ponti F, Narciso-Schiavon JL, Procopet B
S-Editor: Tian YL **L-Editor:** A **E-Editor:** Liu SQ



Skin cancer in immunosuppressed transplant patients: Vigilance matters

Ozan Unlu, Emir Charles Roach, Alexis Okoh, May Olayan, Bulent Yilmaz, Didem Uzunaslari, Abdullah Shatnawei

Ozan Unlu, Didem Uzunaslari, Department of Pathobiology, Cleveland Clinic, Cleveland, OH 44106, United States

Emir Charles Roach, Abdullah Shatnawei, Department of Gastroenterology, Cleveland Clinic, Cleveland, OH 44106, United States

Alexis Okoh, Department of Endocrine Surgery, Cleveland Clinic, Cleveland, OH 44106, United States

May Olayan, Department of Internal Medicine, Fairview Hospital, Cleveland Clinic, Cleveland, OH 44106, United States

Bulent Yilmaz, Department of Gastroenterology, Hacettepe University School of Medicine, 06230 Ankara, Turkey

Author contributions: All authors contributed significantly to the work, had read and revised the manuscript.

Ethics approval: Cleveland Clinic Publication Guidance for IRB review and HIPAA Compliance allow researchers and physician publish case reports involving three or less patients without and IRB review as long as all the patients involved in the study provide written informed consent forms.

Informed consent: The patient in this study provided informed written consent prior to study enrollment.

Conflict-of-interest: We certify that there is no conflict of interest with any commercial, personal, political, intellectual, religious or any other kind of organization regarding the material discussed in the manuscript.

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

Correspondence to: Emir Charles Roach, MD, Department of Gastroenterology, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44106, United States. roache@ccf.org

Telephone: +1-216-8488870

Received: November 10, 2014

Peer-review started: November 11, 2014

First decision: December 12, 2014

Revised: December 27, 2014

Accepted: January 15, 2015

Article in press: January 19, 2015

Published online: April 8, 2015

Abstract

Liver transplantation (LT) is a widely-accepted, definitive therapy of irreversible liver diseases including hepatitis C, alcoholic liver disease and metabolic liver disease. After transplantation, patients generally use a variety of immunosuppressive medications for the rest of their lives to prevent rejection of transplanted liver. Mortality after LT is mainly caused by recurrence of alcoholic hepatitis which is mostly seen in the patients who resume heavy drinking. On the other hand, *de-novo* malignancies after LT are not seldom. Skin cancers make up 13.5% of the *de-novo* malignancies seen in these patients. Malignancies tend to affect survival earlier in the course with a 53% risk of death at 5 years after diagnosis. We aimed to report a case who underwent LT secondary to alcoholic liver disease and developed squamous cell carcinoma of the skin eighteen years after transplantation. In summary, transplant recipients are recommended to be educated on self examination for skin cancer; health care providers should be further suspicious during routine dermatological examinations of the transplant patients and biopsies of possible lesions for skin cancer is warranted even many years after transplantation.

Key words: Alcoholic liver disease; Skin cancer; Non-squamous; Liver transplantation; Sirolimus

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: We presented a case who underwent liver transplantation due to alcoholic liver disease and developed a skin cancer after 18 years of follow-up, which is exceptionally rare as malignancies tend to

affect survival earlier in the course with a 53% risk of death at 5 years after diagnosis.

Unlu O, Roach EC, Okoh A, Olayan M, Yilmaz B, Uzunaslani D, Shatnawei A. Skin cancer in immunosuppressed transplant patients: Vigilance matters. *World J Hepatol* 2015; 7(4): 717-720 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/717.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i4.717>

INTRODUCTION

Alcoholic liver disease (ALD) is known to be the number one cause of cirrhosis in western countries. Twenty percent of the liver transplants in United States and forty per-cent in Europe are performed due to ALD which makes it the second most common indication for liver transplantation (LT), a definitive treatment option for patients with cirrhosis and end stage liver disease^[1-3].

Patient survival rates after LT for alcoholic cirrhosis have been reported to be 73%-86% at 5 years after diagnosis^[4,5]. Mortality after LT is mainly caused by recurrence of ALD and alcoholic hepatitis which are mostly seen in the patients who resume heavy drinking. Other causes of mortality in patients who stay abstinent are cardiovascular diseases or events, infection and malignancies. Of note is the high incidence of *de-novo* malignancies after LT. This has been associated with the use of post LT immunosuppressants and the history of heavy alcohol use. Other reported risk factors for *de-novo* malignancies include older age, male gender, and Epstein Barr virus reactivation or infection for lymphoproliferative malignancy, and exposure to sun for non-melanoma skin cancer. Skin cancers make up the 13.5% of the *de-novo* malignancies seen in the patients with LT^[6-8]. Malignancies tend to affect survival early after LT with a 53% risk of death at 5 years after diagnosis^[9].

Herein, we present a case underwent LT (ALD etiology) who is followed up for 18 years without development of any malignancy. The patient consequently developed skin cancer at different sites 7 years after change of immunosuppressive regimen.

A high index of clinical suspicion together with routine dermatological examinations and biopsies of possible lesions for skin cancer is warranted in LT patients even eighteen years after transplant.

CASE REPORT

This is a 74-year-old male patient, who was under follow up for LT due to ALD at our center for 18 years. He was first diagnosed with cirrhosis secondary to ALD in 1993 and had a liver transplant two years later. Early post-transplant immunosuppressive treatment regimen included mycophenolate and tacrolimus for which he showed moderate clinical response. Afterwards, he

developed calcineurin inhibitor induced end stage renal insufficiency and switched to sirolimus from tacrolimus in 2003 and a combination therapy with mycophenolate was continued.

Previous medical and surgical history included uncomplicated diabetes mellitus, hyperlipidemia and hypertension for 15 years and aortic valve replacement due to vascular thrombosis that occurred over the course of treatment. His co-morbidities were under control with antihypertensives and lipid lowering statins and his renal insufficiency related anemia was managed with erythropoietin.

Two years after treatment with sirolimus was started, the patient had recurring acneiform eruptions with pustules on his face, ears, and scalp. He was therefore referred to a dermatology unit for further evaluation and follow up.

A definitive diagnosis consistent with squamous cell carcinoma of the skin was made five years later. In September 2010, the patient underwent a resection of a tumor from his left temple, sacrificing the frontal branch of left facial nerve. He had multiple surgeries including left superficial parotidectomy due to relapses at different sites such as left auricle, preauricular area and occipitofrontal region of the scalp. Histological evaluations after each surgery revealed surgical margins wider than 6 mm. Identification of atypical tumor cells and keratinous pearls in microscopical evaluation supported the diagnosis. Despite multiple surgeries, chemotherapy and radiotherapy, patient was diagnosed with stage 4 squamous cell carcinoma due to metastatic lesions in his brain in 2012, and died in February 2013.

DISCUSSION

Patient survival rates after LT for alcoholic cirrhosis have been reported to be 81%-92%, 78%-86%, and 73%-86% at 1, 3, and 5 years respectively^[4,5]. In an European Liver Transplant Registry which enrolled patients between 1988 and 2009, survival rates were reported as 73% and 59% for 5 and 10 years of follow-up respectively. These rates were shown to be higher compared to non-alcoholic etiology associated liver transplants, thus confirming ALD as an acceptable indication for LT^[4,5,10].

Malignancy has been shown to significantly affect survival in LT patients with about 38% and 53% risk of death at 1 and 5 years after diagnosis^[9]. Among LT recipients who survive the first year after transplantation, *De novo* malignancy is reported to account for 30%-40% of all deaths^[9,11]. Although, intensive surveillance protocols in the post-transplant period have been shown to improve survival by detection of malignancy, clear guidelines including the frequency of work-up have not been developed yet. In this report, we describe the case of an LT patient who was followed up for 18 years after LT due to ALD. Renal insufficiency that occurred secondary to immunosuppressive therapy with

Table 1 Causes of death after liver transplantation *n* (%)

Years postimplantation	1	2	3	4	5	6	7	8	9	10	> 10	Total
Patient at risk (<i>n</i>)	4000	2940	2665	2478	2261	2018	1732	1511	1238	958	735	
Infection (bacterial, viral, fungal)	372	38	13	16	4	6	8	1	3	1	2	464 (28.4)
Malignancy (recurrent/ <i>de novo</i>)	42	45	28	18	11	19	12	6	3		6	190 (11.6)
Cardiovascular	42	14	6	1	13	17	13	9	6	5	9	135 (8.3)
Respiratory	37	20	14	7	8	3	3	4	5	4	9	114 (7.0)
Intraoperative	99	4	1	2	4	2		1				113 (6.9)
Multisystem organ failure	45	16	9	5	6	9	5	7		1	3	109 (6.7)
Liver failure (recurrent)	21	15	15	7	10	6	3	2	1	2		82 (5.9)
Gastrointestinal	31	6	2	4	5	1		1	1		1	52 (3.2)
Central nervous system	20	2	2	4	1	5	3		1		4	42 (2.6)
PTLD	8		5	6	2	3	1	1	1		2	29 (1.8)
Renal failure				8	5	1		3			1	18 (1.1)
Rejection (acute/chronic)	4	2	1	1	1	2	2		1		4	18 (1.1)
Primary nonfunction	13		1									14 (1.1)
Miscellaneous	27	15	7	12	10	12	8	10	7	5	5	118 (6.1)
Unknown	55	20	18	5	5	7	4	5	4	1	11	135 (8.3)
Total	816 (20.4)	197 (6.7)	122 (4.5)	96 (3.8)	85 (3.7)	93 (4.6)	62 (3.5)	50 (3.3)	36 (2.9)	19 (1.9)	57 (7.7)	1633

PTLD: Posttransplant lymphoproliferative disease.

tacrolimus required a switch to sirolimus. Unfortunately, the patient consequently developed squamous cell carcinoma of the skin at multiple sites. In spite of surgical, chemo and radiation therapy, the patient died due to metastatic lesions in the brain.

Non melanoma skin cancer is the most common malignancy (NMSC) among the LT recipients with an overall incidence of 16% to 22.5%. Previous studies have shown NMSC as a factor effecting mortality^[9,11]. The factors that alter the risk of skin cancer are patient's age, skin type, lifetime sun exposure and male sex^[12]. Immunosuppressant agents were shown to increase the risk of skin cancers, with no evidence of superiority over one another. On the other hand, there are randomized controlled trials suggesting antitumor effects of sirolimus on skin cancer in renal transplant patients. Although studies on sirolimus in LT recipients had high discontinuation rates, similar results with studies on renal transplant patients are anticipated^[13,14].

Jain *et al.*^[15] demonstrated that LT recipients with non-alcoholic liver disease had significantly longer survival rates compared to those with an ALD history. Moreover, another review suggested that while 5 year survival rates in ALD patients were similar between the ones who resumed drinking and those who didn't, 10 year survival rates were significantly different (45.1% vs 85.5%, respectively)^[8]. In these studies, mortality was mainly caused by cardiovascular events and *de novo* neoplasms. This may indicate that there are factors causing malignant changes other than immunosuppressants.

Management of short and long term complications of LT is challenging and the choice of immunosuppressant agent is controversial. The causes of mortality may alter in short and long term follow-up after LT^[16] (Table 1). In order to decide which immunosuppressant should be used as a first choice, further controlled randomized

data are needed.

LT recipients are recommended to be educated on self examination for skin cancer and health care providers should be further suspicious during patients' routine dermatological examinations even many years after transplantation^[6].

ALD is a good indication for LT. In the long term follow up, *de novo* malignancies and particularly skin cancer are long term complications of LT. Therefore, there is a need for particular vigilance of LT recipients. Further investigation into effects of immunosuppressant agents on *de novo* malignancies after LT is warranted to clarify the first choice of therapy.

COMMENTS

Case characteristics

This is a 74-year-old male patient, who was under follow up for 18 years for liver transplantation due to alcoholic liver disease. He presented with recurring acneiform eruptions with pustules on his face, ears, and scalp.

Clinical diagnosis

Physical examination was normal for all systems except that the patient had acneiform eruptions with pustules on his face, ears, and scalp.

Differential diagnosis

Keratoacanthoma, Basal Cell Carcinoma, Malignant melanoma, Solar (actinic) keratosis. A definitive diagnosis of squamous cell carcinoma was made with an excisional biopsy.

Laboratory diagnosis

Laboratory diagnosis was not necessary for the definitive diagnosis of squamous cell carcinoma.

Imaging diagnosis

Computed tomography scan revealed multiple metastatic lesions in the brain.

Pathological diagnosis

Pathology revealed atypical tumor cells, keratinous pearls and surgical margins wider than 6 mm.

Treatment

He took sirolimus and a combination therapy with mycophenolate for immunosuppression. His co-morbidities were under control with antihypertensives and lipid lowering statins. His renal insufficiency related anemia was managed with erythropoietin. After the definitive diagnosis of squamous cell carcinoma, he

had multiple surgeries and took chemotherapy and radiotherapy.

Related reports

De novo malignancies and non melanoma skin cancers were shown to develop in liver transplant patients under immunosuppressive therapy. However, the case is highly unique to develop a squamous cell carcinoma after 18 years of follow up.

Term explanation

A focus of central keratinization found within concentric layers of abnormal squamous cells, occurring in squamous cell carcinoma. Also called epithelial pearl.

Experiences and lessons

Transplant recipients are recommended to be educated on self examination for skin cancer; health care providers should be further suspicious during routine dermatological examinations of the transplant patients and biopsies of possible lesions for skin cancer is warranted even many years after transplantation.

Peer-review

The article reports a case of a patient who developed a squamous cell carcinoma 7 years after liver transplant following the change of immunosuppressive therapy. It is interesting.

REFERENCES

- 1 **Berg CL**, Steffick DE, Edwards EB, Heimbach JK, Magee JC, Washburn WK, Mazariegos GV. Liver and intestine transplantation in the United States 1998-2007. *Am J Transplant* 2009; **9**: 907-931 [PMID: 19341415 DOI: 10.1111/j.1600-6143.2009.02567.x]
- 2 **Burra P**, Senzolo M, Adam R, Delvart V, Karam V, Germani G, Neuberger J. Liver transplantation for alcoholic liver disease in Europe: a study from the ELTR (European Liver Transplant Registry). *Am J Transplant* 2010; **10**: 138-148 [PMID: 19951276 DOI: 10.1111/j.1600-6143.2009.02869.x]
- 3 **Singal AK**, Chaha KS, Rasheed K, Anand BS. Liver transplantation in alcoholic liver disease current status and controversies. *World J Gastroenterol* 2013; **19**: 5953-5963 [PMID: 24106395 DOI: 10.3748/wjg.v19.i36.5953]
- 4 **Adam R**, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, Castaing D, Neuhaus P, Jamieson N, Salizzoni M, Pollard S, Lerut J, Paul A, Garcia-Valdecasas JC, Rodríguez FS, Burroughs A. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol* 2012; **57**: 675-688 [PMID: 22609307 DOI: 10.1016/j.jhep.2012.04.015]
- 5 **Singal AK**, Guturu P, Hmoud B, Kuo YF, Salameh H, Wiesner RH. Evolving frequency and outcomes of liver transplantation based on etiology of liver disease. *Transplantation* 2013; **95**: 755-760 [PMID: 23370710 DOI: 10.1097/TP.0b013e31827afb3a]
- 6 **Chandok N**, Watt KD. Burden of de novo malignancy in the liver transplant recipient. *Liver Transpl* 2012; **18**: 1277-1289 [PMID: 22887956 DOI: 10.1002/lt.23531]
- 7 **Ducroux E**, Boillot O, Ocampo MA, Decullier E, Roux A, Dumortier J, Kanitakis J, Jullien D, Euvrard S. Skin cancers after liver transplantation: retrospective single-center study on 371 recipients. *Transplantation* 2014; **98**: 335-340 [PMID: 24621534 DOI: 10.1097/TP.0000000000000051]
- 8 **Iruzubieta P**, Crespo J, Fábrega E. Long-term survival after liver transplantation for alcoholic liver disease. *World J Gastroenterol* 2013; **19**: 9198-9208 [PMID: 24409048 DOI: 10.3748/wjg.v19.i48.9198]
- 9 **Aberg F**, Pukkala E, Höckerstedt K, Sankila R, Isoniemi H. Risk of malignant neoplasms after liver transplantation: a population-based study. *Liver Transpl* 2008; **14**: 1428-1436 [PMID: 18825704 DOI: 10.1002/lt.21475]
- 10 **Varma V**, Webb K, Mirza DF. Liver transplantation for alcoholic liver disease. *World J Gastroenterol* 2010; **16**: 4377-4393 [PMID: 20845504]
- 11 **Herrero JI**, España A, Quiroga J, Sangro B, Pardo F, Álvarez-Cienfuegos J, Prieto J. Nonmelanoma skin cancer after liver transplantation. Study of risk factors. *Liver Transpl* 2005; **11**: 1100-1106 [PMID: 16123952]
- 12 **Mithoefer AB**, Supran S, Freeman RB. Risk factors associated with the development of skin cancer after liver transplantation. *Liver Transpl* 2002; **8**: 939-944 [PMID: 12360438]
- 13 **Alberú J**, Pascoe MD, Campistol JM, Schena FP, Rial Mdel C, Polinsky M, Neylan JF, Korth-Bradley J, Goldberg-Alberts R, Maller ES. Lower malignancy rates in renal allograft recipients converted to sirolimus-based, calcineurin inhibitor-free immunotherapy: 24-month results from the CONVERT trial. *Transplantation* 2011; **92**: 303-310 [PMID: 21792049 DOI: 10.1097/TP.0b013e3182247ae2]
- 14 **Mathew T**, Kreis H, Friend P. Two-year incidence of malignancy in sirolimus-treated renal transplant recipients: results from five multicenter studies. *Clin Transplant* 2004; **18**: 446-449 [PMID: 15233824]
- 15 **Jain A**, DiMartini A, Kashyap R, Youk A, Rohal S, Fung J. Long-term follow-up after liver transplantation for alcoholic liver disease under tacrolimus. *Transplantation* 2000; **70**: 1335-1342 [PMID: 11087149]
- 16 **Kashyap R**, Jain A, Reyes J, Demetris AJ, Elmagd KA, Dodson SF, Marsh W, Madariaga V, Mazariegos G, Geller D, Bonham CA, Cacciarelli T, Fontes P, Starzl TE, Fung JJ. Causes of death after liver transplantation in 4000 consecutive patients: 2 to 19 year follow-up. *Transplant Proc* 2001; **33**: 1482-1483 [PMID: 11267383]

P- Reviewer: Negosanti L, Qin JM S- Editor: Ji FF

L- Editor: A E- Editor: Liu SQ



First jejunal artery, an alternative graft for right hepatic artery reconstruction

Bibek Aryal, Teruo Komokata, Jun Kadono, Hiroyuki Motodaka, Tetsuya Ueno, Akira Furoi, Yutaka Imoto

Bibek Aryal, Jun Kadono, Hiroyuki Motodaka, Tetsuya Ueno, Yutaka Imoto, Cardiovascular and Gastroenterological Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima-shi, Kagoshima 890-8520, Japan

Teruo Komokata, Department of Surgery, Kagoshima Medical Center, National Hospital Organization, Kagoshima-shi, Kagoshima 892-0853, Japan

Akira Furoi, Department of Surgery, Kirishima Medical Center, Kirishima-shi, Kagoshima 899-5112, Japan

Author contributions: Aryal B, Komokata T and Kadono J followed the case and designed the report; Motodaka H involved in in-patient care and collected the patient's clinical data; Ueno T assisted the vascular reconstruction; Aryal B wrote the paper; Kadono J, Komokata T, Furoi A and Imoto Y reviewed the paper; Imoto Y approved the paper.

Conflict-of-interest: None of the authors in the manuscript has any conflict of interest to declare.

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

Correspondence to: Teruo Komokata, MD, PhD, Department of Surgery, Kagoshima Medical Center, National Hospital Organization, 8-1 Shiroyamacho, Kagoshima-shi, Kagoshima 892-0853, Japan. komokata@kagomc2.hosp.go.jp
Telephone: +81-99-2231151

Fax: +81-99-269246

Received: December 17, 2014

Peer-review started: December 18, 2014

First decision: December 27, 2014

Revised: January 9, 2015

Accepted: February 10, 2015

Article in press: February 12, 2015

Published online: April 8, 2015

safe reconstruction of the artery with suitable graft is essential. Arterial reconstruction with autologous saphenous vein graft is the preferred method practiced routinely. However the right hepatic artery reconstruction has also been carried out with several other vessels like gastroduodenal artery, right gastroepiploic artery or the splenic artery. We report a case of 63-year-old man presenting with history of progressive jaundice, pruritus and impaired appetite. Following various imaging modalities including computed tomography, endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography, intra-ductal ultrasound extrahepatic bile duct cancer was diagnosed; however, none of those detected vessel invasion. Intraoperatively, right hepatic artery invasion was revealed. Right hepatic artery was resected and reconstructed with a graft harvested from the first jejunal artery (JA). Postoperative outcome was satisfactory with a long-term graft patency. First JA can be a reliable graft option for right hepatic artery reconstruction.

Key words: Common bile duct cancer; Right hepatic artery; Arterial reconstruction; Jejunal artery; Arterial graft

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Common bile duct (CBD) cancers frequently invade the surrounding vessels like the right hepatic artery (RHA). The arterial excision with tumor free margins followed by the reconstruction remains the mainstay treatment for a better outcome and long-term survival. Various grafts including saphenous vein, splenic artery, right gastroepiploic artery or gastroduodenal artery have been practiced for RHA reconstruction. In our case, the RHA invasion by CBD cancer was detected intra-operatively. We performed the RHA reconstruction using the autologous first jejunal artery (JA) graft. The use of first JA graft during RHA reconstruction seems

Abstract

Common bile duct cancer invading right hepatic artery is sometimes diagnosed intraoperatively. Excision and

to be technically feasible leading to an acceptable outcome.

Aryal B, Komokata T, Kadono J, Motodaka H, Ueno T, Furoi A, Imoto Y. First jejunal artery, an alternative graft for right hepatic artery reconstruction. *World J Hepatol* 2015; 7(4): 721-724 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i4/721.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i4.721>

INTRODUCTION

Common bile duct (CBD) cancers invading hepatic artery are sometimes diagnosed intraoperatively. Resection of the artery should be considered with efforts to perform a safe reconstruction procedure. A good reconstruction procedure is expected to preserve the blood flow and ensure long-term patency. Arterial reconstruction with an autologous saphenous vein graft (SVG) has been the method of choice for decades. Hepatic artery reconstruction has also been carried out with gastroduodenal artery^[1], right gastroepiploic artery (RGEPA)^[2,3] or splenic artery^[4].

Here, we report a case of advanced extrahepatic bile duct cancer invading right hepatic artery (RHA). Subtotal stomach preserving pancreaticoduodenectomy (SSPPD) accompanied by resection of RHA was performed. Arterial reconstruction was performed with the autologous first jejunal artery (JA) graft. Postoperative course was satisfactory with no complications. The first JA can be considered as an alternative graft for RHA reconstruction.

CASE REPORT

A 63-year-old male patient presented to a private hospital with a history of progressive jaundice, pruritus, and loss of appetite. He underwent computed tomography (CT) scan where bile duct stenosis from superior (Bs) to inferior (Bi) duct was evident. The patient was referred to our department for further evaluation and management.

We carried out diagnostic imagings; endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance cholangiopancreatography (MRCP), intraductal ultrasound (IDUS). MRCP displayed bile duct cancer from Bs to middle duct. ERCP showed an irregular stricture extending from Bs to Bi (Figure 1A). IDUS revealed pancreatic invasion of the tumor. However, there was no evidence of tumor invasion to the portal vein and RHA (Figure 1B) nor the CT showed any signs of RHA invasion (Figure 2). Preoperative diagnosis of CBD cancer with positive pancreatic invasion was made and SSPPD was proposed.

Following opening of the peritoneal cavity, ascites, liver metastasis and peritoneal dissemination were ruled out. We could palpate the hard tumor in the CBD. While scrutinizing around the hepatoduodenal

ligament, we perceived that the bile duct cancer had directly invaded RHA. No invasion or metastasis to duodenum, portal vein, gall bladder, common hepatic artery or other surrounding structures were observed. After dividing RHA and CBD, we resected RHA, 2.8 cm in length. Frozen section revealed cancer cells in adventitia of RHA extending to 15 mm in depth with grossly negative stump's margins.

The back flow from the cut end of the RHA was noted to be poor, so the collateral circulation between RHA and left hepatic artery in the hilar plate was considered to be inadequate. Preoperatively we were not prepared for RHA resection; hence, saphenous vein had not been arranged for reconstruction. We selected the first JA as an alternate graft. A graft of 2 cm from first JA was harvested after transection of jejunum. We interposed the JA in an end-to-end fashion with 7-0 prolene using interrupted suturing technique (Figure 3). Doppler blood flow study displayed a good flow in the graft at a rate of 120 mL/min. SSPPD was completed with pancreatogastrotomy and hepatico-jejunostomy. Total intraoperative blood loss was 2420 mL and time of reconstruction of RHA with first JA was about an hour.

Anticoagulant therapy with heparin was started from fourth postoperative day for a week, and aspirin was continued until 6 mo after operation. Histological examination showed moderately differentiated tubular adenocarcinoma with pancreatic invasion; stage III-B (T3N1M0) (Union for International Cancer Control/UICC classification). No postoperative complications were noted. Following operation, the patient was put on adjuvant chemotherapy with Gemcitabine. After 2 years of surgery, the JA graft was noted to be patent on CT examination with no signs of stenosis. Unfortunately, the patient had evidence of recurrence with multiple para aortic lymph node metastases and died 3 years after the surgery.

DISCUSSION

The pancreatic or biliary cancers potentially invade hepatic artery either by direct extension or by lymphatic metastasis, and the invasion is sometimes detected intraoperatively. Additionally, during radical resection of the pancreatic or biliary tumors the hepatic artery may require excision to achieve tumor-free margins. Various methods for reconstruction of the hepatic artery have already been described: interposition of venous [greater saphenous vein (GSV)] and prosthetic grafts; transposition of the native arteries to the distal stump of the hepatic artery, *i.e.*, splenic artery, right gastroepiploic artery, and even primary repair to avoid prosthetics with combined major visceral resections^[5-9]. The suitable technique should be adapted in regard to the specific situation posed by the resection.

In our case, preoperative imagings including IDUS didn't clue the hepatic artery invasion, and by the

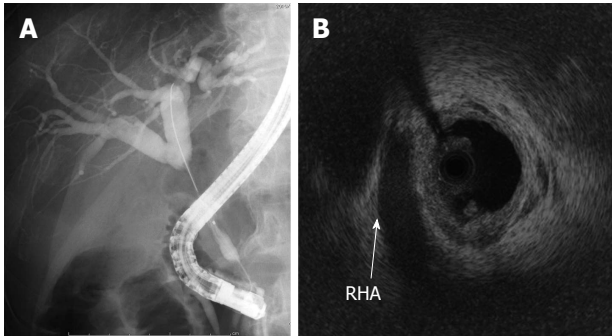


Figure 1 Imaging findings suggestive of bile duct cancer. A: Endoscopic retrograde cholangiopancreatography shows an irregular stenosis from superior to inferior bile duct; B: Intraductal ultrasound showing no cancer invasion to the right hepatic artery (arrow). RHA: Right hepatic artery.



Figure 2 Computed tomography showing right hepatic artery with no signs of cancer invasion. RHA: Right hepatic artery.

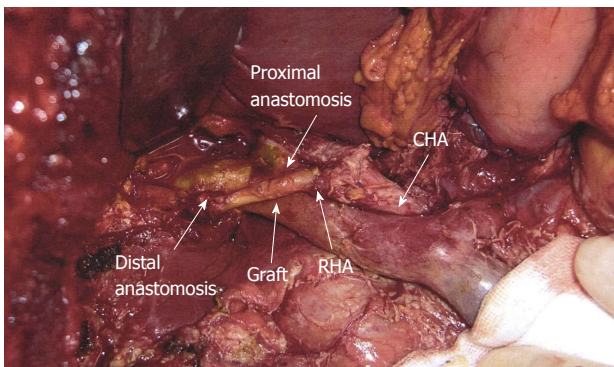


Figure 3 First jejunal artery graft interposed to the right hepatic artery. RHA: Right hepatic artery; CHA: Common hepatic artery.

time RHA invasion was detected, RGEPA had already been resected. We decided to use the first JA for RHA reconstruction. Our concern regarding the anastomosis was a relatively small caliber of the JA. However, no broad caliber discrepancy between the graft and recipient artery made it acceptable. To our knowledge, this is the first case in the literature where JA was used as a free graft in reconstruction of hepatic artery.

The histological disparities between the wall of artery and vein with the long-term patency have been frequently discussed in context of coronary artery bypass

grafting (CABG). Radial artery (RA) was introduced in 1971^[10], and the use was soon discouraged for higher occlusion rates as compared to SVG. The propensity of the RA to go into spasm along with severe intimal hyperplasia contributed to poor results obtained with RA grafting^[11]. At the same time the use of SVG is limited with its poor patency following intimal hyperplasia and accelerated atherosclerosis. There are growing evidences and it is now clear that SVG deteriorates with time and the occlusion rate reaches up to 50% in 10 years after CABG, mainly due to atherosclerosis in the graft called “vein graft disease”^[12]. Subsequently internal mammary artery^[13], internal thoracic artery^[14] and RGEPA^[15] grafts emerged as better choices for their markedly superior long-term patency.

Specific to JA, one review has demonstrated JA of Roux-en-Y limb being used in hepatic artery reconstruction during living related liver transplantation as an in-situ graft. The report together presents successful HA reconstruction with the JA of the Roux-en-Y limb (in pediatric living donor liver transplantation)^[16]. Likewise, use of JA graft with anastomosis to neck vessels like carotid artery during pharyngo-jejunostomy has been practiced^[17].

This is the first report to describe the utility of isolated JA graft in arterial reconstruction for malignant invasion. The postoperative course was uneventful, and the graft had been patent for more than 2 years.

When the use of SVG is restricted in conditions like varicose vein, vasculitis, or it has already been used for bypass conduit, as an interposition graft, or patch graft, JA can be considered as a preferable graft of choice for the RHA reconstruction. JA can also be preferred in the conditions precluding the use of other candidate vessel RGEPA, such as prior resection for gastrectomy or lymph node metastasis around RGEPA. JA graft is readily accessible, is of adequate length to access the hepatic artery, and has an adequate diameter to facilitate the anastomoses. In cases like ours when the RHA invasion is detected intraoperatively, the JA graft can ensure a fast, easy, and safe reconstruction procedure with an extendable patency. Meantime, the caliber discrepancy between the RHA and the JA should always be considered during the reconstruction.

Although SVG has been the preferred graft, this report concludes that the first JA may provide a local autologous graft for RHA reconstruction when an alternative is required.

COMMENTS

Case characteristics

A 63-year-old male with a history of progressive jaundice presented with pruritus and loss of appetite.

Differential diagnosis

Pancreatic carcinoma, gallbladder cancer.

Laboratory diagnosis

White blood count: 5300/uL; Hemoglobin: 14.20 gm/dL; Total bilirubin: 1.2 mg/dL; Direct bilirubin: 0.6 mg/dL; Gamma-glutamyl transferase: 168 IU/L; Aspartate transaminase: 18 IU/L; Alanine transaminase: 21 IU/L; Albumin: 4.1 g/dL;

Carcinoembryonic antigen: 2.1 ng/mL; CA19-9: 20 IU/mL.

Imaging diagnosis

Magnetic resonance cholangiopancreatography showed bile duct cancer from superior (Bs) to middle duct. Endoscopic retrograde cholangiopancreatography showed an irregular stricture extending from Bs to inferior. Intraductal ultrasound revealed pancreatic invasion of the tumor.

Pathological diagnosis

Histological examination showed moderately differentiated tubular adenocarcinoma with pancreatic invasion.

Treatment

Subtotal stomach preserving pancreaticoduodenectomy accompanied by resection of right hepatic artery.

Related reports

Jejunal artery (JA) of Roux-en-y limb has been previously used for hepatic artery reconstruction.

Experiences and lessons

This case report presents the applicability of first JA in right hepatic artery reconstruction. The hepatic artery reconstruction with graft from first JA should be a feasible and promising procedure.

Peer-review

This article demonstrates the use of first JA graft in right hepatic artery reconstruction.

REFERENCES

- Sarmiento JM**, Panneton JM, Nagorney DM. Reconstruction of the hepatic artery using the gastroduodenal artery. *Am J Surg* 2003; **185**: 386-387 [PMID: 12657395 DOI: 10.1016/S0002-9610(02)01416-2]
- Kusano T**, Furukawa M, Nakata T, Kusaba E, Yamauchi H, Tsukasa T, Tsuchiya R. [Hepatic artery reconstruction grafting with the right gastroepiploic artery for surgical treatment of upper bile duct cancer]. *Nihon Geka Gakkai Zasshi* 1990; **91**: 1749-1751 [PMID: 2277623]
- Ikegami T**, Kawasaki S, Hashikura Y, Miwa S, Kubota T, Mita A, Iijima S, Terada M, Miyagawa S, Furuta S. An alternative method of arterial reconstruction after hepatic arterial thrombosis following living-related liver transplantation. *Transplantation* 2000; **69**: 1953-1955 [PMID: 10830238 DOI: 10.1097/00007890-200005150-00036]
- Figueras J**, Parés D, Aranda H, Rafecas A, Fabregat J, Torras J, Ramos E, Lama C, Lladó L, Jaurrieta E. Results of using the recipient's splenic artery for arterial reconstruction in liver transplantation in 23 patients. *Transplantation* 1997; **64**: 655-658 [PMID: 9293883 DOI: 10.1097/00007890-199708270-00020]
- Hamazaki K**, Mimura H, Kobayashi T, Kim H, Sakagami K, Orita K. Hepatic artery reconstruction after resection of the hepatoduodenal ligament. *Br J Surg* 1991; **78**: 1366-1367 [PMID: 1662104 DOI: 10.1002/bjs.1800781131]
- Garcia-Valdecasas JC**, Grande L, Rimola A, Fuster J, Lacy A, Visa J. The use of the saphenous vein for arterial reconstruction in orthotopic liver transplant. *Transplant Proc* 1990; **22**: 2376-2377 [PMID: 2219406]
- Takenaka H**, Iwase K, Ohshima S, Hiranaka T. A new technique for the resection of gastric cancer: modified Appleby procedure with reconstruction of hepatic artery. *World J Surg* 1992; **16**: 947-951 [PMID: 1462635 DOI: 10.1007/BF02066997]
- Itabashi Y**, Hakamada K, Narumi S, Toyoki Y, Totsuka E, Umehara Y, Aoki K, Sasaki M. A case of living-related partial liver transplantation using the right gastroepiploic artery for hepatic artery reconstruction. *Hepatogastroenterology* 2000; **47**: 512-513 [PMID: 10791224]
- Ahn CS**, Hwang S, Moon DB, Song GW, Ha TY, Park GC, Namgoong JM, Yoon SY, Jung SW, Jung DH, Kim KH, Park YH, Park HW, Lee HJ, Park CS, Lee SG. Right gastroepiploic artery is the first alternative inflow source for hepatic arterial reconstruction in living donor liver transplantation. *Transplant Proc* 2012; **44**: 451-453 [PMID: 22410041 DOI: 10.1016/j.transproceed.2012.01.057]
- Carpentier A**, Guernonprez JL, Deloche A, Frechette C, DuBost C. The aorta-to-coronary radial artery bypass graft. A technique avoiding pathological changes in grafts. *Ann Thorac Surg* 1973; **16**: 111-121 [PMID: 4582222 DOI: 10.1016/S0003-4975(10)65825-0]
- Curtis JJ**, Stoney WS, Alford WC, Burrus GR, Thomas CS. Intimal hyperplasia. A cause of radial artery aortocoronary bypass graft failure. *Ann Thorac Surg* 1975; **20**: 628-635 [PMID: 1082316 DOI: 10.1016/S0003-4975]
- Taggart DP**. Current status of arterial grafts for coronary artery bypass grafting. *Ann Cardiothorac Surg* 2013; **2**: 427-430 [PMID: 23977618 DOI: 10.3978/j.issn.2225-319X.2013.07.21]
- Endo M**, Nishida H, Tomizawa Y, Kasanuki H. Benefit of bilateral over single internal mammary artery grafts for multiple coronary artery bypass grafting. *Circulation* 2001; **104**: 2164-2170 [PMID: 11684625 DOI: 10.1161/hc4301.098283]
- Raja SG**, Dreyfus GD. Internal thoracic artery: to skeletonize or not to skeletonize? *Ann Thorac Surg* 2005; **79**: 1805-1811 [PMID: 15854993 DOI: 10.1016/j.athoracsur.2004.05.053]
- Suma H**. Gastroepiploic artery graft in coronary artery bypass grafting. *Ann Cardiothorac Surg* 2013; **2**: 493-498 [PMID: 23977628 DOI: 10.3978/j.issn.2225-319X.2013.06.04]
- Wakiya T**, Sanada Y, Mizuta K, Umehara M, Urahashi T, Egami S, Hishikawa S, Nakata M, Hakamada K, Yasuda Y, Kawarasaki H. Hepatic artery reconstruction with the jejunal artery of the Roux-en-Y limb in pediatric living donor liver re-transplantation. *Pediatr Transplant* 2012; **16**: E86-E89 [PMID: 21496191 DOI: 10.1111/j.1399-3046.2010.01442.x]
- Lee HS**, Park SY, Jang HJ, Kim MS, Lee JM, Zo JI. Free jejunal graft for esophageal reconstruction using end-to-side vascular anastomosis and extended pharyngo-jejunosomy. *Ann Thorac Surg* 2012; **93**: 1850-1854 [PMID: 22440367 DOI: 10.1016/j.athoracsur.2012.01.068]

P-Reviewer: Solinas A, Zhang Q **S-Editor:** Tian YL
L-Editor: A **E-Editor:** Liu SQ





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>

