ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2015 June 28; 7(12): 1595-1724

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

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

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 Repoublic (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).

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 Waye, *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 Repoublic

Kamil Vyslouzil, *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 Siahanidou, *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, *Florencee*
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, *Kashihara*

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, *Wonu*
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*

World Journal of Hepatology

Contents

Three issues per month Volume 7 Number 12 June 28, 2015

World Journal of Hepatology
Volume 7 Number 12 June 28, 2015

Contents

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)

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

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

PUBLICATION DATE
June 28, 2015

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm

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

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1595

World J Hepatol 2015 June 28; 7(12): 1595-1600
ISSN 1948-5182 (online)

EDITORIAL

Liver ultrasound elastography: More than staging the disease

George S Gherlan

George S Gherlan, "Dr. Victor Babes" Center for Diagnostics and Treatment, 030303 Bucharest, Romania

Author contributions: Gherlan GS solely contributed to this manuscript.

Conflict-of-interest: None to declare.

Correspondence to: George S Gherlan, MD, PhD, "Dr. Victor Babes" Center for Diagnostics and Treatment, 281 Mihai Bravu Street, 030303 Bucharest, Romania. gherlanus@gmail.com
Telephone: +40-21-3179503
Fax: +40-21-3179502

Received: January 7, 2015
Peer-review started: January 8, 2015
First decision: February 7, 2015
Revised: February 22, 2015
Accepted: April 16, 2015
Article in press: May 6, 2015
Published online: June 28, 2015

Abstract

Ultrasound elastography is perhaps the most important breakthrough in the evolution of ultrasonography in the last 15 years. Since transient elastography was introduced, many other methods have been developed and became more and more widely available. The value of ultrasound elastography in staging a chronic liver disease has been established by numerous studies. There have been many studies that have shown that using liver elastography it is possible to predict the presence of the complications of cirrhosis: portal hypertension, presence of esophageal varices (and even their risk of bleeding) and hepatocellular carcinoma. It has been shown that liver elastography can predict the progression of liver fibrosis and also the survival (hepatic events - free) of the patients with chronic liver diseases. These are the real quests of the clinicians, this is the ultimate scope of any medical investigation - to predict the outcome of a patient and to help making therapeutic decisions. I brought together only a small amount of the data that has already been written on this subject to support my affirmation that liver ultrasound elastography is more than a tool for staging the liver disease, but it is also comparable to a crystal ball which in the hands of a skilled clinician can reveal the future of the patient and can help to improve this future.

Key words: Liver ultrasound elastography; Transient elastography; Fibrosis; Hepatitis; Survival; Cirrhosis

Core tip: In this editorial I brought together data from the literature in the support of the affirmation that liver ultrasound elastography is more than a tool for staging the disease, that it can also be used to predict the presence of the complications of cirrhosis: portal hypertension, presence of esophageal varices (and even their risk of bleeding), ascites and hepatocellular carcinoma. Studies shown that liver elastography can predict the progression of liver fibrosis and also the survival (hepatic events - free) of the patients with chronic liver diseases, being therefore a helpful tool in the hands of a skilled clinician.

Gherlan GS. Liver ultrasound elastography: More than staging the disease. *World J Hepatol* 2015; 7(12): 1595-1600 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1595.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1595

INTRODUCTION

Ultrasound elastography is perhaps the most important breakthrough in the evolution of ultrasonography in the last 15 years. Liver elastography in particular has seen an unprecedented development in the last 10 years since transient elastography (TE) was introduced in 2003 as a tool to assess the liver fibrosis. Since then, many approaches have been tried with the same purpose: to evaluate the stiffness of the liver tissue and thus to appreciate the extension of the liver damage, to correctly identify the stage of the fibrosis.

The main idea behind elastography is that the elasticity of the analyzed tissue can offer information on the health of that particular organ. A stiffer liver tissue usually indicates the presence of the consequence of any chronic liver disease: the fibrosis. There can be some interferences (inflammation, steatosis, meal consumption prior the examination)[1-5], but the increase of the stiffness of the liver is mostly due to fibrosis.

Liver ultrasound elastography techniques are based on the principle that the speed of a wave that propagates through the liver is influenced by the stiffness of the tissue. Basically, the stiffer the liver, the faster the wave passes through.

TE (Fibroscan/Echosens) uses a mechanical wave generated by a special transducer, while acoustic radiation force impulse imaging (ARFI, Siemens) and shear wave elastography (SWE, Supersonic Imaging) use sound waves. Other ultrasound elastography techniques have been also developed, but TE and ARFI are the subjects of most researches, these two techniques being the oldest in use.

The value of ultrasound elastography in staging a chronic liver disease has been established by numerous studies[6-14]. But staging the disease is just one step towards what the clinician actually wants to achieve: to glimpse into the future of the patient, to see how the disease is going to evolve, what complications and when are they going to occur.

Liver elastography has some limitations - TE cannot be performed or the results may be influenced in the presence of the obesity, ascites, narrow intercostal spaces. ARFI overcomes most of these limitations, the rate of unsuccessful or unreliable measurements being significantly lower than with TE.

The predictive value of the liver ultrasound elastography is the subject of this editorial. If not otherwise mentioned, the following information refers to TE.

USE OF LIVER ULTRASOUND ELASTOGRAPHY FOR THE PREDICTION OF THE PATIENT'S PROGNOSIS

There have been many studies that have shown that using liver elastography it is possible to predict the presence of the complications of cirrhosis: portal hypertension (PH), presence of esophageal varices (EV) and their risk of rupture, ascites and hepatocellular carcinoma (HCC). It has been shown that liver elastography can predict the progression of liver fibrosis and can also predict the survival of patients with chronic liver diseases.

LIVER ELASTOGRAPHY IN THE PREDICTION OF PH

PH is traditionally evaluated by measuring (invasively) the hepatic vein portal gradient (HVPG) and is defined as the HVPG of over 5 mmHg. PH becomes clinically significant when HVPG value is over 10 mmHg as it is more often associated with the presence of the varices[15,16]. A value of over 12 mmHg predicts a high risk of variceal bleeding[15,16]. HVPG measurement is recommended to all patients newly diagnosed with cirrhosis for the evaluation of risk and establishment of prognosis[17] and is also a good tool to monitor the response to treatment and achievement of endpoints (over 20% HVPG decrease as compared to baseline and/or HVPG < 12 mmHg)[17].

Both liver stiffness and spleen stiffness have shown to be predictors for detecting PH[18-21]. Liver stiffness measurement (LSM) has a good correlation with HVPG $r = 0.81$, $P < 0.0001$ when using TE[18], and $r = 0.611$, $P < 0.0001$ with SWE[20]. One study that compared LS with spleen stiffness (SS) assessed both by SWE found that the diagnostic performance of LSM was significantly better than that of SS for the diagnosis of clinically significant PH (area under the receiver operating characteristic curve of 0.87 *vs* 0.64, $P = 0.003$).

LSM has also a good correlation with the stage of cirrhosis, increasing along with HVPG as the Child stage increases[22]. A meta analysis made on 18 studies which included 3644 patients found an overall specificity of 90% (95%CI: 0.81-0.95) and a sensitivity of 79% (95%CI: 0.58-0.91) for LSM by TE in the detection of significant PH[23]. The study of Zhang in 2014 showed that a value of over 13.6 kPa at TE predicts significant PH with a specificity of 72.53% and a sensitivity of 83.87%[21]. Another study comparing TE with ARFI found that both are well correlated with PH: $r = 0.765$; $P < 0.001$ for TE and $r = 0.646$; $P < 0.001$ for ARFI[24]. At the optimal cut-off (2.58 m/s), the sensitivity and specificity for ARFI (AUROC: 0.855) were 71.4% and 87.5%, respectively[24]. In the study by Carrión *et al*[25], there was a close correlation of TE with HVPG ($r = 0.84$, $P < 0.001$). The optimal liver stiffness cutoff value for diagnosis of PH (HVPG 6 mmHg) was 8.74 kPa, with a sensitivity of 90%, specificity 81%, positive predictive value 81%, and negative predictive value of 90%[25].

Predicting clinically significant PH is one step towards the prediction of the presence of EV and their risk of bleeding.

LIVER ELASTOGRAPHY IN THE PREDICTION OF THE PRESENCE OF EV AND THEIR RISK OF RUPTURE

Liver stiffness measured by TE showed good results in detecting the presence of EV, with AUROC's ranging between 0.76 and 0.88[18,26-28]. The cut-offs mentioned by the above studies were 17.6, 21.5, 19 kPa and respectively 19.2 kPa and for these cut-offs the sensitivities were 0.9, 0.76, 0.84 and 0.85 while the specificities were 0.43, 0.78, 0.7 and 0.87.

Studies have also shown a correlation between LSM and the size of the EV[27,29,30]. Thus, LSM may be of help in the selection of patients for endoscopic screening for EV and their complications.

Liver stiffness may also predict the risk of variceal bleeding by predicting large grade EV (Paquet grade higher or equal to 2), AUROC = 0.85 (95%CI: 0.75-0.94)[28]. Another study found an AUROC of 0.58 (95%CI: 0.48-0.67) for ARFI and 0.53 (95%CI: 0.44-0.63) for TE for predicting variceal bleeding[31] sowing that the two analyzed methods have similar value for this purpose. Elastography may be helpful to screen and identify patients who are at high risk of having large grade EV, which predict variceal bleeding and, therefore, need endoscopic screening.

Liver elastography can also be used in combination with other markers (such as spleen diameter and platelet count) to identify more precisely the patients with higher risk for EV bleeding[32].

LIVER ELASTOGRAPHY IN THE PREDICTION OF THE PRESENCE OF HCC

Prognosis of patients with chronic liver disease is determined by the extent and progression of liver fibrosis, which may lead to the development of HCC.

Liver stiffness is significantly higher in patients with HCC than in patients without HCC[33-35]. However, most of the studies found that liver stiffness alone is insufficient to predict the presence or absence of HCC and that it should be associated in a score with other markers. A score developed by Wong *et al*[33] based on liver stiffness, age, serum albumin and hepatitis B virus DNA level was found to have AUROC's of 0.83 to 0.89 in the identification of the HCC patients and a very good negative (99.4%-100%) for the exclusion of HCC in patients. In the study conducted by Feier *et al*[34], LS was significantly higher (42 kPa *vs* 27 kPa, $P < 0.0001$) in the HCC group than in the non-HCC group, but other 3 parameters (alanine-aminotransferase, alpha-fetoprotein and interquartile range of the LSMs) were added to elastography in a score and the resulted model combining the four variables showed a good diagnostic performance in both training and validation groups, with AUROCs of 0.86 and 0.8, respectively[34].

Jung *et al*[36] has shown that liver stiffness is also useful as a part of a predictive model that identifies patients that are at risk for late recurrence after curative resection of HCC. On multivariate analysis, patients with older age, male sex, heavy alcohol consumption (> 80 g/d), lower serum albumin, HBe antigen positivity and LSM > 8 kPa were at a significantly greater risk of HCC development.

LIVER ELASTOGRAPHY IN THE PREDICTION OF THE SURVIVAL OF THE PATIENTS WITH CHRONIC LIVER DISEASES

Liver stiffness, expressing the severity of the liver damage, is correlated with hepatic events and death. It has been shown by many studies that measuring liver elasticity one can predict the survival of a patient[37-40].

In the study conducted by Wong *et al*[37], they found age, Hui index and liver stiffness to be independent predictors of hepatic event - free survival. The same study showed that the worsening of the liver stiffness and Hui index at a follow up visit compared to baseline predicted a hepatic event.

Pang *et al*[38] found that liver stiffness by TE was an independent predictor of complications (hazard ratio 1.05 per kPa; 95%CI: 1.03-1.06), with the 2-year incidence rates of death or hepatic complications of 2.6%, 9%, 19%, and 34% in patients with liver stiffness < 10 kPa, 10-19.9 kPa, 20-39.9 kPa, and ⩾ 40 kPa, respectively ($P < 0.00005$).

de Lédinghen *et al*[39] showed that survival in patients with chronic B hepatitis was significantly decreased in patients diagnosed with severe fibrosis, no matter if liver elastography was used ($P < 0.0001$) or liver biopsy ($P = 0.02$) for the staging of fibrosis.

The study conducted by Vergniol *et al*[40] also showed that in patients with chronic C hepatitis, noninvasive tests for liver fibrosis (measurement of liver stiffness or FibroTest) can predict 5-year survival.

The fact that liver stiffness can predict survival may help clinicians in their decision-making process for establishing therapeutic options for the patient and even liver transplantation indication.

CONCLUSION

In the past 10 years, liver ultrasound elastography struggled and succeeded to partially replace liver biopsy for the purpose of staging the liver diseases regardless of their etiology. However, as the method became more widely available and because the actual quest of the clinician is to evaluate as completely as possible the extent of the liver damage, its complications and if possible, even to predict an outcome, LSM was studied recently for these purposes also.

It is now known that cirrhosis has a complex and dynamic pathologic spectrum. The average risk of progressing from compensated to decompensated cirrhosis is 6%-9% per year[41]. Survival in the com-

Table 1 Use of ultrasound elastography to predict liver disease related complications

Ref.	Method	Cut-off value	Sensitivity	Specificity	AUROC
Elastography to predict significant portal hypertension					
Zhang *et al*[21]	TE	13.6 kPa	83.87%	72.53%	0.83
Salzl *et al*[24]	TE	16.8 kPa	89.75%	75%	0.87
Salzl *et al*[24]	ARFI	2.58 m/s	71.4%	87.5%	0.85
Carrión *et al*[25]	TE	8.74 kPa	90%	81%	0.94
Elastography to predict the presence of esophageal varices					
Vizzutti *et al*[18]	TE	17.6 kPa	90%	43%	0.76
Castéra *et al*[26]	TE	21.5 kPa	76%	78%	0.86
Kazemi *et al*[27]	TE	19 kPa	84%	70%	0.84
Pár *et al*[28]	TE	19.2 kPa	85%	87%	0.88
Elastography to predict hepatocellular carcinoma					
Feier *et al*[34]	TE	38 kPa	51.7%	90.4%	0.68
Wong *et al*[33]	TE (part of a score)	-	-	-	0.83-0.89
Elastography to predict the liver related death of the patients with liver cirrhosis					
				Rate of death	
Pang *et al*[38]	TE	40 kPa		34%/3 yr	
de Lédinghen *et al*[39]	TE	< 9 kPa		2.9%/5 yr	
		> 20 kPa		38.5%/5 yr	

TE: Transient elastography (fibroscan); ARFI: Acoustic radiation force impulse imaging.

pensated state is of an average of 12 years, while in the decompensated state the median survival is of only 2 years[41]. Thus identifying patients in early stages of a liver disease (even in the compensated state of cirrhosis) is crucial for the outcome of the patient. Besides, identifying patients with complications and establishing their survival prognostic is of more help in the treatment decision and in the monitoring plan for the future.

We now have noninvasive means to precisely stage the fibrosis. Particularly, as shown above, liver ultrasound elastography (with many methods developed by now for the same purpose - TE, ARFI or SWE) is also a useful tool for identifying patients with a higher risk of having complications like PH, EV and even HCC. With the use of elastography the clinician can also appreciate the risk of the patient of having an unfavorable course and develop complications like EV rupture, decompensation of the cirrhosis and even death (Table 1).

Therefore, used rationally, liver ultrasound is more than a tool for staging the disease, is a kind of crystal ball that in the hand of a skilled clinician can reveal the future of the patient and contribute to the improvement of this future.

REFERENCES

1 **Chan HL**, Wong GL, Choi PC, Chan AW, Chim AM, Yiu KK, Chan FK, Sung JJ, Wong VW. Alanine aminotransferase-based algorithms of liver stiffness measurement by transient elastography (Fibroscan) for liver fibrosis in chronic hepatitis B. *J Viral Hepat* 2009; **16**: 36-44 [PMID: 18673426 DOI: 10.1111/j.1365-2893.2008.01037.x]

2 **Roulot D**, Czernichow S, Le Clésiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. *J Hepatol* 2008; **48**: 606-613 [PMID: 18222014 DOI: 10.1016/j.jhep.2007.11.020]

3 **Corpechot C**, El Naggar A, Poujol-Robert A, Ziol M, Wendum D, Chazouillères O, de Lédinghen V, Dhumeaux D, Marcellin P, Beaugrand M, Poupon R. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; **43**: 1118-1124 [PMID: 16628644 DOI: 10.1002/hep.21306]

4 **Arena U**, Lupsor Platon M, Stasi C, Moscarella S, Assarat A, Bedogni G, Piazzolla V, Badea R, Laffi G, Marra F, Mangia A, Pinzani M. Liver stiffness is influenced by a standardized meal in patients with chronic hepatitis C virus at different stages of fibrotic evolution. *Hepatology* 2013; **58**: 65-72 [PMID: 23447459 DOI: 10.1002/hep.26343]

5 **Berzigotti A**, De Gottardi A, Vukotic R, Siramolpiwat S, Abraldes JG, García-Pagan JC, Bosch J. Effect of meal ingestion on liver stiffness in patients with cirrhosis and portal hypertension. *PLoS One* 2013; **8**: e58742 [PMID: 23520531 DOI: 10.1371/journal.pone.0058742]

6 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713 [PMID: 14698338 DOI: 10.1016/j.ultrasmedbio.2003.07.001]

7 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546 DOI: 10.1053/j.gastro.2004.11.018]

8 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]

9 **Tsochatzis EA**, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; **54**: 650-659 [PMID: 21146892 DOI: 10.1016/j.jhep.2010.07.033]

10 **Cardoso AC**, Carvalho-Filho RJ, Stern C, Dipumpo A, Giuily N, Ripault MP, Asselah T, Boyer N, Lada O, Castelnau C, Martinot-Peignoux M, Valla DC, Bedossa P, Marcellin P. Direct comparison of diagnostic performance of transient elastography in patients with chronic hepatitis B and chronic hepatitis C. *Liver Int* 2012; **32**: 612-621 [PMID: 22103765 DOI: 10.1111/j.1478-3231.2011.02660.x]

11 **Sporea I**, Sirli R, Deleanu A, Tudora A, Popescu A, Curescu M, Bota S. Liver stiffness measurements in patients with HBV vs HCV chronic hepatitis: a comparative study. *World J Gastroenterol* 2010; **16**: 4832-4837 [PMID: 20939112 DOI: 10.3748/wjg.v16.i38.4832]

12 **Chon YE**, Choi EH, Song KJ, Park JY, Kim do Y, Han KH, Chon

CY, Ahn SH, Kim SU. Performance of transient elastography for the staging of liver fibrosis in patients with chronic hepatitis B: a meta-analysis. *PLoS One* 2012; **7**: e44930 [PMID: 23049764 DOI: 10.1371/journal.pone.0044930]

13 **Mahadeva S**, Mahfudz AS, Vijayanathan A, Goh KL, Kulenthran A, Cheah PL. Performance of transient elastography (TE) and factors associated with discordance in non-alcoholic fatty liver disease. *J Dig Dis* 2013; **14**: 604-610 [PMID: 23859493 DOI: 10.1111/1751-2980.12088]

14 **Kumar R**, Rastogi A, Sharma MK, Bhatia V, Tyagi P, Sharma P, Garg H, Chandan Kumar KN, Bihari C, Sarin SK. Liver stiffness measurements in patients with different stages of nonalcoholic fatty liver disease: diagnostic performance and clinicopathological correlation. *Dig Dis Sci* 2013; **58**: 265-274 [PMID: 22790906 DOI: 10.1007/s10620-012-2306-1]

15 **Bosch J**, Garcia-Pagán JC, Berzigotti A, Abraldes JG. Measurement of portal pressure and its role in the management of chronic liver disease. *Semin Liver Dis* 2006; **26**: 348-362 [PMID: 17051449]

16 **Groszmann RJ**, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, Garcia-Pagan 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]

17 **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]

18 **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]

19 **Elkrief L**, Rautou PE, Ronot M, Lambert S, Dioguardi Burgio M, Francoz C, Plessier A, Durand F, Valla D, Lebrec D, Vilgrain V, Castéra L. Prospective comparison of spleen and liver stiffness by using shear-wave and transient elastography for detection of portal hypertension in cirrhosis. *Radiology* 2015; **275**: 589-598 [PMID: 25469784]

20 **Procopet B**, Berzigotti A, Abraldes JG, Turon F, Hernandez-Gea V, García-Pagán JC, Bosch J. Real-time shear-wave elastography: Applicability, reliability and accuracy for clinically significant portal hypertension. *J Hepatol* 2015; **62**: 1068-1075 [PMID: 25514554 DOI: 10.1016/j.jhep.2014.12.007]

21 **Zhang W**, Wang L, Wang L, Li G, Huang A, Yin P, Yang Z, Ling C, Wang L. Liver stiffness measurement, better than APRI, Fibroindex, Fib-4, and NBI gastroscopy, predicts portal hypertension in patients with cirrhosis. *Cell Biochem Biophys* 2015; **71**: 865-873 [PMID: 25417057 DOI: 10.1007/s12013-014-0275-z]

22 **Reiberger T**, Ferlitsch A, Payer BA, Pinter M, Homoncik M, Peck-Radosavljevic M. Non-selective β-blockers improve the correlation of liver stiffness and portal pressure in advanced cirrhosis. *J Gastroenterol* 2012; **47**: 561-568 [PMID: 22170417 DOI: 10.1007/s00535-011-0517-4]

23 **Shi KQ**, Fan YC, Pan ZZ, Lin XF, Liu WY, Chen YP, Zheng MH. Transient elastography: a meta-analysis of diagnostic accuracy in evaluation of portal hypertension in chronic liver disease. *Liver Int* 2013; **33**: 62-71 [PMID: 22973991 DOI: 10.1111/liv.12003]

24 **Salzl P**, Reiberger T, Ferlitsch M, Payer BA, Schwengerer B, Trauner M, Peck-Radosavljevic M, Ferlitsch A. Evaluation of portal hypertension and varices by acoustic radiation force impulse imaging of the liver compared to transient elastography and AST to platelet ratio index. *Ultraschall Med* 2014; **35**: 528-533 [PMID: 24871695 DOI: 10.1055/s-0034-1366506]

25 **Carrión JA**, Navasa M, Bosch J, Bruguera M, Gilabert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl* 2006; **12**: 1791-1798 [PMID: 16823833 DOI: 10.1002/lt.20857]

26 **Castéra L**, Le Bail B, Roudot-Thoraval F, Bernard PH, Foucher J, Merrouche W, Couzigou P, de Lédinghen V. Early detection in routine clinical practice of cirrhosis and oesophageal varices in chronic hepatitis C: comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. *J Hepatol* 2009; **50**: 59-68 [PMID: 19013661 DOI: 10.1016/j.jhep.2008.08.018]

27 **Kazemi F**, Kettaneh A, N'kontchou G, Pinto E, Ganne-Carrie N, Trinchet JC, Beaugrand M. Liver stiffness measurement selects patients with cirrhosis at risk of bearing large oesophageal varices. *J Hepatol* 2006; **45**: 230-235 [PMID: 16797100 DOI: 10.1016/j.jhep.2006.04.006]

28 **Pár G**, Trosits A, Pakodi F, Szabó I, Czimmer J, Illés A, Gódi S, Bajor J, Sarlós P, Kenyeres P, Miseta A, Vincze A, Pár A. [Transient elastography as a predictor of oesophageal varices in patients with liver cirrhosis]. *Orv Hetil* 2014; **155**: 270-276 [PMID: 24509356 DOI: 10.1556/OH.2014.29824]

29 **Bureau C**, Metivier S, Peron JM, Selves J, Robic MA, Gourraud PA, Rouquet O, Dupuis E, Alric L, Vinel JP. Transient elastography accurately predicts presence of significant portal hypertension in patients with chronic liver disease. *Aliment Pharmacol Ther* 2008; **27**: 1261-1268 [PMID: 18397389 DOI: 10.1111/j.1365-2036.2008.03701.x]

30 **Li F**, Yan T, Shao Q, Ji D, Li B, Li Z, Chen G. [Clinical study of FibroScan efficiency for diagnosing size of oesophageal varices in liver cirrhosis patients]. *Zhonghua Gan Zang Bing Za Zhi* 2014; **22**: 600-603 [PMID: 25243961 DOI: 10.3760/cma.j.issn.1007-3418.2014.08.010]

31 **Vermehren J**, Polta A, Zimmermann O, Herrmann E, Poynard T, Hofmann WP, Bojunga J, Sarrazin C, Zeuzem S, Friedrich-Rust M. Comparison of acoustic radiation force impulse imaging with transient elastography for the detection of complications in patients with cirrhosis. *Liver Int* 2012; **32**: 852-858 [PMID: 22222050 DOI: 10.1111/j.1478-3231.2011.02736.x]

32 **Kim BK**, Kim do Y, Han KH, Park JY, Kim JK, Paik YH, Lee KS, Chon CY, Ahn SH. Risk assessment of esophageal variceal bleeding in B-viral liver cirrhosis by a liver stiffness measurement-based model. *Am J Gastroenterol* 2011; **106**: 1654-1652, 1730 [PMID: 21691339 DOI: 10.1038/ajg.2011.160]

33 **Wong GL**, Chan HL, Wong CK, Leung C, Chan CY, Ho PP, Chung VC, Chan ZC, Tse YK, Chim AM, Lau TK, Wong VW. Liver stiffness-based optimization of hepatocellular carcinoma risk score in patients with chronic hepatitis B. *J Hepatol* 2014; **60**: 339-345 [PMID: 24128413 DOI: 10.1016/j.jhep.2013.09.029]

34 **Feier D**, Lupsor Platon M, Stefanescu H, Badea R. Transient elastography for the detection of hepatocellular carcinoma in viral C liver cirrhosis. Is there something else than increased liver stiffness? *J Gastrointestin Liver Dis* 2013; **22**: 283-289 [PMID: 24078985]

35 **Wong GL**. Transient elastography: Kill two birds with one stone? *World J Hepatol* 2013; **5**: 264-274 [PMID: 23717737 DOI: 10.4254/wjh.v5.i5.264]

36 **Jung KS**, Kim JH, Kim SU, Song K, Kim BK, Park JY, Kim do Y, Ahn SH, Moon do C, Song IJ, Choi GH, Park YN, Han KH. Liver stiffness value-based risk estimation of late recurrence after curative resection of hepatocellular carcinoma: development and validation of a predictive model. *PLoS One* 2014; **9**: e99167 [PMID: 24910997 DOI: 10.1371/journal.pone.0099167]

37 **Wong GL**, Chan HL, Yu Z, Wong CK, Leung C, Ho PP, Chan CY, Chung VC, Chan ZC, Tse YK, Chim AM, Lau TK, Chan HY, Tse CH, Wong VW. Noninvasive assessments of liver fibrosis with transient elastography and Hui index predict survival in patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2015; **30**: 582-590 [PMID: 25252074 DOI: 10.1111/jgh.12779]

38 **Pang JX**, Zimmer S, Niu S, Crotty P, Tracey J, Pradhan F, Shaheen AA, Coffin CS, Heitman SJ, Kaplan GG, Swain MG, Myers RP. Liver stiffness by transient elastography predicts liver-related complications and mortality in patients with chronic liver disease. *PLoS One* 2014; **9**: e95776 [PMID: 24755824 DOI: 10.1371/journal.pone.0095776]

39 **de Lédinghen V**, Vergniol J, Barthe C, Foucher J, Chermak F, Le Bail B, Merrouche W, Bernard PH. Non-invasive tests for fibrosis and liver stiffness predict 5-year survival of patients chronically infected with hepatitis B virus. *Aliment Pharmacol Ther* 2013; **37**: 979-988 [PMID: 23557139 DOI: 10.1111/apt.12307]

40 **Vergniol J**, Foucher J, Terrebonne E, Bernard PH, le Bail B, Merrouche W, Couzigou P, de Ledinghen V. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. *Gastroenterology* 2011; **140**: 1970-1979, 1979.e1-3 [PMID: 21376047 DOI: 10.1053/j.gastro.2011.02.058]

41 **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]

P- Reviewer: Cao GW, Lisotti A **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1601

World J Hepatol 2015 June 28; 7(12): 1601-1605
ISSN 1948-5182 (online)

EDITORIAL

Treatment of ectopic varices with portal hypertension

Takahiro Sato

Takahiro Sato, Department of Gastroenterology, Sapporo Kosei General Hospital, Sapporo 060-0033, Japan

Author contributions: Sato T solely contributed to this paper.

Conflict-of-interest: The author declared no conflicts of interest.

Correspondence to: Takahiro Sato, MD, Department of Gastroenterology, Sapporo Kosei General Hospital, Kita 3 Higashi 8, Chuo-ku, Sapporo 060-0033, Japan. taka.sato@ja-hokkaidoukouseiren.or.jp
Telephone: +81-11-2615331
Fax: +81-11-2616040

Received: January 16, 2015
Peer-review started: January 18, 2015
First decision: February 7, 2015
Revised: March 20, 2015
Accepted: April 10, 2015
Article in press: April 14, 2015
Published online: June 28, 2015

Abstract

Ectopic varices are unusual with portal hypertension and can involve any site along the digestive tract outside the gastroesophageal region. Hemorrhage from ectopic varices generally are massive and life threatening. Diagnosis of ectopic varices is difficult and subsequent treatment is also difficult; the optimal treatment has not been established. Recently, interventional radiology and endoscopic treatments have been carried out successfully for hemorrhage from ectopic varices.

Key words: Portal hypertension; Endoscopic injection sclerotherapy; Balloon-occluded retrograde transvenous obliteration; Ectopic varices; Endoscopic band ligation; Percutaneous transhepatic obliteration; Transjugular intrahepatic portosystemic shunts

Core tip: Ectopic varices with portal hypertension are considered to be the cause of hemorrhage presenting as lower gastrointestinal bleeding. Recently, interventional radiology and endoscopic procedures have been performed successfully as a treatment option for ectopic varices.

Sato T. Treatment of ectopic varices with portal hypertension. *World J Hepatol* 2015; 7(12): 1601-1605 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1601.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1601

INTRODUCTION

Portal hypertension results from an increase in the resistance of blood flow in the intrahepatic portal vein and can cause the reopening of embryonic channels[1]. Portal venous pressure is critical in the liver function of cirrhosis and esophageal varices are the most common complication of cirrhosis. Ectopic varices are portosystemic collaterals along the digestive tract outside the gastroesophageal region and are unusual[2,3]. Endoscopic procedures such as endoscopic injection sclerotherapy (EIS) and endoscopic band ligation (EBL) have been carried out for treating esophageal varices[4,5]. Balloon-occluded retrograde transvenous obliteration (B-RTO) is an angiographic technique for fundic varices of stomach[6]. But, the optimal procedure has not been defined for hemorrhage from ectopic varices.

Here, we review the options in the treatment of

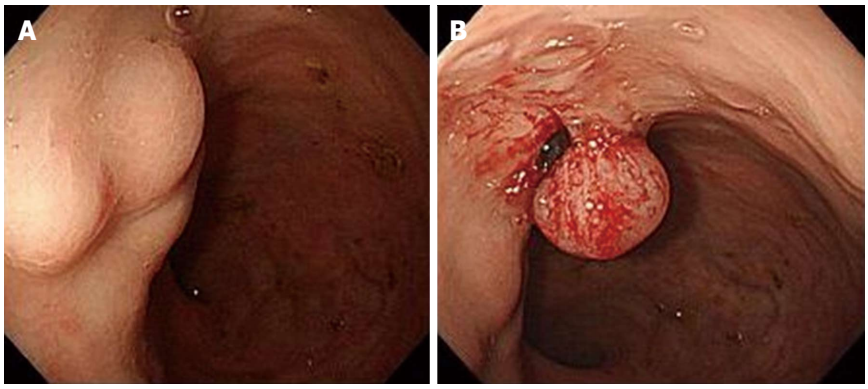

Figure 1 Endoscopy showed enlarged and tortuous, erosion positive rectal varices in a cirrhotic patient with anal bleeding (A) and endoscopic band ligation was performed successfully (B).

hemorrhaging ectopic varices.

ECTOPIC VARICES

The majority of portosystemic collaterals with portal hypertension are located in the esophagus and stomach and, especially, esophageal varices are the most common site. Ectopic variceal bleeding is massive and serious condition. Other sites of ectopic varices are the duodenum, small intestine, colon, rectum, and the peritoneum[7]. A current survey of ectopic varices in Japan has been reported and the most frequent sites of ectopic varices are the rectum in 44.5%, followed by the duodenum in 32.9%[8].

Rectal varices

Rectal varices are a consequence of portosystemic collaterals from the superior rectal veins to the middle inferior rectal veins. Endoscopy can detect the discrete dilated submucosal varices in the rectum and some investigators have reported that rectal varices occur at a high frequency[9-11]. Hemorrhage from rectal varices occurs at a low frequency from 0.5% to 3.6%[12-14].

Several medical procedures have been performed for controlling rectal variceal bleeding, however, a standard treatment has not been established.

Surgical procedures such as portosystemic shunting, ligation, and under-running suturing, also have been reported[9]. Interventional radiologic techniques, including transjugular intrahepatic portosystemic shunts (TIPS), have been performed successfully for bleeding rectal varices[15-17].

As an endoscopic treatment, Wang *et al*[18] used EIS for rectal variceal bleeding. EBL has been performed as an effective procedure for rectal varices[19-21] and Levine *et al*[19] used EBL successfully for the rectal varices remaining after EIS. EBL is a safe and effective treatment for rectal varices. However, a retrospective study comparing EIS and EBL concluded that recurrence rate tended to be greater with EBL[22] and stated that 5% ethanolamine oleate of the sclerosant should be carefully injected using fluoroscopy, avoiding injection into the systemic circulation. Sato *et al*[22] evaluated the efficacy of EIS for rectal varices and reported that EIS was a useful and safe treatment for rectal varices with regard to effectiveness and complication[23].

Case presentation

EBL for rectal varices: Endoscopic finding of liver cirrhosis with anal bleeding revealed enlarged and tortuous, erosion positive rectal varices (Figure 1A), and endoscopic band ligation was performed successfully (Figure 1B).

EIS for rectal varices: EIS was performed using a flexible gastrointestinal endoscope, with a transparent hood attached to the tip, and the injection needle was placed into the varices (Figure 2A). Fluoroscopic observation with infusion of 5% ethanolamine oleate with iopamidol was made to determine the extent of the varices (Figure 2B).

Duodenal varices

Hemorrhage from duodenal varices is low frequent, however, it is often serious condition[24]. The hemodynamics of duodenal varices involved the development of collateral veins between the portal vein trunk or superior mesenteric vein and the inferior vena cava[25]. Hemorrhage from duodenal varices is generally massive and fatal[25,26]. The diagnosis of duodenal varices is done ordinary by endoscopic examination, however, it is often difficult to observe hemorrhaging duodenal varices. The common site of duodenal varices is the duodenal bulb[26], ranked next the second portion of the duodenum[27]. In the United States and Europe, varices of the duodenal bulb occur most frequently because of extrahepatic portal obstruction, causing portal hypertension. On the other hand, in Japan, common site of duodenal varices is the second portion of the duodenum. In addition, hemorrhage from duodenal varices in the distal third portion is very rare[28,29].

Recently, interventional radiology and endoscopic treatments have been performed successfully for duodenal varices. EBL is useful for obtaining hemostasis[30,31],

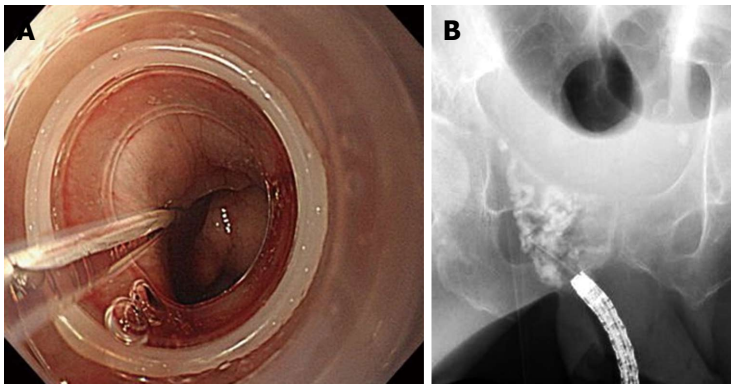

Figure 2 Endoscopic injection sclerotherapy was performed using a transparent hood attached to the tip and injection needle placed into the varices (A) and fluoroscopic observation with infusion of 5% ethanolamine oleate with iopamidol was made to determine the extent of the varices (B).

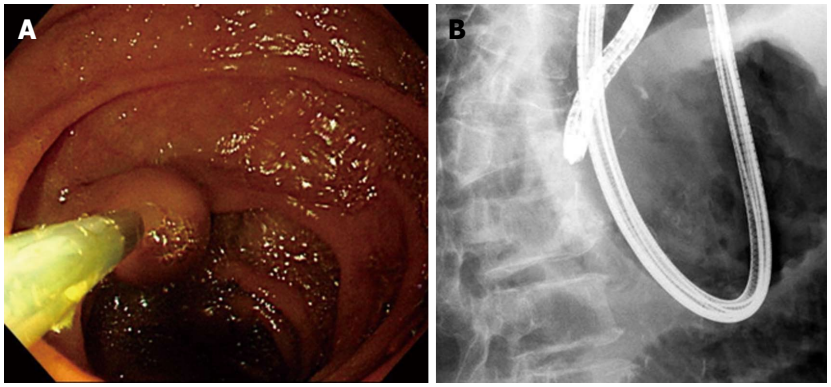

Figure 3 Endoscopy revealed tortuous duodenal varices in the anterior wall of the second portion (A) and N-butyl-2-cyanoacrylate was injected with contrast medium into the duodenal varices on fluoroscopic observation (B).

however, re-bleeding of varices is an weak point with EBL. EIS has been performed successfully for the treatment of duodenal variceal bleeding[32,33] but there were serious problem of re-bleeding of the varices[34,35]. N-butyl-2-cyanoacrylate is a tissue glue monomer; upon contact with blood, it immediately polymerizes and solidifies, resultings in hemostasis of variceal bleeding. Endoscopic therapy using N-butyl-2-cyanoacrylate is also very useful for massive duodenal variceal bleeding[29,30] because of the high blood velocity and blood flow. Interventional radiologic techniques such as TIPS, B-RTO, and percutaneous transhepatic obliteration (PTO) are options for hemorrhaging duodenal varices and successful treatments have been reported, including TIPS[26] and B-RTO[35-37]. B-RTO is able to obliterate the afferent and efferent veins and may be considered as a treatment option for duodenal varices. PTO also has been used successfully in some cases[38,39].

Case presentation

Endoscopic treatment using cyanoacrylate for duodenal varices: Endoscopy of liver cirrhosis with massive melena revealed tortuous duodenal varices in the anterior wall of the second portion (Figure 3A). N-butyl-2-cyanoacrylate was injected with contrast medium into the duodenal varices on fluoroscopic observation (Figure 3B).

Small intestinal varices

Bleeding from jejunal and ileal varices may be massive and serious and it is difficult to achieve early diagnosis. Most cases are detected following intra-abdominal surgery. The development of collateral circulation *via* the post-operative adhesions is a risk factor of small intestinal varices in patients with portal hypertension.

Many literatures of hemorrhaging jejunal[40-45] and ileal varices[46-54] have been described. Bleeding small intestinal varices are as follows: portal hypertension, hematochezia without hematemesis, and previous abdominal surgery[55]. Several treatments are available for jejunal varices, such as surgery[40], portal venous stenting[42,44,45] and percutaneous embolization[40,43], and surgery has been used successfully for bleeding ileal varices[50,51,56,57]. In ileal variceal patients with a serious condition, angiographic techniques including TIPS have been used successfully as a non-surgical approach[3,52,54]. B-RTO is also practical for treating ileal varices[58,59] and it also may be used for patients in a serious condition.

CONCLUSION

Ectopic varices in patients with portal hypertension are

considered to be the cause of hemorrhage presenting with lower gastrointestinal bleeding, and recently, their frequency has been increasing.

The diagnosis of ectopic varices and subsequent treatment are difficult. Recently, interventional radiology and endoscopic treatments have been performed successfully for ectopic varices. More investigations are necessary in a large number of patients.

REFERENCES

1 **Walsh G**, Williams MP. Case report: giant pararectal varices--computed tomographic appearances. *Br J Radiol* 1995; **68**: 203-204 [PMID: 7735753 DOI: 10.1259/0007-1285-68-806-203]

2 **Kinkhabwala M**, Mousavi A, Iyer S, Adamsons R. Bleeding ileal varicosity demonstrated by transhepatic portography. *AJR Am J Roentgenol* 1977; **129**: 514-516 [PMID: 409211 DOI: 10.2214/ajr.129.3.514]

3 **Norton ID**, Andrews JC, Kamath PS. Management of ectopic varices. *Hepatology* 1998; **28**: 1154-1158 [PMID: 9755256 DOI: 10.1002/hep.510280434]

4 Prophylactic sclerotherapy for esophageal varices in men with alcoholic liver disease. A randomized, single-blind, multicenter clinical trial. The Veterans Affairs Cooperative Variceal Sclerotherapy Group. *N Engl J Med* 1991; **324**: 1779-1784 [PMID: 2038367 DOI: 10.1056/NEJM199106203242505]

5 **Goff JS**, Reveille RM, Van Stiegmann G. Endoscopic sclerotherapy versus endoscopic variceal ligation: esophageal symptoms, complications, and motility. *Am J Gastroenterol* 1988; **83**: 1240-1244 [PMID: 3263792 DOI: 10.1111/j.0002-9270.1988.8311-1240]

6 **Kanagawa H**, Mima S, Kouyama H, Gotoh K, Uchida T, Okuda K. Treatment of gastric fundal varices by balloon-occluded retrograde transvenous obliteration. *J Gastroenterol Hepatol* 1996; **11**: 51-58 [PMID: 8672742 DOI: 10.1111/jgh.12766_2]

7 **Lebrec D**, Benhamou JP. Ectopic varices in portal hypertension. *Clin Gastroenterol* 1985; **14**: 105-121 [PMID: 3872747]

8 **Watanabe N**, Toyonaga A, Kojima S, Takashimizu S, Oho K, Kokubu S, Nakamura K, Hasumi A, Murashima N, Tajiri T. Current status of ectopic varices in Japan: Results of a survey by the Japan Society for Portal Hypertension. *Hepatol Res* 2010; **40**: 763-776 [PMID: 20649816 DOI: 10.1111/j1872-034X.2010.00690.x]

9 **Hosking SW**, Smart HL, Johnson AG, Triger DR. Anorectal varices, haemorrhoids, and portal hypertension. *Lancet* 1989; **1**: 349-352 [PMID: 2563507 DOI: 10.1016/S0140-6736(89)91724-8]

10 **Wang TF**, Lee FY, Tsai YT, Lee SD, Wang SS, Hsia HC, Lin WJ, Lin HC, Lai KH, Chan CY. Relationship of portal pressure, anorectal varices and hemorrhoids in cirrhotic patients. *J Hepatol* 1992; **15**: 170-173 [PMID: 1506636 DOI: 10.1016/0168-8278(92)90031-j]

11 **Chawla Y**, Dilawari JB. Anorectal varices--their frequency in cirrhotic and non-cirrhotic portal hypertension. *Gut* 1991; **32**: 309-311 [PMID: 2013427 DOI: 10.1136/gut.32.3.309]

12 **McCormack TT**, Bailey HR, Simms JM, Johnson AG. Rectal varices are not piles. *Br J Surg* 1984; **71**: 163 [PMID: 6692118 DOI: 10.1002/bjs.1800710236]

13 **Johansen K**, Bardin J, Orloff MJ. Massive bleeding from hemorrhoidal varices in portal hypertension. *JAMA* 1980; **244**: 2084-2085 [PMID: 6968834 DOI: 10.1001/jama.1980.03310180050035]

14 **Wilson SE**, Stone RT, Christie JP, Passaro E. Massive lower gastrointestinal bleeding from intestinal varices. *Arch Surg* 1979; **114**: 1158-1161 [PMID: 314792 DOI: 10.1001/archsurg.1979.01370340064011]

15 **Katz JA**, Rubin RA, Cope C, Holland G, Brass CA. Recurrent bleeding from anorectal varices: successful treatment with a transjugular intrahepatic portosystemic shunt. *Am J Gastroenterol* 1993; **88**: 1104-1107 [PMID: 8317414 DOI: 10.1111/j.0002-9270.1993.8807-1104]

16 **Shibata D**, Brophy DP, Gordon FD, Anastopoulos HT, Sentovich SM, Bleday R. Transjugular intrahepatic portosystemic shunt for treatment of bleeding ectopic varices with portal hypertension. *Dis Colon Rectum* 1999; **42**: 1581-1585 [PMID: 10613477]

17 **Fantin AC**, Zala G, Risti B, Debatin JF, Schöpke W, Meyenberger C. Bleeding anorectal varices: successful treatment with transjugular intrahepatic portosystemic shunting (TIPS). *Gut* 1996; **38**: 932-935 [PMID: 8984036 DOI: 10.1136/gut.38.6.932]

18 **Wang M**, Desigan G, Dunn D. Endoscopic sclerotherapy for bleeding rectal varices: a case report. *Am J Gastroenterol* 1985; **80**: 779-780 [PMID: 3876025 DOI: 10.1111/j.0002-9270.1985.8010-0779]

19 **Levine J**, Tahiri A, Banerjee B. Endoscopic ligation of bleeding rectal varices. *Gastrointest Endosc* 1993; **39**: 188-190 [PMID: 8495844]

20 **Firoozi B**, Gamagaris Z, Weinshel EH, Bini EJ. Endoscopic band ligation of bleeding rectal varices. *Dig Dis Sci* 2002; **47**: 1502-1505 [PMID: 12141807]

21 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Suga T. Two cases of rectal varices treated by endoscopic variceal ligation. *Dig Endosc* 1999; **11**: 66-69 [DOI: 10.1111/j.1443-1661.1999.tb00196.x]

22 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Suga T. The value of the endoscopic therapies in the treatment of rectal varices: a retrospective comparison between injection sclerotherapy and band ligation. *Hepatol Res* 2006; **34**: 250-255 [PMID: 16540369 DOI: 10.1016/j.hepres.2006.01.007]

23 **Sato T**. Treatments for rectal varices with portal hypertension. *J Gastroint Dig Syst* 2013: **S6** [DOI: 10.4172/2161-069X.S6-005]

24 **D'Imperio N**, Piemontese A, Baroncini D, Billi P, Borioni D, Dal Monte PP, Borrello P. Evaluation of undiluted N-butyl-2-cyanoacrylate in the endoscopic treatment of upper gastrointestinal tract varices. *Endoscopy* 1996; **28**: 239-243 [PMID: 8739740 DOI: 10.1055/s-2007-1005435]

25 **Khouqeer F**, Morrow C, Jordan P. Duodenal varices as a cause of massive upper gastrointestinal bleeding. *Surgery* 1987; **102**: 548-552 [PMID: 3498234]

26 **Jonnalagadda SS**, Quiason S, Smith OJ. Successful therapy of bleeding duodenal varices by TIPS after failure of sclerotherapy. *Am J Gastroenterol* 1998; **93**: 272-274 [PMID: 9468260 DOI: 10.1111/j.1572-0241.1998.270-3x]

27 **Wang CS**, Jeng LB, Chen MF. Duodenal variceal bleeding--successfully treated by mesocaval shunt after failure of sclerotherapy. *Hepatogastroenterology* 1995; **42**: 59-61 [PMID: 7782038]

28 **Shiraishi M**, Hiroyasu S, Higa T, Oshiro S, Muto Y. Successful management of ruptured duodenal varices by means of endoscopic variceal ligation: report of a case. *Gastrointest Endosc* 1999; **49**: 255-257 [PMID: 9925710 DOI: 10.1016/s0016-5107(99)70498-0]

29 **Yoshida Y**, Imai Y, Nishikawa M, Nakatukasa M, Kurokawa M, Shibata K, Shimomukai H, Shimano T, Tokunaga K, Yonezawa T. Successful endoscopic injection sclerotherapy with N-butyl-2-cyanoacrylate following the recurrence of bleeding soon after endoscopic ligation for ruptured duodenal varices. *Am J Gastroenterol* 1997; **92**: 1227-1229 [PMID: 9219810]

30 **Ota K**, Shirai Z, Masuzaki T, Tanaka K, Higashihara H, Okazaki M, Arakawa M. Endoscopic injection sclerotherapy with n-butyl-2-cyanoacrylate for ruptured duodenal varices. *J Gastroenterol* 1998; **33**: 550-555 [PMID: 9719241 DOI: 10.1007/s005350050131]

31 **Selçuk H**, Boyvat F, Eren S, Korkmaz M, Gür G, Yilmaz U, Boyacioğlu S. Duodenal varices as an unusual cause of gastrointestinal bleeding due to portal hypertension: a case report. *Turk J Gastroenterol* 2004; **15**: 104-107 [PMID: 15334321]

32 **Gertsch P**, Blumgart LH. Cure of a bleeding duodenal varix by sclerotherapy. *Br J Surg* 1988; **75**: 717 [PMID: 3262004 DOI: 10.1002/bjs.1800750731]

33 **Barbish AW**, Ehrinpreis MN. Successful endoscopic injection sclerotherapy of a bleeding duodenal varix. *Am J Gastroenterol* 1993; **88**: 90-92 [PMID: 8420280 DOI: 10.1111/j.0002-9270.1993.8801-0090]

34 **Hashiguchi M**, Tsuji H, Shimono J, Azuma K, Fujishima M.

Ruptured duodenal varices: an autopsy case report. *Hepatogastroenterology* 1999; **46**: 1751-1754 [PMID: 10430337]
35 **Ohta M**, Yasumori K, Saku M, Saitsu H, Muranaka T, Yoshida K. Successful treatment of bleeding duodenal varices by balloon-occluded retrograde transvenous obliteration: a transjugular venous approach. *Surgery* 1999; **126**: 581-583 [PMID: 10486613 DOI: 10.1016/s0039-6060(99)70102-9]
36 **Akazawa Y**, Murata I, Yamao T, Yamakawa M, Kawano Y, Nomura N, Isomoto H, Mizuta Y, Murase K, Kohno S. Successful management of bleeding duodenal varices by endoscopic variceal ligation and balloon-occluded retrograde transvenous obliteration. *Gastrointest Endosc* 2003; **58**: 794-797 [PMID: 14595327 DOI: 10.1016/S0016-5107(03)02008-X]
37 **Sonomura T**, Horihata K, Yamahara K, Dozaiku T, Toyonaga T, Hiroka T, Sato M. Ruptured duodenal varices successfully treated with balloon-occluded retrograde transvenous obliteration: usefulness of microcatheters. *AJR Am J Roentgenol* 2003; **181**: 725-727 [PMID: 12933468 DOI: 10.2214/ajr.181.3.1810725]
38 **Menu Y**, Gayet B, Nahum H. Bleeding duodenal varices: diagnosis and treatment by percutaneous portography and transcatheter embolization. *Gastrointest Radiol* 1987; **12**: 111-113 [PMID: 3493935 DOI: 10.1007/bf01885117]
39 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Akaike J, Kuwata Y, Suga T. Efficacy of color Doppler ultrasonography for the diagnosis of duodenal varices successfully treated with percutaneous transhepatic obliteration. *J Med Ultrasonics* 2007; **34**: 59-63 [DOI: 10.1007/s10396-006-0129-4]
40 **Lim LG**, Lee YM, Tan L, Chang S, Lim SG. Percutaneous paraumbilical embolization as an unconventional and successful treatment for bleeding jejunal varices. *World J Gastroenterol* 2009; **15**: 3823-3826 [PMID: 19673028 DOI: 10.3748/wjg.15.3823]
41 **Yuki N**, Kubo M, Noro Y, Kasahara A, Hayashi N, Fusamoto H, Ito T, Kamada T. Jejunal varices as a cause of massive gastrointestinal bleeding. *Am J Gastroenterol* 1992; **87**: 514-517 [PMID: 1553940]
42 **Sakai M**, Nakao A, Kaneko T, Takeda S, Inoue S, Yagi Y, Okochi O, Ota T, Ito S. Transhepatic portal venous angioplasty with stenting for bleeding jejunal varices. *Hepatogastroenterology* 2005; **52**: 749-752 [PMID: 15966197]
43 **Sasamoto A**, Kamiya J, Nimura Y, Nagino M. Successful embolization therapy for bleeding from jejunal varices after choledochojejunostomy: report of a case. *Surg Today* 2010; **40**: 788-791 [PMID: 20676866 DOI: 10.1007/s00595-009-4149-z]
44 **Hiraoka K**, Kondo S, Ambo Y, Hirano S, Omi M, Okushiba S, Katoh H. Portal venous dilatation and stenting for bleeding jejunal varices: report of two cases. *Surg Today* 2001; **31**: 1008-1011 [PMID: 11766071 DOI: 10.1007/s005950170013]
45 **Ota S**, Suzuki S, Mitsuoka H, Unno N, Inagawa S, Takehara Y, Sakaguchi T, Konno H, Nakamura S. Effect of a portal venous stent for gastrointestinal hemorrhage from jejunal varices caused by portal hypertension after pancreatoduodenectomy. *J Hepatobiliary Pancreat Surg* 2005; **12**: 88-92 [PMID: 15754107 DOI: 10.1007/s00534-004-0941-4]
46 **Falchuk KR**, Aiello MR, Trey C, Costello P. Recurrent gastrointestinal bleeding from ileal varices associated with intraabdominal adhesions: case report and review of the literature. *Am J Gastroenterol* 1982; **77**: 859-860 [PMID: 6982615]
47 **Arst HF**, Reynolds JD. Acute ileal variceal hemorrhage secondary to esophageal sclerotherapy. *J Clin Gastroenterol* 1986; **8**: 603-604 [PMID: 3491132]
48 **Højhus JH**, Pedersen SA. Cirrhosis and bleeding ileal varices without previous intraabdominal surgery. A case report. *Acta Chir Scand* 1986; **152**: 479-480 [PMID: 3490103]
49 **Lewis P**, Warren BF, Bartolo DC. Massive gastrointestinal haemorrhage due to ileal varices. *Br J Surg* 1990; **77**: 1277-1278 [PMID: 2253011 DOI: 10.1002/bjs.1800771126]
50 **Ohtani T**, Kajiwara E, Suzuki N, Kawasaki A, Sadoshima S, Sakata H, Sasaguri Y, Onoyama K. Ileal varices associated with recurrent bleeding in a patient with liver cirrhosis. *J Gastroenterol* 1999; **34**: 264-268 [PMID: 10213130 DOI: 10.1007/s005350050255]
51 **Kobayashi K**, Yamaguchi J, Mizoe A, Isomoto I, Koshiishi T, Izawa K, Kanematsu T. Successful treatment of bleeding due to ileal varices in a patient with hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 2001; **13**: 63-66 [PMID: 11204813]
52 **Guth E**, Katz MD, Hanks SE, Teitelbaum GP, Ralls P, Korula J. Recurrent bleeding from ileal varices treated by transjugular intrahepatic portosystemic shunt: value of Doppler ultrasonography in diagnosis and follow-up. *J Ultrasound Med* 1996; **15**: 67-69 [PMID: 8667487]
53 **Varanasi RV**, Fleisher AS, Darwin PE, King CE, Haluszka O. Colonoscopic sclerotherapy of ileal varices. *Gastrointest Endosc* 2000; **52**: 109-111 [PMID: 10882977 DOI: 10.1067/mge.2000.106538]
54 **López-Benítez R**, Seidensticker P, Richter GM, Stampfl U, Hallscheidt P. [Case report: massive lower intestinal bleeding from ileal varices: treatment with transjugular intrahepatic portosystemic shunt (TIPSS)]. *Radiologe* 2007; **47**: 407-410 [PMID: 16249924 DOI: 10.1007/s00117-005-1279-x]
55 **Cappell MS**, Price JB. Characterization of the syndrome of small and large intestinal variceal bleeding. *Dig Dis Sci* 1987; **32**: 422-427 [PMID: 3549204]
56 **Ueda J**, Yoshida H, Mamada Y, Taniai N, Mizuguchi Y, Shimizu T, Matsumoto S, Kakinuma D, Ishikawa Y, Kanda T, Akimaru K, Teranishi N, Naito Z, Tajiri T. Successful emergency enterectomy for bleeding ileal varices in a patient with liver cirrhosis. *J Nippon Med Sch* 2006; **73**: 221-225 [PMID: 16936448 DOI: 10.1272/jnms.73.221]
57 **Mashimo M**, Hara J, Nitta A, Oohira M, Yamaguchi S, Yamada S, Tanaka H, Higuchi K, Arakawa T. [A case of ruptured ileal varices associated with alcoholic liver cirrhosis]. *Nihon Shokakibyo Gakkai Zasshi* 2007; **104**: 561-567 [PMID: 17409666]
58 **Haruta I**, Isobe Y, Ueno E, Toda J, Mitsunaga A, Noguchi S, Kimura T, Shimizu K, Yamauchi K, Hayashi N. Balloon-occluded retrograde transvenous obliteration (BRTO), a promising nonsurgical therapy for ectopic varices: a case report of successful treatment of duodenal varices by BRTO. *Am J Gastroenterol* 1996; **91**: 2594-2597 [PMID: 8946993 DOI: 10.1111/j.0002-9270.1996.9112-2594]
59 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Akaike J. Ileal varices treated with balloon-occluded retrograde transvenous obliteration. *Gastroenterol Res* 2009; **2**: 122-5 [DOI: 10.4021/gr2009.04.1286]

P- Reviewer: Genesca J, Ong HT **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1606

World J Hepatol 2015 June 28; 7(12): 1606-1616
ISSN 1948-5182 (online)

REVIEW

Treatment of chronic hepatitis C in liver transplant candidates and recipients: Where do we stand?

Chrysoula Pipili, Evangelos Cholongitas

Chrysoula Pipili, Division of Nephrology, Royal Infirmary of Edinburgh, Scotland EH16 4SA, United Kingdom
Evangelos Cholongitas, 4th Department of Internal Medicine, Medical, Medical School of Aristotle University, Hippokration General Hospital of Thessaloniki, 54642 Thessaloniki, Greece

Author contributions: Pipili C and Cholongitas E contributed equally in writing and editing of the article.

Conflict-of-interest: None.

Correspondence to: Evangelos Cholongitas, Assistant Professor of Internal Medicine, 4th Department of Internal Medicine, Medical School of Aristotle University, Hippokration General Hospital of Thessaloniki, 49, Konstantinopoleos Street, 54642 Thessaloniki, Greece. cholongitas@yahoo.gr
Telephone: +30-23-10892110
Fax: +30-23-10992940

Received: December 13, 2014
Peer-review started: December 13, 2014
First decision: January 8, 2015
Revised: May 18, 2015
Accepted: June 1, 2015
Article in press: June 2, 2015
Published online: June 28, 2015

Abstract

The first generation direct antiviral agents (DAAs) highlighted substantial prognosis improvement among liver transplant (LT) candidates and recipients with recurrent hepatitis C virus (HCV) infection. During 2014, second generation DAAs are associated with high sustained virological response rates (> 95%), shortened duration courses and relatively few toxicities. In keeping with the currently available data, patients with decompensated cirrhosis awaiting LT is preferable to be treated with interferon-free, new generation DAAs, with or without ribavirin combinations. Although data about the safety of new DAAs combinations in this patient population are limited, sofosbuvir and daclatasvir pharmacokinetics do not appear to change significantly in moderate or severe liver impairment, while other new DAAs (simeprevir, asunaprevir) seem to be contraindicated in patients with severe liver impairment (Child-Pugh class C). On the other hand, sofosbuvir should not be given in patients with glomerular filtration rate $\leqslant$ 30 mL/min, but ongoing trials will clarify better this issue. With the objective that newer antiviral combinations will yield safer and more efficient manipulation of HCV recurrence post-transplant, the European Association for the Study of the Liver has recently updated its recommendations towards this direction. Nevertheless the new antivirals' high cost may be the biggest challenge to their implementation worldwide.

Key words: Liver transplantation; Decompensated cirrhosis; Hepatitis C; New antiviral agents; Sofosbuvir; Simeprevir; Daclatasvir; Recurrent hepatitis C

Core tip: Treatment landscape for liver transplant (LT) candidates and recipients with chronic hepatitis C is rapidly shifting due to novel updated data on direct antiviral agents (DAAs); Patients with decompensated cirrhosis awaiting LT should be treated with the interferon (IFN)-free, new generation DAAs, with or without ribavirin (RBV) regimens; IFN-free combinations of sofosbuvir with other novel DAAs with or without RBV led to remarkable on-treatment virological response

along with minimal adverse effects "on difficult to treat" LT recipients with recurrent hepatitis C - those with chronic hepatitis C genotype 1, decompensated cirrhosis with Child-Pugh stage B and C as well as previously intolerant or non responsive to IFN therapy; Evidence regarding the efficacy and safety of novel combinative treatments although are very promising, refer still to patients case series.

Pipili C, Cholongitas E. Treatment of chronic hepatitis C in liver transplant candidates and recipients: Where do we stand? *World J Hepatol* 2015; 7(12): 1606-1616 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1606.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1606

INTRODUCTION

Hepatitis C virus (HCV) infection is the leading liver cause of death worldwide and the main indication for liver transplantation (LT)[1-3]. It also diminishes overall and graft survival in LT recipients resulting in shorter survival comparing with other LT recipients[4,5]. Ideally, viral load elimination prior to LT hampers graft loss from chronic hepatitis C (CHC) recurrence[6]. In the absence of an HCV vaccine to prevent infection and with therapy until very recently limited to interferon (IFN)-based regimens, most HCV-infected candidates for LT patients remained untreated.

Direct antiviral agents (DAAs) represent a striking innovation in HCV treatment. The first generation DAAs, boceprevir and telaprevir, act mainly as NS3-4A serine protease inhibitors and have been in use since 2011. When combined with pegylated IFN (Peg-IFN) and ribavirin (RBV) lead to 30% increase of sustained virological response (SVR) on CHC patients with genotype 1 treated for the first time and to 50%-60% increase on those who experienced at least one episode of relapse compared to the standard of care (Peg-IFN plus RBV)[7]. However, first generation DAAs present significant draw backs such as limited efficacy on HCV genotype 1, absolute co-administration with Peg-IFN and RBV, frequent drug-to drug interactions and toxicity, particularly in patients with underlying cirrhosis[7]. Patients with decompensated cirrhosis who are on the transplant list should be considered for IFN-free, ideally RBV-free therapy because both Peg-IFN and RBV are associated with poor tolerability and many side effects mainly due to the splenomegaly-related pancytopenia[6].

Other classes of DAAs targeting different steps of HCV replication have been approved more recently, including nucleotide polymerase (NUC-NS5B) inhibitors, non-nucleotide polymerase (non-NUC-NS5B) inhibitors and NS5A inhibitors. Several dual and triple DAA regimens are in clinical development in patients before and after LT suggesting that these regimens will dramatically reduce the impact of recurrent HCV post LT[8].

Within 2014, three new DAAs were licensed for use in combined therapies against HCV infection in the European Union. These include sofosbuvir, (Sovaldi, Gilead Sciences) which represents the first NS5B HCV polymerase inhibitor that has pangenotypic antiviral activity and a high genetic barrier to resistance[9]; simeprevir (Olysio, Janssen), a second-wave NS3/4A protease inhibitor; and daclatasvir (Daklinza, BMS), an NS5A inhibitor[6]. In addition, other DAAs are currently under consideration and their final approval is waiting in the first half of 2015 (Table 1). All these DAAs have been evaluated with or without Peg-IFN ± RBV. Although scarce data is available in LT candidates and recipients, novel DAAs combinations seem more efficacious and better-tolerated option. Nevertheless, cost limitations across various health insurances may avert their use. The current review updates treatment for patients with CHC before and after LT.

HCV positive LT candidates

The aim of antiviral therapy before LT is the undetectable HCV RNA at the time of LT or SVR before LT[10]. Peg-IFN and RBV was the standard of care for patients with CHC up to 2011. Indeed they reduced cirrhosis driven complications such as HCC and improved the abnormal histological changes[10,11], but in an inadequate percentage of patients[12-16]. Generally, SVR rates are lower in patients with cirrhosis ranging 40%-50% for Child-Pugh (CP) class A and 7%-26% for CP class C[17-20] and in patients with genotypes 1 and 4 compared with genotype 2 and 3 patients (51% *vs* 61%)[20] while a reduction on SVR is evident regarding the level of fibrosis (Tables 2 and 3).

Peg-IFN-based treatment has also been associated with poor tolerability and lots of adverse effects[3,5]. These include neuropsychiatric disorders and mainly pancytopenia[20], requiring erythropoietin and granulocyte colony- stimulating factors[21,22]. Of note, LT candidates with HCV compensated cirrhosis are more prone to Peg-IFN- and RBV-related hematologic toxicities, because splenomegaly caused by portal hypertension enhances the risk for cytopenias[23]. Therefore, Peg-IFN and RBV dose adjustment and close monitoring is recommendable. More specifically, patients with CP score B require treatment tailoring decisions focused on type of genotype, level of viral load and first or repeat antiviral treatment[10].

A triple therapy with the addition of first generation DAAs is the preferred choice for patients with genotype 1 when newer DAAs are not available[6]. Boceprevir and telabrevir are better therapeutic choice for patients with genotype 1 ensuring excellent efficacy and prognosis compared to Peg-IFN and RBV treatment. Data showed that triple therapy increased SVR rates in naïve and previously treated patients with genotype 1 between 68%-75% and 59%-88% respectively[24-28]. Boceprevir therapy also offers the possibility of shortened duration therapy for rapid responders, defined by an undetectable plasma HCV RNA level at treatment week 8[24]. One should notice that DAAs should not be applied as monotherapy and their dose should not be reduced,

Table 1 Direct acting antivirals commenced on patients with decompensated cirrhosis or recipients after liver transplantation

Approved protease inhibitors	
Boceprevir	NS3-4A serine protease inhibitors
Telaprevir	Second-wave NS3/4A protease inhibitor
Simeprevir	
Approved NS5A inhibitors	
Daclatasvir	NS5A inhibitor
Approved NS5B RNA-dependent RNA polymerase nucleotide inhibitor	
Sofosbuvir	NS5B RNA-dependent RNA polymerase nucleotide inhibitor
Newer DAAs evaluated in combination regimens without Peg-IFN	
Ledipasvir (formerly GS-5885)	NS5A inhibitor
ABT-450	NS3/4A protease inhibitor
Ombitasvir (formerly ABT-267)	NS5A inhibitor
Dasabuvir (formerly ABT-333)	Non-nucleoside NS5B polymerase inhibitor

Peg-IFN: Pegylated interferon.

Table 2 Doses of antivirals in liver transplant candidates and recipients

Peg-IFN-α 2a	180 μg/wk, subcutaneous	Renal adjustment
Peg-IFN-α 2b	Weight-based 1.5 μg/kg per week, subcutaneous	No CNI adjustment
Ribavirin	Weight-based, 1000 mg in patients < 75 kg, 1200 mg in patients ⩾ 75 kg, orally twice a day	
Boceprevir	800 mg orally three times a day	No renal adjustment
Telaprevir	1125 mg orally twice a day	CNI adjustment
Sofosbuvir	400 mg daily, orally	
Daclatasvir	60 mg daily, orally	No renal adjustment[1]
Simeprevir	150 mg daily, orally	No CNI adjustment[2]

[1]Sofosbuvir only in patients with glomerular filtration rate > 30 mL/min; [2]Simeprevir should not be given with cyclosporine. CNI: Calcineurin inhibitor; Peg-IFN: Pegylated interferon.

stopped and then restarted[29]. However, high rates of serious adverse events, discontinuation and deaths have been recorded[30]. The adverse effects included anaemia, metallic taste, infection and rash which were more evident in patients with cirrhosis[24-28]. For this reason the administration of the triple therapy remains of high risk in patients with cirrhosis. Anaemia is considered positive predictor of SVR in patients receiving the triple regimen and should be corrected with erythropoietin and reduction of RBV dose[31-33] without any change in boceprevir or telaprevir administration.

The data regarding the efficacy and safety of triple therapy to HCV patient with decompensated cirrhosis are very limited. Verna *et al*[34] treated 29 patients with cirrhosis CP class A (62%) or B (38%) while waiting on the transplant list with telaprevir (93%) or boceprevir-based (7%) triple therapy for a median (range) of 27 (3-50) wk, including a Peg-IFN and RBV lead-in phase in 18%. Overall SVR at 12 wk was 52%, including patients who completed a full course of therapy, but 28% of patients require hospital admission because of treatment complications including decompensation of liver disease, variceal hemorrhage, cholecystits, optic neuritis and anemia requiring transfusion[34]. The efficacy and the safety of triple therapy have been tested in patients with mildly decompensated cirrhosis as well[35]. SVR at 12 wk was achieved by 35% of patients with CP ⩾ 6 *vs* 54% of those with CP = 5[35]. Furthermore, 25% of patients with compensated cirrhosis discontinued treatment early due to adverse events, while high proportion required drug dose reductions and hospitalizations due to side effects[35]. Although a French multicenter phase II trial is being assessing the safety and the efficacy of boceprevir-based triple therapy in patients with genotype 1 and a baseline MELD score 18 on the waiting list[36], it is apparent that IFN-free therapies are highly desirable for patients with decompensated disease[34,35].

In fact, the treatment chances of critical CHC candidates for LT will be very high should second generation DAAs is applicable. Sofosbuvir, a nucleotide HCV polymerase inhibitor, is now available and offers better tolerability and efficacy across all HCV genotypes making sustained clearance of HCV deliverable to a much larger number of infected individuals[8]. We are at the beginning of an era where combinations of DAAs may pave the way for IFN-free regimens, even improving the viral clearance rate to near 100%. Evidence comes from the following four trials[37-40]. In the open label phase II study of Curry *et al*[37] 61 CHC candidates with HCC and normal liver function tests (CP score ⩽ 7) commenced on sofosbuvir (400 mg/d) and RBV (based on their weight) until LT for a maximum of 48 wk. Thirty six out of the 40 patients who underwent LT achieved viral load below 25 IU/mL pre LT. In the view of excellent tolerability, 96% had unidentifiable HCV RNA and no viral recurrence at four weeks post LT. Nevertheless, 69% achieved SVR and 27% had HCV recurrence, 12 wk post LT. Curiously, one patient undergone second LT died and one presented anemia related to RBV.

Given the high mortality due to HCV recurrence after LT, the evidence that sofosbuvir and RBV before LT prevented the recurrence of CHC after LT in 70% of patients who had undetectable HCV RNA prior to LT, provide great hope for patients in need. In the randomized open label study of Afdhal *et al*[38] 50 CHC with genotypes 1-4 either commenced on sofosbuvir plus RBV for 12 mo or were observed for 6 mo and then treated. Patients' characteristics included portal hypertension plus/or minus decompensated liver disease, CP score 5-10 and MELD score > 13. Undetectable HCV-RNA was achieved in 100% for CP class A and 93% for CP class B after 6 mo of treatment. No patient experienced treatment breakthroughs. Ultimately, in the study of Gane *et al*[39] 20 patients with CHC genotype 1 and CP class B received sofovusvir ± RBV combined with the newer NS5A inhibitor ledipasvir for four weeks

Table 3 Treatment of liver transplant candidates with hepatitis C[6]

Cirrhosis CP A, all HCV genotypes		
Sofosbuvir + RBV until LT	Peg-IFN + RBV + sofosbuvir for 12 wk	RBV + sofosbuvir + daclatasvir for 12 wk
Cirrhosis CP B and C, all HCV genotypes		
Sofosbuvir + RBV until LT	IFN contraindicated	RBV + sofosbuvir + daclatasvir for 12 wk

CP: Child-Pugh; HCV: Hepatitis C virus; LT: Liver transplant; RBV: Ribavirin; Peg-IFN: Pegylated interferon.

Table 4 Major studies of interferon-free regimens for treatment of hepatitis C virus positive liver transplant candidates reported in 2014

Ref.	*n*	CP score	Antiviral scheme	Virological response
Curry *et al*[37]	61	⩽ 7	SOF + RBV for 48 wk or until LT	69% (12 wk after LT)
Gane *et al*[39]	20	7-9	SOF ± RBV + ledipasvir for 12 wk	89% (SVR 4 wk)
Flamm *et al*[40]	108	Decompensated cirrhosis (range: 7-12)	SOF + RBV + ledipasvir for 12 or 24 wk	87% and 89%, respectively (SVR 12 wk)
Afdhal *et al*[38]	50	5-10	SOF + RBV for 48 wk	100% CP class A 93% CP class B at 24 wk under treatment

n: Number of patients; CP: Child-Pugh; SOF: Sofosbuvir; RBV: Ribavirin; SVR: Sustained virological response.

(preliminary data for the ongoing Electron Study). High percentage (89%) achieved SVR so far (at four weeks) with good safety and tolerance profile (there were no treatment discontinuations). Similarly, sofosbuvir, ledipasvir and RBV for 3 or 6 mo were well tolerated and resulted in high SVR (87% and 89%, respectively) in 108 patients with decompensated cirrhosis CP class B or C[40]. Interestingly, Donato *et al*[41] reported undetectable HCV RNA for 24 wk after treatment discontinuation in a LT recipient treated prior LT - on the waiting list- over LT and post LT for a total period of 24 wk with the combination of sofosbuvir and RBV (Table 4).

Based on the currently available data, patients with decompensated cirrhosis awaiting LT should be treated with the IFN-free, new generation DAAs, with or without RBV regimens. Although more data are needed about the safety of new DAAs drug combinations in this patient population, it seems that the pharmacokinetics of sofosbuvir and daclatasvir do not appear to change significantly in moderate or severe liver impairment[42], while other new DAAs (simeprevir, asunaprevir) seem to be contraindicated in patients with severe liver impairment (CP class C). On the other hand, sofosbuvir should not be given in patients with GFR ⩽ 30 mL/min, but ongoing trials will clarify better this issue. With the objective that newer drug combinations will yield safer and more efficient prevention of HCV recurrence post-transplant and the - so far poorly treated - patients with low MELD scores and HCC will benefit most[6], treatment recommendations for LT candidates are updated as below: Patients with conserved liver function (CP class A) in whom the indication is HCC for LT is strongly recommended to be treated with daily weight-based RBV (1000 or 1200 mg in patients < 75 kg or ⩾ 75 kg, respectively) and daily sofosbuvir until LT. Otherwise, they should be offered a 12-wk treatment prior to LT with the combination of Peg-IFN, RBV and sofosbuvir or the combination of daily weight-based RBV, daily sofosbuvir and daily daclatasvir[6]. Candidates with more advanced decompensated cirrhosis (CP class B and C), should be commenced only in IFN-free regimes; *i.e.*, daily weight-based RBV and daily sofosbuvir for minimization post-LT HCV recurrence; for genotypes 1 to 4 the triple combination of daily weight-based RBV, daily sofosbuvir and daily daclatasvir until LT in experienced centres under close monitoring[6]. Both sofosbuvir and daclatasvir do not need dose adjustments in patients with CP class B or C disease. Their most frequently reported side effects were fatigue, headache, nausea and anemia, the latter attributable to the co-administration of RBV[6,43].

HCV positive LT recipients

Reinfection of liver allograft is universal resulting in acceleration of HCV recurrence compared with non-LT patients leading in 70% decompensation *vs* 10% in other immunocompetent groups three years post-transplant[44]; graft loss and lower survival rates[45,46]. HCV recurrence is the most frequent cause of death and accounts for the two thirds of graft failures in patients transplanted for HCV infection[39]. Fibrosing cholestatic hepatitis is the most severe aggressive form of HCV recurrence affecting 5%-8% of LT recipients[47]. It is characterized by high viral load levels and extensive dense portal fibrosis extending into the sinusoidal spaces, ductular proliferation, cholestasis and mononuclear inflammation[48]. Its prognosis is very poor if recipients do not respond to antiviral treatment (Tables 2 and 5).

Several strategies, including the new generation of antivirals, the optimal donor and the immunosuppressant selection, have been applied to improve outcomes post LT. Negative predictive factors associated with aggressive recurrent HCV infection and graft loss are the high HCV RNA levels in both serum and liver at the time of or early post-LT, female gender and older donor age, steatosis of the graft as well as the degree

Table 5 Treatment of hepatitis C virus recurrence post liver transplant

IFN-free: Sofosbuvir + RBV (HCV genotype 2 for 12-24 wk); sofosbuvir + daclatasvir ± RBV (HCV genotypes 1, 3-6 for 12-24 wk); sofosbuvir + simeprevir ± RBV (HCV genotypes 1, 4 for 12 -24 wk)		
In case of restriction applying first generation DAAs		
Fibrosis metavir stage 3-4	Genotype 2/3	Genotype 1
Genotype 1a		Fibrosis metavir stage 2
IL-28B polymorphism		Predictors of poor response
Fibrosing cholestatic hepatitis	Peg-IFN plus RBV	Peg-IFN plus RBV plus boceprevir or telaprevir
Non responders to previous treatments	Monitor closely Hb, WBC, PLTs CNI levels, renal function	
	Consider	
	Administration of blood transfusions, EPO, CSGF	
	Decrease of RBV dose	
	Renal dose adjustment	
	Decrease of CNI dose	

IFN: Interferon; RBV: Ribavirin; CNI: Calcineurin inhibitor; HCV: Hepatitis C virus; Peg-IFN: Pegylated interferon; DAA: Direct antiviral agent; WBC: White cells blood count; PLTs: Platelets; EPO: Erythropoietin; CSGF: Granulocyte-colony stimulating factor.

of human leukocyte antigen matching of the donor and recipient[49,50]. Moreover, immunosuppressant selection is considered a major factor contributing to acceleration of HCV recurrence. While methyl prednisolone pulses for treatment of acute rejection could drive aggressive HCV recurrence ending up to graft loss, sirolimus could result on HCV RNA elimination without additional antiviral treatment[51,52]. Ultimately, everolimus may offer a benefit for posttransplant HCV-related fibrosis progression[53]. Cyclosporine is considered to have advantage over the other immunosuppressants, because of potential antiviral action (the target protein of cyclosporine, cyclophilin A is involved in the HCV replication)[54]; the enhancement of SVR in LT recipients treated with the dual therapy[55] and the fewer interactions with the triple regimen. Steroid free regimens have been also tried; although they were safe and effective, they did not influence HCV recurrence[56,57]. Nevertheless, many of these issues might have limited impact in the era of the newer DAAs, since the majority of patients with HCV recurrence can effectively be treated.

Preemptive prophylaxis for CHC recurrence post LT is not recommendable so far with Peg-IFN-based regimens, because as determined by randomized trials the therapeutic cost is high, tolerability is poor and no additional therapeutic effect is gained[58-60]. Nevertheless, new DAAs clinical application this may change this therapeutic strategy in the near future. The presence of significant fibrosis or portal hypertension one year after transplantation is predictive of rapid disease progression and graft loss, requiring urgent antiviral treatment[61,62]. Fibrosing cholestatic hepatitis and significant fibrosis[63] determined by METAVIR score > F1[64], liver stiffness > 8.7 kPa[65] and hepatic venous gradient > 6[60] are additional indications for antiviral treatment. Of note is the fact that patients with fibrosis level > 3 cannot tolerate Peg-IFN-based therapy[66]. Timely diagnosis of HCV recurrence based on histology is also recommendable, but should be properly differentiated with acute liver rejection since pathology is similar but treatment distinct[67]. Simultaneous use of regular protocol biopsies, transient elastography and fibrosis markers are necessary to determine the progression of rejection and/or fibrosis[22].

The therapeutic strategy for treatment of HCV recurrence post LT continues to be updated. Initially, Peg-IFN combined with RBV was the only therapeutic choice. SVR stabilization has been achieved in only 30% of recipients[62-71] and IFN on the top of intense immunosuppression lead to multiple episodes of sepsis, acute and chronic rejection and plasma cell hepatitis[10,63,68]. Secondly, the addition of first generation DAAs on the classical Peg-IFN and RBV regimen was applied to LT recipients with genotype 1, non-responders to dual therapy, cases of METAVIR fibrosis stage 2, and cholestatic hepatitis[63,72]. The results were satisfactory and encouraging[63]. Seven studies[73-79] (including four multicenter) suggested that triple therapy was effective in LT recipients, particularly those experiencing severe recurrence, demonstrating SVR in 50%-91% when administered for 12 to 66 wk (Table 6). One major benefit of the triple therapy was the success in treating recipients with fibrosing cholestatic hepatitis[73]. Nevertheless, serious adverse and few fatal events were recorded. The most common side effect was anaemia (40%-50%) required red blood cell transfusions, erythropoietin and/or RBV dose reduction. The mechanism of anaemia was the combination of hemolysis with suppression of bone marrow. In cirrhotic non-responding patients severe infections have been marked in the context of neutropenia (pneumocystosis, aspergilosis, urinary tract infections and erysipelas have been noted). On this ground, severe liver disease may be a contraindication for triple regimen or may require antibiotic prophylaxis and colony-stimulating factors[73]. Kidney insufficiency has also been reported in 13%-38%[73,74].

Furthermore drug-drug interactions remain a crucial clinical issue. Close monitoring of daily calcineurin inhibitors (CNIs) levels and reduction of CNIs dose was required[80]. CNI trough level is increased when first generation DAAs are initiated because they may inhibit cytochrome P450 3A4 and P-glucoprotein. With boceprevir the average reductions were about 2-fold and 5-fold with cyclosporine and tacrolimus respectively, while

Table 6 Major studies tested the efficacy of first generation direct antiviral agents combined with peg-interferon and ribavirin in liver transplant recipients

Ref.	Year of publication	No. of patients	Sustained virological response
Coilly *et al*[73]	2013	37	50% (20% for telaprevir and 71% for boceprevir)
Pungpapong *et al*[74]	2013	60	56% (67% for telaprevir and 45% for boceprevir)
Werner *et al*[75]	2012	9	89%
Stravitz *et al*[76]	2013	122	28%
Ann Brown *et al*[77]	2013	46	60%
Faisal *et al*[78]	2014	76	59.5%
Werner *et al*[79]	2014	14	50%

with telaprevir the interactions were more potent around 3-fold and 23-fold with cyclosporine and tacrolimus respectively[73]. Great attention is needed at the time of DAAs discontinuation, increasing the CNI dose the next day and measuring CNI levels at least every 48 h until steady state to be achieved. mammalian target of rapamycin inhibitors are also metabolized by CYP3A4; the clearance of everolimus was reported to decrease by 55% when boceprevir is commenced[80]. No information on the interactions between mycophenolate mofetil and DAAs is available. In healthy individuals prednisone area under the curve was increased by 22%[81].

So far, triple antiviral therapy with first generation DAAs was less effective in patients with genotype 1a, TT/CT interleukin-28B polymorphism and non responders to previous peg-IFN/RBV regimen[82]. However, the approval of sofosbuvir headed forward the care of naive and treatment experienced LT recipients with CHC recurrence with excellent tolerability, without interaction with the immunosuppressive regimen and potent antiviral activity across a broad range of HCV genotypes[43,83]. Particularly, in the single-arm open-label pilot study of Samuel *et al*[83], 40 LT recipients with CHC recurrence after 6 mo of LT treated with sofosbuvir plus RBV for maximum of 6 mo. Thirty-three (83%) patients had genotype 1, 16 (40%) were cirrhotic and 9 (23%) patients were previously on telaprevir or boceprevir combined regimens. All patients (100%) had undetectable HCV RNA after a month of treatment and 70% achieved SVR. Rejection and CNIs dose adjustment were not needed. Only fatigue, anemia, arthralgia and diarrhea were of note. Forns *et al*[43] demonstrated significant antiviral efficacy associated with disease amelioration in 87 LT recipients [72 (83%) presented HCV genotype 1] with severe CHC recurrence including fibrosing cholestatic hepatitis. Fifty-seven (65%) were treated with sofosbuvir combined only with RBV, while 30 (35%) received additional Peg-IFN for up to 48 wk. SVR at 12 wk was achieved in 47 (54%) of LT recipients treated with sofosbuvir plus RBV and in 40 (44%) of LT recipients treated with sofosbuvir plus RBV plus Peg-IFN. No drug side effects were reported. Importantly, 53 (70%) of patients had improved on treatment, 10 (13%) had stabilized and 13 (17%) died with all deaths attributed to progression of liver disease or associated complications.

More recently, very promising are the IFN-free combinations of sofosbuvir with other novel DAAs with or without RBV after LT (Table 7). Although these new DAAs may have interactions with several drugs, they have no contraindication for CNIs co-administration (only simeprevir should not be given with cyclosporine) (Tables 2 and 8). Preliminary data[84-89] showed that they led to excellent on treatment virological response and SVR rates along with minimal adverse effects on difficult to treat LT recipients - those with CHC genotype 1, cirrhosis CP stage B and C as well as previously intolerant or non responsive to IFN therapy. More specifically, sofosbuvir plus simeprevir presented 91% SVR in LT recipients with genotype 1[85]; Sofosbuvir and daclatasvir showed 85% on treatment virological response in LT recipients with genotype 1[86] and major clinical improvement in 71% along with HCV RNA < 15 IU/mL in 95% at 12 wk under treatment in LT recipients with fibrosing cholestatic hepatitis[87]; ledipasvir plus sofosbuvir plus RBV presented 96%-98% SVR in LT recipients with CHC recurrence[88].

Ultimately, preliminary data from Coral I study[89] showed breakthrough results in 34 LT recipients with CHC genotype 1 treated for 24 wk with the following four-drug combination: Paritaprevir (potent NS3/4A protease inhibitor identified by AbbVie and Enanta, formely ABT-450) with ritonavir (which increases overall drug exposures of paritaprevir and enables once daily dosing) combined with Ombitasvir (potent NS5A inhibitor, formerly ABT-267) and Dasabuvir (non-nucleoside NS5B polymerase inhibitor, formerly ABT-333) and RBV. More specifically, 97.1% (33/34) achieved SVR at 4 wk and 97% (33/34) achieved SVR at 12 wk. The regimen was generally well tolerated with 1 patient discontinuing study drug due to adverse events. No deaths, no graft losses, or episodes of rejection were recorded. CNI dosing was manageable over the period of the study (Table 7).

Although data are preliminary, current EASL[6] guidelines recommend the following therapeutic regimens to treat HCV recurrence after LT: sofosbuvir and RBV for LT recipients HCV genotype 2; sofosbuvir and daclatasvir plus/minus RBV for LT recipients HCV genotypes 1, 3-6 and sofosbuvir and simeprevir plus/minus RBV for genotypes 1, 4. All regimens should be continued for 12-24 wk and no dose adjustments are required for CNI inhibitors.

Table 7 Major studies of sofosbuvir with other novel direct antiviral agents with or without ribavirin for treatment of hepatitis C virus positive liver transplant recipients reported in 2014

Ref.	*n*	Patient characteristics	Antiviral scheme	Virological response	SVR	Duration (wk)
Pungpapong *et al*[85]	55	Fibrosis 3-4 (29%) Decompensated cirrhosis (4%) Cholestatic recurrence (15%)	SOF + simeprevir ± RBV	98% (EOT)	91%	12
Leroy *et al*[87] (CUPILT)	21	Fibrosing cholestatic hepatitis	SOF + daclatasvir ± RBV (*n* = 13) SOF + RBV (*n* = 6)	95% HCV RNA < 15 IU/mL 81% were not detectable (at week 12 under treatment)	-	24
Conti *et al*[86]	55	Fibrosis 3-4 (33%) Fibrosing cholestatic hepatitis (7%)	SOF + daclatasvir	85% (at week 8 under treatment)	-	24
Kwo *et al*[89] (CORAL I)	34	Fibrosis 0-2	Paritaprevir + ritonavir + ombitasvir + dasabuvir + RBV	100% (EOT)	97%	24
Reddy *et al*[88]	223	Fibrosis 0-3, CP A-C	SOF + ledipasvir + RBV	-	96%-98% (CP A: 9% CP B: 83%-85% CP C: 60%-67%)	12-24

n: Number of patients included in the study; RBV: Ribavirin; SOF: Sofosbuvir; EOT: End of treatment; SVR: Sustained virological response; CP: Child-Pugh; HCV: Hepatitis C virus.

Table 8 Major drug-drug interactions of the newer direct acting antivirals for hepatitis C

DAA	Co-administration should be avoided
Sofosbuvir	P-glycoprotein inducers Anticonvulsants: Carbamazepine, oxcarbazepine, phenobarbital, phenytoin; antimycobacterials: Rifampin, rifabutin, rifapentin; St. John's wort; HIV drugs: Tipranavir/ritonavir
Simeprevir	Inhibitors or inducers of CYP3A4 Antifungals: Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole; Antibiotics: Clarithromycin, erythromycin, telithromycin; Dexamethasone; Cicapride; HIV drugs: Cobicistat, efavirenz, delavirdine, etravirine, nevirapine, ritonavir and any HIV protease inhibitor P-glycoprotein inducers
Daclatasvir	Strong inducers of CYP3A4 and/or P-glycoprotein *e.g.*, phenytoin, carbamazepine, oxcarbazepine, phenobarbital, rifampicin, rifabutin, rifapentine, dexamethasone, St. John's wort; HIV drugs: darunavir, lopinavir, etravirine
Sofosbuvir/ledipasvir	P-glycoprotein inducers, rosuvastatin, simeprevir

DAA: Direct acting antiviral; HIV: Human immunodeficiency virus.

CONCLUSION

Until 2011, the standard of care treatment for genotype 1 HCV was dual therapy with Peg-IFN and RBV. Unfortunately the success rate was less than 50%, and treatment was frequently associated with significant toxicity. For this reason, much effort has been invested in the development of new treatment for HCV, leading to the approval of the first generation DAAs. Triple therapy with Peg-IFN, RBV and boceprevir or telaprevir was associated with 68% SVR rates in treatment-naïve patients. Whilst the addition of these first generation DAAs marked a bright new era for the prognosis amelioration of LT candidates and recipients with recurrent HCV, there remains the potential for side effects and drug-drug interactions. During 2014, different IFN-free regimens, combinations of second generations DAAs associated with high SVR rates (> 95%), shortened duration courses and relatively few toxicities. However, the efficacy of IFN-free regimens in patients with advanced decompensated cirrhosis waiting for LT is not yet established and their high cost may be the biggest challenge to their implementation worldwide. Immune therapies and therapeutic vaccines are very promising but still in progress. Based on the current related published data and the EASL therapeutic recommendations it was presented the rapidly shifting treatment landscape for CHC LT candidates and recipients. In the context of that treatment decisions continue to be individualized.

REFERENCES

1 **Kim WR**. The burden of hepatitis C in the United States. *Hepatology* 2002; **36**: S30-S34 [PMID: 12407574 DOI: 10.1053/jhep.2002.36791]
2 **Wiesner RH**, Sorrell M, Villamil F. Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. *Liver Transpl* 2003; **9**: S1-S9 [PMID: 14586888 DOI: 10.1053/jlts.2003.50268]
3 **Adam R**, Del Gaudio M. Evolution of liver transplantation for

hepatocellular carcinoma. *J Hepatol* 2003; **39**: 888-895 [PMID: 14642602 DOI: 10.1016/j.jhep.2003.10.003]
4 **Garcia-Retortillo M**, Forns X, Feliu A, Moitinho E, Costa J, Navasa M, Rimola A, Rodes J. Hepatitis C virus kinetics during and immediately after liver transplantation. *Hepatology* 2002; **35**: 680-687 [PMID: 11870384 DOI: 10.1053/jhep.2002.31773]
5 **Vinaixa C**, Rubín A, Aguilera V, Berenguer M. Recurrence of hepatitis C after liver transplantation. *Ann Gastroenterol* 2013; **26**: 304-313 [PMID: 24714603]
6 **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]
7 **Alexopoulou A**, Papatheodoridis GV. Current progress in the treatment of chronic hepatitis C. *World J Gastroenterol* 2012; **18**: 6060-6069 [PMID: 23155334 DOI: 10.3748/wjg.v18.i42.6060]
8 **Gane EJ**, Agarwal K. Directly acting antivirals (DAAs) for the treatment of chronic hepatitis C virus infection in liver transplant patients: "a flood of opportunity". *Am J Transplant* 2014; **14**: 994-1002 [PMID: 24730431 DOI: 10.1111/ajt.12714]
9 **Herbst DA**, Reddy KR. Sofosbuvir, a nucleotide polymerase inhibitor, for the treatment of chronic hepatitis C virus infection. *Expert Opin Investig Drugs* 2013; **22**: 527-536 [PMID: 23448131]
10 **Roche B**, Samuel D. Hepatitis C virus treatment pre- and post-liver transplantation. *Liver Int* 2012; **32** Suppl 1: 120-128 [PMID: 22212582 DOI: 10.1111/j.1478-3231.2011.02714.x]
11 **Parshutin NP**, Korsakov SG. [Comparative analysis of the data of acupuncture electrodiagnosis and hormonal status of women with oligomenorrhea]. *Akush Ginekol* (Mosk) 1990; (**6**): 26-29 [PMID: 2221258]
12 **Bzowej N**, Nelson DR, Terrault NA, Everson GT, Teng LL, Prabhakar A, Charlton MR. PHOENIX: A randomized controlled trial of peginterferon alfa-2a plus ribavirin as a prophylactic treatment after liver transplantation for hepatitis C virus. *Liver Transpl* 2011; **17**: 528-538 [PMID: 21506241 DOI: 10.1002/lt.22271]
13 **Chalasani N**, Manzarbeitia C, Ferenci P, Vogel W, Fontana RJ, Voigt M, Riely C, Martin P, Teperman L, Jiao J, Lopez-Talavera JC. Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized, controlled trials. *Hepatology* 2005; **41**: 289-298 [PMID: 15660392 DOI: 10.1002/hep.20560]
14 **Mazzaferro V**, Tagger A, Schiavo M, Regalia E, Pulvirenti A, Ribero ML, Coppa J, Romito R, Burgoa L, Zucchini N, Urbanek T, Bonino F. Prevention of recurrent hepatitis C after liver transplantation with early interferon and ribavirin treatment. *Transplant Proc* 2001; **33**: 1355-1357 [PMID: 11267325 DOI: 10.1016/S0041-1345(00)02508-2]
15 **Shergill AK**, Khalili M, Straley S, Bollinger K, Roberts JP, Ascher NA, Terrault NA. Applicability, tolerability and efficacy of preemptive antiviral therapy in hepatitis C-infected patients undergoing liver transplantation. *Am J Transplant* 2005; **5**: 118-124 [PMID: 15636619 DOI: 10.1111/j.1600-6143.2004.00648.x]
16 **Sugawara Y**, Makuuchi M, Matsui Y, Kishi Y, Akamatsu N, Kaneko J, Kokudo N. Preemptive therapy for hepatitis C virus after living-donor liver transplantation. *Transplantation* 2004; **78**: 1308-1311 [PMID: 15548968 DOI: 10.1097/01.TP.0000142677.12473.E5]
17 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]
18 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749 DOI: 10.1016/S0140-6736(01)06102-5]
19 **Hadziyannis SJ**, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355 [PMID: 14996676 DOI: 10.7326/0003-4819-140-5-200403020-]
20 **Bruno S**, Shiffman ML, Roberts SK, Gane EJ, Messinger D, Hadziyannis SJ, Marcellin P. Efficacy and safety of peginterferon alfa-2a (40KD) plus ribavirin in hepatitis C patients with advanced fibrosis and cirrhosis. *Hepatology* 2010; **51**: 388-397 [PMID: 19918980 DOI: 10.1002/hep.23340]
21 **Pipili C**, Cholongitas E. Management of patients with hepatitis B and C before and after liver and kidney transplantation. *World J Hepatol* 2014; **6**: 315-325 [PMID: 24868325 DOI: 10.4254/wjh.v6.i5.315]
22 **Rubín A**, Aguilera V, Berenguer M. Liver transplantation and hepatitis C. *Clin Res Hepatol Gastroenterol* 2011; **35**: 805-812 [PMID: 21963086]
23 **Vezali E**, Aghemo A, Colombo M. A review of the treatment of chronic hepatitis C virus infection in cirrhosis. *Clin Ther* 2010; **32**: 2117-2138 [PMID: 21316532 DOI: 10.1016/S0149-2918(11)00022-1]
24 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
25 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
26 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
27 **Zeuzem S**, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]
28 **Sherman KE**, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, Fried MW, Adler M, Reesink HW, Martin M, Sankoh AJ, Adda N, Kauffman RS, George S, Wright CI, Poordad F. Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med* 2011; **365**: 1014-1024 [PMID: 21916639 DOI: 10.1056/NEJMoa1014463]
29 **Joshi D**, Carey I, Agarwal K. Review article: the treatment of genotype 1 chronic hepatitis C virus infection in liver transplant candidates and recipients. *Aliment Pharmacol Ther* 2013; **37**: 659-671 [PMID: 23432320 DOI: 10.1111/apt.12260]
30 **Hezode C**, Dorival C, Zoulim F: Safety and efficacy of telaprevir or boceprevir in combination with peginterferon alfa/ribavirin, in 455 cirrhotic non responders. Week 16 analysis of the French early access program (ANRS O20-CUPIC) in real-life setting. *Hepatology* 2012; **56**: S4 Abstract 51, 217A
31 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]
32 **Sulkowski MS**, Poordad F, Manns MP, Bronowicki JP, Rajender Reddy K, Harrison SA, Afdhal NH, Sings HL, Pedicone LD, Koury KJ, Sniukiene V, Burroughs MH, Albrecht JK, Brass CA, Jacobson IM. Anemia during treatment with peginterferon Alfa-2b/ribavirin and boceprevir: Analysis from the serine protease inhibitor therapy 2 (SPRINT-2) trial. *Hepatology* 2013; **57**: 974-984 [PMID: 23081753 DOI: 10.1002/hep.26096]

33 **Poordad F**, Lwitz E, Reddy K, Afdhal NH, Hezode C, Zeuzem S, Lee SS, Calleja JL, Brown RS, Craxi A, Wedemeyer H, Deng W, Koury K, Boparai N, Pedicone LD, Burroughs M, Wahl J, Brass CA, Albrecht JK, Sulkowski MS. A randomized trial comparing ribavirin dose reduction versus erythropoietin for anemia management in previously untreated patients with chronic hepatitis C receiving boceprevir plus peginterferon/ribavirin. *J Hepatol* 2012; **56**: S549-560 Abstract 1419 [DOI: 10.1016/S0168-8278(12)61430-5]

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

35 **Saxena V**, Manos MM, Yee HS, Catalli L, Wayne E, Murphy RC, Shvachko VA, Pauly MP, Chua J, Monto A, Terrault NA. Telaprevir or boceprevir triple therapy in patients with chronic hepatitis C and varying severity of cirrhosis. *Aliment Pharmacol Ther* 2014; **39**: 1213-1224 [PMID: 24654657 DOI: 10.1111/apt.12718]

36 **French National Agency for Research on AIDS and Viral Hepatitis**. Clinical Trial to Evaluate the Efficacy of Pegylated Interferon-Ribavirin-Boceprevir Triple Therapy in Patients Infected With Genotype 1 HCV With Cirrhosis and Awaiting Liver Transplantation (ANRS HC 29 BOCEPRETRANSPLANT). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: http://clinicaltrials.gov/ct2/show/NCT01463956 NLM Identifier: NCT01463956

37 **Curry MP**, Forns X, Chung RT, Terrault NA, Brown R, Fenkel JM, Gordon F, O'Leary J, Kuo A, Schiano T, Everson G, Schiff E, Befeler A, Gane E, Saab S, McHutchison JG, Subramanian GM, Symonds WT, Denning J, McNair L, Arterburn S, Svarovskaia E, Moonka D, Afdhal N. Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology* 2015; **148**: 100-107.e1 [PMID: 25261839 DOI: 10.1053/j.gastro.2014.09.023]

38 **Afdhal N**, Everson G, Calleja J,McCaughan G, Symonds WT, Denning J, McNair L, McHutchison JG, Arterburn S, Charlton M, Reddy R, Asselah T, Gane E, Forns X. Sofosbuvir and ribavirin for the treatment of chronic hepatitis C with cirrhosis and portal hypertension with or without decompensation: early virologic response and safety. *J Hepatol* 2014; **60**: S23 [DOI: 10.1016/S0168-8278(14)60070-2]

39 **Gane EJ**, Hyland RH, An D, Pang PS, Symonds WT, Mchutchison JG, Stedman CA: Sofosbuvir/Ledipasvir fixed dose combination is safe and effective in -difficult- to treat populations including genotype-3 patients, decompasated type 3 patients and genotype 1 patients with prior sofosbuvir treatment experience. *J Hepatol* 2014; **60**: S1-22 [DOI: 10.1016/S0168-8278(14)60008-8]

40 **Flamm S**, Everson G, Charlton M, Denning J, Arterbur S, Brandt-Sarif, Pang P, McHutchisonJ, Reddy K, Afdahal N: Ledipasvir/Sofosbuvir with Ribavirin for the treatment of HCV in Patients with Decompensated Cirrhosis: Preliminary Results of a Prospective, Multicenter study. *Hepatology* 2014; **60** (Suppl 4): 321A

41 **Donato MF**, Monico S, Malinverno F, Aghemo A, Maggioni M, Reggiani P, Colombo M. Bridging all oral DAA therapy from wait time to post-liver transplant to improve HCV eradication? *Liver Int* 2015; **35**: 1-4 [PMID: 25074044 DOI: 10.1111/liv.12646]

42 **Lens S**, Gambato M, Londoño MC, Forns X. Interferon-free regimens in the liver-transplant setting. *Semin Liver Dis* 2014; **34**: 58-71 [PMID: 24782259 DOI: 10.1055/s-0034-1371011]

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

44 **Berenguer M**, Prieto M, Rayón JM, Mora J, Pastor M, Ortiz V, Carrasco D, San Juan F, Burgueño MD, Mir J, Berenguer J. Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. *Hepatology* 2000; **32**: 852-858 [PMID: 11003634]

45 **Gane EJ**, Portmann BC, Naoumov NV, Smith HM, Underhill JA, Donaldson PT, Maertens G, Williams R. Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med* 1996; **334**: 815-820 [PMID: 8596547]

46 **Forman LM**, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology* 2002; **122**: 889-896 [PMID: 11910340 DOI: 10.1053/gast.2002.32418]

47 **Antonini TM**, Sebagh M, Roque-Afonso AM, Teicher E, Roche B, Sobesky R, Coilly A, Vaghefi P, Adam R, Vittecoq D, Castaing D, Samuel D, Duclos-Vallée JC. Fibrosing cholestatic hepatitis in HIV/HCV co-infected transplant patients-usefulness of early markers after liver transplantation. *Am J Transplant* 2011; **11**: 1686-1695 [PMID: 21749638 DOI: 10.1111/j.1600-6143.2011.03608.x]

48 **Berenguer M**, McCaughan G. Hepatitis C virus-associated cholestatic hepatitis: we cannot seem to agree on diagnostic criteria. *Liver Transpl* 2013; **19**: 115-117 [PMID: 23197377]

49 **Di Bisceglie AM**, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghany MG, Morishima C, Snow KK, Dienstag JL. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; **359**: 2429-2441 [PMID: 19052125]

50 **Di Bisceglie AM**, Stoddard AM, Dienstag JL, Shiffman ML, Seeff LB, Bonkovsky HL, Morishima C, Wright EC, Snow KK, Lee WM, Fontana RJ, Morgan TR, Ghany MG. Excess mortality in patients with advanced chronic hepatitis C treated with long-term peginterferon. *Hepatology* 2011; **53**: 1100-1108 [PMID: 21480316 DOI: 10.1002/hep.24169]

51 **Manousou P**, Samonakis D, Cholongitas E, Patch D, O'Beirne J, Dhillon AP, Rolles K, McCormick A, Hayes P, Burroughs AK. Outcome of recurrent hepatitis C virus after liver transplantation in a randomized trial of tacrolimus monotherapy versus triple therapy. *Liver Transpl* 2009; **15**: 1783-1791 [PMID: 19938143 DOI: 10.1002/lt.21907]

52 **Samonakis DN**, Cholongitas E, Triantos CK, Griffiths P, Dhillon AP, Thalheimer U, Patch DW, Burroughs AK. Sustained, spontaneous disappearance of serum HCV-RNA under immunosuppression after liver transplantation for HCV cirrhosis. *J Hepatol* 2005; **43**: 1091-1093 [PMID: 16239045 DOI: 10.1016/j.jhep.2005.08.005]

53 **Ganschow R**, Pollok JM, Jankofsky M, Junge G. The role of everolimus in liver transplantation. *Clin Exp Gastroenterol* 2014; **7**: 329-343 [PMID: 25214801 DOI: 10.2147/CEG.S41780]

54 **Chatterji U**, Bobardt M, Selvarajah S, Yang F, Tang H, Sakamoto N, Vuagniaux G, Parkinson T, Gallay P. The isomerase active site of cyclophilin A is critical for hepatitis C virus replication. *J Biol Chem* 2009; **284**: 16998-17005 [PMID: 19380579 DOI: 10.1074/jbc.M109.007625]

55 **Cescon M**, Grazi GL, Cucchetti A, Vetrone G, Ravaioli M, Ercolani G, Morelli MC, Piscaglia F, Tamè M, Pinna AD. Predictors of sustained virological response after antiviral treatment for hepatitis C recurrence following liver transplantation. *Liver Transpl* 2009; **15**: 782-789 [PMID: 19562715 DOI: 10.1002/lt.21760]

56 **Klintmalm GB**, Davis GL, Teperman L, Netto GJ, Washburn K, Rudich SM, Pomfret EA, Vargas HE, Brown R, Eckhoff D, Pruett TL, Roberts J, Mulligan DC, Charlton MR, Heffron TG, Ham JM, Douglas DD, Sher L, Baliga PK, Kinkhabwala M, Koneru B, Abecassis M, Millis M, Jennings LW, Fasola CG. A randomized, multicenter study comparing steroid-free immunosuppression and standard immunosuppression for liver transplant recipients with chronic hepatitis C. *Liver Transpl* 2011; **17**: 1394-1403 [PMID: 21850690 DOI: 10.1002/lt.22417]

57 **Neumann U**, Samuel D, Trunečka P, Gugenheim J, Gerunda GE, Friman S. A Randomized Multicenter Study Comparing a Tacrolimus-Based Protocol with and without Steroids in HCV-Positive Liver Allograft Recipients. *J Transplant* 2012; **2012**: 894215 [PMID: 22690326 DOI: 10.1155/2012/894215]

58 **Verna EC**, Brown RS. Hepatitis C virus and liver transplantation.

Clin Liver Dis 2006; **10**: 919-940 [PMID: 17164125 DOI: 10.1016/j.cld.2006.08.012]

59 **Fagiuoli S**, Ravasio R, Lucà MG, Baldan A, Pecere S, Vitale A, Pasulo L. Management of hepatitis C infection before and after liver transplantation. *World J Gastroenterol* 2015; **21**: 4447-4456 [PMID: 25914454]

60 **Gurusamy KS**, Tsochatzis E, Xirouchakis E, Burroughs AK, Davidson BR. Antiviral therapy for recurrent liver graft infection with hepatitis C virus. *Cochrane Database Syst Rev* 2010; (**1**): CD006803 [PMID: 20091608]

61 **Blasco A**, Forns X, Carrión JA, García-Pagán JC, Gilabert R, Rimola A, Miquel R, Bruguera M, García-Valdecasas JC, Bosch J, Navasa M. Hepatic venous pressure gradient identifies patients at risk of severe hepatitis C recurrence after liver transplantation. *Hepatology* 2006; **43**: 492-499 [PMID: 16496308 DOI: 10.1002/hep.21090]

62 **Neumann UP**, Berg T, Bahra M, Seehofer D, Langrehr JM, Neuhaus R, Radke C, Neuhaus P. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. *J Hepatol* 2004; **41**: 830-836 [PMID: 15519657 DOI: 10.1016/j.jhep.2004.06.029]

63 **Coilly A**, Roche B, Samuel D. Current management and perspectives for HCV recurrence after liver transplantation. *Liver Int* 2013; **33** Suppl 1: 56-62 [PMID: 23286847 DOI: 10.1111/liv.12062]

64 **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]

65 **Carrión JA**, Torres F, Crespo G, Miquel R, García-Valdecasas JC, Navasa M, Forns X. Liver stiffness identifies two different patterns of fibrosis progression in patients with hepatitis C virus recurrence after liver transplantation. *Hepatology* 2010; **51**: 23-34 [PMID: 19839063 DOI: 10.1002/hep.23240]

66 **Roche B**, Sebagh M, Canfora ML, Antonini T, Roque-Afonso AM, Delvart V, Saliba F, Duclos-Vallee JC, Castaing D, Samuel D. Hepatitis C virus therapy in liver transplant recipients: response predictors, effect on fibrosis progression, and importance of the initial stage of fibrosis. *Liver Transpl* 2008; **14**: 1766-1777 [PMID: 19025933 DOI: 10.1002/lt.21635]

67 **Gierej B**, Kobryń K, Gierej P, Górnicka B. C4d in acute rejection after liver transplantation and its usefulness in differential diagnosis between acute liver rejection and hepatitis C recurrence. *Ann Transplant* 2014; **19**: 373-381 [PMID: 25082343 DOI: 10.12659/AOT.890234]

68 **Selzner N**, Guindi M, Renner EL, Berenguer M. Immune-mediated complications of the graft in interferon-treated hepatitis C positive liver transplant recipients. *J Hepatol* 2011; **55**: 207-217 [PMID: 21145865]

69 **Berenguer M**. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. *J Hepatol* 2008; **49**: 274-287 [PMID: 18571272 DOI: 10.1016/j.jhep.2008.05.002]

70 **Wang CS**, Ko HH, Yoshida EM, Marra CA, Richardson K. Interferon-based combination anti-viral therapy for hepatitis C virus after liver transplantation: a review and quantitative analysis. *Am J Transplant* 2006; **6**: 1586-1599 [PMID: 16827859 DOI: 10.1111/j.1365-2893.2008.01019]

71 **Xirouchakis E**, Triantos C, Manousou P, Sigalas A, Calvaruso V, Corbani A, Leandro G, Patch D, Burroughs A. Pegylated-interferon and ribavirin in liver transplant candidates and recipients with HCV cirrhosis: systematic review and meta-analysis of prospective controlled studies. *J Viral Hepat* 2008; **15**: 699-709 [PMID: 18673428]

72 **Ramachandran P**, Fraser A, Agarwal K, Austin A, Brown A, Foster GR, Fox R, Hayes PC, Leen C, Mills PR, Mutimer DJ, Ryder SD, Dillon JF. UK consensus guidelines for the use of the protease inhibitors boceprevir and telaprevir in genotype 1 chronic hepatitis C infected patients. *Aliment Pharmacol Ther* 2012; **35**: 647-662 [PMID: 22296568 DOI: 10.1111/j.1365-2036.2012.04992.x]

73 **Coilly A**, Roche B, Dumortier J, Leroy V, Botta-Fridlund D, Radenne S, Pageaux GP, Si-Ahmed SN, Guillaud O, Antonini TM, Haïm-Boukobza S, Roque-Afonso AM, Samuel D, Duclos-Vallée JC. Safety and efficacy of protease inhibitors to treat hepatitis C after liver transplantation: a multicenter experience. *J Hepatol* 2014; **60**: 78-86 [PMID: 23994384 DOI: 10.1016/j.jhep.2013.08.018]

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

75 **Werner CR**, Egetemeyr DP, Lauer UM, Nadalin S, Königsrainer A, Malek NP, Berg CP. Telaprevir-based triple therapy in liver transplant patients with hepatitis C virus: a 12-week pilot study providing safety and efficacy data. *Liver Transpl* 2012; **18**: 1464-1470 [PMID: 22941516 DOI: 10.1002/lt.23542]

76 **Stravitz TLJ**, Dodge J, Saxena V, Burton J, Verna E, O'Leary J, Parikh N, Everson G, Brown R, Trotter J, Terrault N: Higher Sustained Virologic Response (SVR-12) Achievable in Liver Transplant (LT) Recipients with Hepatitis C (HCV) Treated with Protease Inhibitor (PI) Triple Therapy (TT). *Hepatology* 2013: **58** (4 suppl)

77 **Ann Brown K**, Russo M, Levitsky J, Yoshida E, Vargas H, Bsharat M, Rubin R, Brown R. Twice -daily Telaprevir in combination with Peginterferon alfa 2α/ribavirin in genotype 1 HCV Liver Transplant recipients: Interim week 16 Safety and Efficacy Results on the Prospective, Multicenter REFRESH Study. *Hepatology* 2013; **58**: 4

78 **Faisal N**, Yoshida EM, Bilodeau M, Wong P, Ma M, Burak KW, Al-Judaibi B, Renner EL, Lilly LB. Protease inhibitor-based triple therapy is highly effective for hepatitis C recurrence after liver transplant: a multicenter experience. *Ann Hepatol* 2014; **13**: 525-532 [PMID: 25152985]

79 **Werner CR**, Egetemeyr DP, Nadalin S, Königsrainer A, Malek NP, Lauer UM, Berg CP. Treatment of recurrent genotype 1 hepatitis C post-liver transplantation: single center experience with telaprevir-based triple therapy. *Z Gastroenterol* 2014; **52**: 27-34 [PMID: 24420796 DOI: 10.1055/s-0033-1356345]

80 **Coilly A**, Furlan V, Roche B, Barau C, Noël C, Bonhomme-Faivre L, Antonini TM, Roque-Afonso AM, Samuel D, Taburet AM, Duclos-Vallée JC. Practical management of boceprevir and immunosuppressive therapy in liver transplant recipients with hepatitis C virus recurrence. *Antimicrob Agents Chemother* 2012; **56**: 5728-5734 [PMID: 22908172]

81 **Jumes P**. Pharmakokinetic interaction between the HCV protease inhibitor boceprevir and prednisolone in healthy volunteers. *Hepatology* 2012; **56** (S1): 10676A

82 **Coilly A**, Roche B, Duclos-Vallée JC, Samuel D. Management of HCV transplant patients with triple therapy. *Liver Int* 2014; **34** Suppl 1: 46-52 [PMID: 24373078 DOI: 10.1111/liv.12406]

83 **Samuel D**, Charlton E, Gane E, Brown JR, Curry M, Kwo P, Fontana R, GilroyR, Teperman L, Muir AJ, McHutchison JG, Symonds WT, Denning J, McNair L, Arterburn S, Terrault N, Forns X, Manns M. Sofosbuvir plus ribavirin for the treatment of recurrent hepatitis C infection after liver transplantation: Results of a prospective, multicenter study. *J Hepatol* 2014; **60**: S49910

84 **Aqel B**, Pungpapong S, Werner KT, Chervenak A, Rakela J, Watt K, Leise M, Murphy J, Henry T, Ryland K. The use of Sofosbuvir and Simeprevir to treat HCV G1 in the liver transplant setting: The experience of three transplant settings. *Hepatology* 2014; **60** (S4): 206A

85 **Pungpapong S**, Werner KT, Aqel B, Leise M, Murphy J, Henry T, Ryland K, Chervenak A,Watt K, Vargas H, Keaveny A. Multicenter Experience using Sofosbuvir and Simeprevir with /without Ribavirin to treat HCV Genotype 1 after Liver Transplantation. *Hepatology* 2014; **60** (S4): 201A

86 **Conti F,** Lebray P, Schielke A, Regnaut H, Thabut D, Eyroad D, Poujol-Robert A, Chazouilleres O, Calmus Y. Sofosbuvir/Daclatasvir therapy for recurrent Hepatitis C after Liver Transplantation:

Preliminary report from the Parisian centers. *Hepatology* 2014; **60** (S4): 208A

87 **Leroy V**, Dumortier J, Coilly A, Sebag M, Fougerou-Leurent C, Radenne S, Botta D, Dourand F, Silvain C, Lebray P.. High rates of virological response and major clinical improvement during sofosbuvir and daclatasvir -based regimens for the treatment of fibrosing cholestatic HCV- recurrence after liver transplantation: The ANRS study CO23 COPILT study. *Hepatology* 2014; **60** (S4): 207A

88 **Reddy K**, Everson G, Flamm S, Denning J, Arterburn S, Brandt-Sarif T, Pang P, McHutchison J, Curry M, Charlton M. Ledipasvir/Sofosbuvir with ribavirin for the treatment of HCV in patients with post transplant recurrence: Preliminary results of a prospective multicenter study. *Hepatology* 2014; **60** (S4): 200A

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

P- Reviewer: Dang SS, Inoue K, Kapoor S **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1617

World J Hepatol 2015 June 28; 7(12): 1617-1631
ISSN 1948-5182 (online)

REVIEW

Predictive factors associated with hepatitis C antiviral therapy response

Lourianne Nascimento Cavalcante, André Castro Lyra

Lourianne Nascimento Cavalcante, André Castro Lyra, Hospital Sao Rafael - Gastro-Hepatology Service, Salvador, Bahia 41253-190, Brazil
André Castro Lyra, Department of Medicine, Federal University of Bahia, Salvador, Bahia 40026-010, Brazil

Author contributions: Cavalcante LN and Lyra AC contributed equally to this work.

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

Correspondence to: Lourianne Nascimento Cavalcante, MD, Hospital Sao Rafael - Gastro-Hepatology Service, Diretoria Científica, 6° andar Sao Rafael Av. 2152 - Sao Marcos, Salvador, Bahia 41253-190, Brazil. lourianne@gmail.com
Telephone: +55-71-32816432
Fax: +55-71-32816855

Received: August 28, 2014
Peer-review started: August 29, 2014
First decision: November 14, 2014
Revised: December 16, 2014
Accepted: May 5, 2015
Article in press: May 6, 2015
Published online: June 28, 2015

Abstract

Hepatitis C virus (HCV) infection may lead to significant liver injury, and viral, environmental, host, immunologic and genetic factors may contribute to the differences in the disease expression and treatment response. In the early 2000s, dual therapy using a combination of pegylated interferon plus ribavirin (PR) became the standard of care for HCV treatment. In this PR era, predictive factors of therapy response related to virus and host have been identified. In 2010/2011, therapeutic regimens for HCV genotype 1 patients were modified, and the addition of NS3/4a protease inhibitors (boceprevir or telaprevir) to dual therapy increased the effectiveness and chances of sustained virologic response (SVR). Nevertheless, the first-generation triple therapy is associated with many adverse events, some of which are serious and associated with death, particularly in cirrhotic patients. This led to the need to identify viral and host predictive factors that might influence the SVR rate to triple therapy and avoid unnecessary exposure to these drugs. Over the past four years, hepatitis C treatment has been rapidly changing with the development of new therapies and other developments. Currently, with the more recent generations of pan-genotipic antiviral therapies, there have been higher sustained virologic rates, and prognostic factors may not have the same importance and strength as before. Nonetheless, some variables may still be consistent with the low rates of non-response with regimens that include sofosbuvir, daclatasvir and ledipasvir. In this manuscript, we review the predictive factors of therapy response across the different treatment regimens over the last decade including the new antiviral drugs.

Key words: Hepatitis C; Direct acting antivirals; Antiviral therapy; Interferon; Sustained virologic response

Core tip: Treatment of chronic hepatitis C has been changing very rapidly in recent years. The chances of cure have increased with the new drugs. Predictive factors of sustained treatment response in the "age" of based-interferon therapy is becoming less important with the arrival of the direct acting antivirals, however, viral genotype, cirrhosis and viral kinetics may still

impact on therapy outcome with the new available drugs.

Cavalcante LN, Lyra AC. Predictive factors associated with hepatitis C antiviral therapy response. *World J Hepatol* 2015; 7(12): 1617-1631 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1617.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1617

INTRODUCTION

Hepatitis C virus (HCV) is an important etiology of chronic hepatitis and cirrhosis and is the leading indication for liver transplantation (LT) in adults around the world[1,2]. Therefore, early recognition and effective management of the disease can modify its natural history. HCV infection may lead to significant liver injury, and viral, environmental and host factors, including immunologic and genetic susceptibilities, may contribute to differences in the disease expression and treatment response[3]. This genetic susceptibility has a significant part in developing of HCV infection, from viral antigen recognition and presentation to the type of immune response developed against the pathogen[3].

The natural history of HCV genotypes 1, 2 and 3 infection appears to be similar, and patients are at risk for developing liver cirrhosis, decompensation of liver disease and hepatocellular carcinoma. HCV genotype 3 is also associated with an increase in hepatic steatosis, which is believed to be related to viral interference in host lipid metabolism[4].

The predictive factors of therapy response are also related to the virus and hosts, and they can be classified as clinical, immunologic and genetic factors. HCV genotype 1, male gender, advanced liver fibrosis, human immunodeficiency virus (HIV) and HBV coinfection, insulin resistance, poor treatment adherence, high viral load (⩾ 600.000 UI/mL) and African ancestry have been related with the failure of interferon (IFN) based therapies, particularly with dual therapy (pegylated interferon and ribavirin)[1,5].

Gene polymorphisms that encode or regulate the host molecular expression may be useful as disease evaluation markers and therapy response predictors; moreover, they could provide helpful information for understanding the complex mechanisms underlying the virus-host interaction and the variations observed in antiviral therapy responses. The interleukin-28B (IL28B) polymorphisms were considered the strongest baseline identified predictors of dual therapy response (pegylated interferon and ribavirin); they are also predictors of viral kinetics and spontaneous clearance in acute HCV infection. IL28B polymorphisms differs among different ethnic backgrounds[6,7].

Along the last four years, hepatitis C treatment has rapidly changed with the development of new therapies and other advancements, and the chances of cure are significantly increasing. Initially, with the first-generation direct acting antivirals (DAA) boceprevir and telaprevir, individuals with HCV genotype 1 achieved sustained virologic response (SVR) rates of nearly 70% or greater, and the viral kinetics and hepatic fibrosis were the main predictors of response[8,9]. Currently, with the more recent generations of pangenotipic antiviral therapies, there have been even higher SVR rates, and prognostic factors may not have the same importance and strength as before. Nevertheless, viral kinetics, the presence of liver cirrhosis and HCV genotype 3 may still be relevant factors that influence the rates of SVR[9].

PROGNOSTIC FACTORS IN DUAL PEGYLATED INTERFERON PLUS RIBAVIRIN THERAPY ERA

Early in 2000s, dual therapy using a combination of pegylated interferon-alpha plus ribavirin (PR) was the standard of care for HCV treatment. HCV genotype 1 infection used to be the most difficult genotype to treat, with relatively low SVR rates compared to current rates, and required an expected duration of therapy of 48 wk that could be extended to 72 wk for partial responders. Conversely, for genotypes 2 and 3 HCV infected patients, the recommended treatment time was 12 to 48 wk, and superior SVR rates were reached compared with HCV genotype 1[1,5,10]. The sustained virological response proportion for HCV genotype 1 infected patients were 40%-50%, whereas for HCV genotype 2 and 3 infected subjects it was, approximately, 75%-80%[1].

In the PR era, predictive factors of therapy response related to virus and host have been identified. Clinical host characteristics possibly correlated with the response to dual regimen are gender, age, dyslipidemia, insulin resistance, liver fibrosis stage, ancestry, 25(OH) vitamin D status, coinfection with HBV and/or HIV. Immunologic elements related with response to PR treatment are cytokines and interferon-gamma inducible protein 10 (IP-10); in addition, genetic factors such as IL28B polymorphisms, other genes associated with JAK-STAT pathway polymorphisms and genetic ancestry markers have also been described as predictors of response[11]. The HCV features associated with therapy antiviral response are genotype, baseline viral load and viral kinetics at specific time-points throughout the treatment. The single most important viral factor that influences the response to antiviral treatment is HCV genotype, and the most important host factors are IL28B genotype and liver fibrosis[12-14].

Pegylated interferon alpha-2a and -2b

Both pegylated interferons may be used during PR therapy. In the Ideal study, which included 3070 HCV genotype 1 infected patients, the SVR rates were similar among the schemes with pegylated interferon alpha 2a or 2b; the SVR rates were 40.9% and 39.8%, respectively, without statistically significant differences[15].

In HCV genotypes 2 and 3, ribavirin doses (800 mg/d compared to 1000-1200 g/d adjusted for weight (kg) did not produce any difference in SVR rates when patients were treated for at least 24 wk[13,16].

On the other hand, a meta-analysis of 26 studies included, 11 were randomized and 15 were non-randomized, with a total number of 18260 patients (8125 patients were treated with PEG-IFN alpha 2a and 10135 were treated with PEG-IFN alpha-2b) and showed that dual therapy with PEG-INF-alpha 2a was associated with a greater SVR than PEG-IFN-alpha 2b in HCV monoinfected patients, particularly for genotypes 1 and 4. An analysis of randomized clinical trials, including HCV-type 1 and 4 patients, showed SVR rates of 43.2% for the PEG-IFN-alpha 2a group and 38.7% for the PEG-IFN-alpha 2b and ribavirin group. In the HCV genotypes 2 and 3 group, the SVR rates among patients treated with PEG-IFN-alpha 2a was 82.6% and 75.5% for the PEG-IFN-alpha 2b and ribavirin group[17].

Ancestry/race and SVR

Ancestry is an important marker of response to IFN-based treatments in people chronically infected by HCV. People of African descent have lower chances of success with dual antiviral therapy compared with Caucasians. These findings were observed in more homogeneous populations, with low rates of racial admixture assessed by self-reported ancestry; similar findings were found in admixed populations when ancestry was assessed using genetic markers[18]. Although people of African descent are most commonly infected by HCV genotype 1, randomized studies ruled out the possibility that HCV genotype infection was the reason for the lower response to antiviral treatment in this group. The immunological and genetic background appear to be the reasons for this suboptimal response because these differences did not occur as a result of therapy adherence, viral genotype, histopathological changes, type of IFN or social or the economic status of the patients[19,20]. The IL28B polymorphisms are most likely influenced by ancestry, and these variables may modify IFN-based therapy outcomes[21].

IL28B polymorphisms

Genome Wide Association Studies (GWAS) described single nucleotide polymorphisms of genes in the area of the IFN-λ as powerful predictors of therapy response with double regimen with peg-IFN PR in patients infected by hepatitis C genotype 1 and of spontaneous viral clearance during acute HCV infection[6,7,18,21-24]. In HCV genotypes 2 and 3 infected individuals, the outcomes were somewhat controversial; some studies showed that IL28B polymorphisms are associated with rapid virologic response (RVR) and not SVR, whereas others showed that IL28B polymorphisms are associated with SVR only in patients who did not get RVR[24].

The most studied IL28B genetic polymorphisms are IL28B (IFN-λIII) rs12979860 single nucleotide polymorphism (SNP) (T > C) and rs8099917 (T > G), which are separated by 4378 nucleotides. In genetic studies with hepatitis C infected patients, both IL28B variants (rs12979860 and rs8099917) are in linkage disequilibrium, and in HCV genotype 1 patients treated with pegylated IFN PR, the IL28B C/C genotype in rs12979860, T/T in rs8099917 and A/A in rs12980275 were associated with sustained virological response[7,25].

According to GWAS, SNP rs12979860 IL28B C/C genotype was strongly related with superior chances of SVR, and it was observed that ancestry had affected the results. Individuals with European ancestry and C/C genotype had two times superior chances of achieving SVR than did subjects with T/T genotype; IL28B C/C African Americans had three times higher chances of achieving SVR than IL28B T/T genotype African Americans, and among Hispanics, the C/C genotype was associated with a twofold higher chance of SVR compared to the T/T genotype[26]. Data revealed that African Americans with chronic hepatitis C genotype 1 and Il28B C/C have better SVR rates compared to European Americans (north American Caucasians) without the IL28B C/C genotype[27]. The C allele is more common in populations of European and Asian ancestries, and this supports the hypothesis that some of the differences in SVR rates among people of African descent and Caucasians can be explained by the variance in the frequency of the C allele in these populations. Asians infected with hepatitis C genotype 1 virus had higher SVR rates compared with Caucasians, African Americans and Hispanics, and the frequency of the IL28B alleles is a possible explanation for this difference[7].

An analysis of the Brazilian admixed population showed that the *IL28B* gene polymorphisms, rs12979860 and rs8099917, were also predictors of SVR to PR dual therapy consistent with results of studies conducted in populations with low levels of racial admixture[18]. However, in the studied admixed population, the association among ancestry, IL28B polymorphisms and therapy response was detected only when ancestry was assessed using genetic markers[18].

Liver fibrosis

Liver fibrosis is a host factor that has consistently been associated with response rates to IFN-based therapies. Patients with advanced liver fibrosis (Metavir F3 or F4) have a lower chance of SVR compared to subjects with milder liver fibrosis. Invasive (liver-biopsy) and non-invasive methods may be used to assess the fibrosis stage[25].

IP-10

The combination of serum IP-10 and IL28B SNPs may increase the predictive value of the treatment response. The IP-10 (IFN - IP-10) is also called CXCL10 and belongs to the CXC chemokine family. IP-10 is a indicator of liver inflammation and fibrosis in individuals with chronic hepatitis C. Low pre-treatment IP-10 levels have been related with SVR and on the other hand, increased levels

have been associated with therapy failure. A baseline IP-10 level > 600 pg/mL was determined to be greatly predictive of an unfavorable therapy outcome[26,27].

Vitamin D

Studies including HCV genotypes 1 and 4 infected patients have revealed that low vitamin D status is related with inferior probabilities of achieving SVR following peg-IFN alpha PR therapy[28,29]. Nevertheless, a recent published systematic review and meta-analysis did not confirm these findings[30]. The authors found no significant association between the baseline mean 25(OH) D level and SVR (OR = 1.44; *P* = 0.11), either in patients infected with HCV genotypes 1, 4, 5 (OR = 1.48; *P* = 0.09) or genotypes 2/3 (OR = 1.51; *P* = 0.65).

Statin use

The role of metabolic factors as well overweight and visceral obesity, hepatic steatosis, insulin resistance and diabetes, in the response to antiviral therapy has been studied widely in the last decade[30]. To assessing the role of statins on HCV response rate to treatment, several studies analyzed the addition of fluvastatin to the HCV treatment (peg-IFN and ribavirin)[31,32]. The use of statins significantly improved SVR (OR = 2.02, 95%CI: 1.38-2.94), RVR (OR = 3.51, 95%CI: 1.08-11.42) and early virologic response (OR = 1.89, 95%CI: 1.20-2.98). The SVR rate substantially improved for HCV genotype 1 (OR = 2.11; 95%CI: 1.40-3.18). There was not an important increase in adverse events reports and withdrawn with the adding of statins.

Gender

Females overall appear to have higher chances of achieving SVR. Nevertheless, several studies have suggested that in HCV genotype 1 infected women, menopause is related with an increased severity of liver fibrosis, and with a lower likelihood of response to therapy with peg-IFN and ribavirin[33-36].

A cohort of HCV patients treated with dual therapy revealed that SVR was independently related with female gender, younger age, IL28B C/C genotype, viral genotype and low baseline levels of serum HCV-RNA[35]. However, females older than 50 years infected with HCV genotype 1 achieved lower rates of SVR. The possible reason was that, at baseline, females older than 50 years included in cohort had high body mass index and visceral obesity, metabolic alterations and severe histological liver damage, findings more frequently observed in the menopause females. In genotype 2 and genotype 3 patients, gender usually does not affect the SVR[37].

RVR

As reported in several studies, RVR (HCV RNA viral load undetectable at week 4) is associated with a notably higher rate of SVR. Some trials have observed that patients infected with HCV genotypes 2 or 3 achieve RVR in higher proportions than patients infected with genotype 1. However, regardless of the HCV genotype, patients who reach RVR have the highest rates of SVR. In the study by Fried *et al*[37] RVR was achieved by 16% of patients with genotype 1, 71% of genotype 2 and 60% of genotype 3. Among individuals who reached RVR, the SVR rate was high across all HCV-genotypes and ranged from 88% to 100% (genotypes 1-4). Baseline predictive factors of RVR comprised genotype, low baseline viral load, high alanina aminotransferase ratio, nonexistence of advanced fibrosis, and younger age. RVR was the most important predictor of SVR based on logistic regression analysis[37]. Among HCV genotype 3 infected patients, if RVR is present, the treatment period may be shortened. In a previous trial, among patients with RVR - week 4, SVR was 81.6% among patients treated for 24 wk and 82.5% among them treated for 12 wk. In patients without RVR, SVR was 52.1% if the treatment duration was 24 wk and 61.7% if the duration was 36 wk. According to this study, HCV genotype 3 patients with RVR may be treated for 12 wk if the appropriate ribavirin doses are used; in patients without RVR, the SVR rates were higher with 36 wk of treatment compared with 24 wk[38].

FIRST WAVE OF DIRECT ACTING ANTIVIRAL (TELAPREVIR AND BOCEPREVIR)

In 2010/2011, therapeutic regimens for HCV genotype 1 patients were modified, and the adding NS3/4a protease inhibitors to dual therapy increased the effectiveness and chances of SVR. The grouping of pegylated IFN, ribavirin and a protease inhibitor (boceprevir or telaprevir) significantly improved the SVR rates compared with dual treatment (approximately 70% to 80% *vs* 40% to 50% SVR, respectively)[9,39,40]. Protease inhibitors should not be used as monotherapy, due to the development of resistance and genetic modification of the host barrier. With triple therapy, there is the possibility of shortening treatment as guided by viral kinetics. Individuals with IL28B (rs12979860) genotype C/C have higher chances of achieving shortened response guided therapy. Randomized trials have suggested that patients with unfavorable IL28B genotypes (C/T and T/T, rs12979860) had significantly improved SVR rates when protease inhibitors were combined with dual therapy[9,39,41-45] (Tables 1 and 2).

Nevertheless, the first generation triple therapy is associated with many adverse events, some of which are serious and associated with death, especially in cirrhotic patients. This led to the need to identify viral and host predictive factors that might influence the SVR rate to triple therapy, and additionally, it was important to determine whether a subdivision of patients might have a higher likelihood of response to dual therapy so that the use of first-generation protease inhibitors with their associated adverse effects and high costs could be

Table 1 Risk factors associated with response (sustained virologic response 12) to first-generation direct acting antivirals in naive patients

	SVR12 rates (%)				
	Boceprevir			Telaprevir	
Predictive variables	PR48	BOCRGT	BOCPR48	PR48	T12PR48
Naïve	40	67	66	44	75
Mild-moderate fibrosis	38	67	67	47	118.5
Advanced fibrosis	38	41	52	33	62
Black race	23	42	53	25	62
HCVRNA viral load < 800.000 IU/mL	64	76	85	36	74
IL28B C/C	78	82	80	64	90
Il28B C/T	28	65	71	23	71
IL28B T/T	27	55	59	25	73
HCV genotype 1a	35	59	63	41	71
HCV genotype 1b	40	66	70	4	79
BMI < 25	47	58	67	44	83
BMI ⩾ 30	33	48	66	41	71
Relapse	22	9	9	28	9

Data obtained, analyzed and adapted from Ref. [8,9,44]. PR48: Standard therapy with pegylated interferon plus ribavirin for 48 wk; BOCRGT: Boceprevir with therapy possibly shortened by response guided therapy; BOCPR48: Boceprevir with treatment fixed time for 48 wk; T12PR48: Telaprevir by 12 wk and standard therapy for 48 wk; IL28B: Interleukin-28B; BMI: Body mass index; HCV: Hepatitis C virus; SVR: Sustained virologic response.

Table 2 Risk factors associated with therapeutic response to first-generation direct acting antivirals in patients previously treated with pegylated interferon and ribavirin

	SVR rates (%)				
	Boceprevir			Telaprevir	
Predictive variables	PR48	BOCRGT	BOCPR48	PR48	T12PR48
Previous relapser	29	69	75	24	83
Previous partial-responder	7	40	52	15	59
Previous null responder				5	29
Mild-moderate fibrosis	23	63	68	16	76
Advanced fibrosis	13	44	68	11	49
Black race	8	61	63	10	55
HCVRNA viral load > 800.000 IU/mL (baseline)					
IL28B C/C	46	79	77	29	79
Il28B C/T	17	61	73	16	60
IL28B T/T	50	55	72	13	61
HCV genotype 1a	24	5	61		
HCV genotype 1b	22	65	73		
BMI < 25	20	6	68		
BMI ⩾ 30	11	56	65		
Relapse	15	59	54		

Data obtained, analyzed and adapted from Ref. [40,41,44,45]. PR48: Standard therapy with pegylated interferon plus ribavirin for 48 wk; BOCRGT: Boceprevir with therapy possibly shortened by response guided therapy; BOCPR48: Boceprevir with treatment fixed time for 48 wk; T12PR48: Telaprevir by 12 wk and standard therapy for 48 wk; IL28B: Interleukin-28B; BMI: Body mass index; HCV: Hepatitis C virus; SVR: Sustained virologic response.

avoided.

SVR rates to treatment regimens containing protease inhibitors vary with the type of prior non-response to treatment. Naïve individuals reach response rates between 67% and 75% (Table 1), and among relapsers to previous dual PR therapy, the response rates vary between 69% and 88%; for previous partial responders, the response rates are between 40% and 59%, and for previous null responders, the SVR rates vary between 23% and 38%[9,39,41,45]. However, the response rates are lower among individuals with liver cirrhosis (SVR = 11%-68%) and are higher among subjects with IL28B genotype C/C (Table 2).

In two phase 3 trials including boceprevir, baseline predictors of SVR in previously treated patients include former treatment response (previous relapse rather than previous partial nonresponse), nonexistence of cirrhosis, use of triple therapy rather than PR, low viral load at baseline and lack of cirrhosis. In previously naive patients, based on multivariate analysis, baseline predictors of SVR were triple therapy, non-black race, low viral load at baseline (< 400.00 UI/mL), age (⩽ 40 years), statin use and absence of cirrhosis. In both studies, a 1 $\log_{10}$ IU/mL decline in HCV-RNA viral load

after the 4-wk (lead-in) on-treatment was the greatest predictor of SVR[8,45,46].

Untreated subjects with unfavorable predictive variables of therapy response to PR treatment have improved the chances of cure with the addition of telaprevir. Patients with advanced fibrosis (bridging fibrosis or cirrhosis), older age, diabetes mellitus, and HCV RNA levels of 800000 IU/mL or higher, black race and C/T and T/T *IL28B* genotypes showed improved chances of an HCV cure[9]. Studies with telaprevir-based triple therapy including previously treated patients evaluated previous partial responders, relapsers and null responders. The SVR rates during treatment were higher in patients who had previous relapse or partial response than in patients who had null response. Based on these analyzes, advanced fibrosis appears to be associated to unsuccessful, especially among patients with no response or a partial response to previous treatment, although there was no such effect on prior relapsers. The lead-in phase with peguilated IFN alpha-2a PR before telaprevir intake did not improve the response rate[41] (Table 2).

Lead in phase as a predictor of SVR

Clinical trials have suggested that the lead-in phase, by evaluating the sensitivity to IFN, is able to predict the efficacy of triple therapy using first generation DAA[47]. Lead-in phase consists of four weeks of pegylated IFN and ribavirin treatment before triple therapy. A viral load decline > 1 log after lead-in was the strongest predictor of SVR in both naïve and previously treated patients with boceprevir[44]. Notably, in individuals with a viral load decline less than 1 log after lead-in phase, the chances of achieving SVR were lower, which may reflect resistance to IFN. In the Sprint-2 trial, patients who achieved more than a 2 log viral load decline after lead-in had an SVR rate greater that 80%.

The rationale for performing a lead-in phase is to avoid adverse effects associated with triple therapy with boceprevir or telaprevir in patients with few chances of SVR, particularly in cirrhotic and previous experimented patients. Poor-IFN sensitive patients without RVR at week 4 after lead-in and with other unfavorable predictors to SVR may avoid the disadvantages of triple therapy with boceprevir or telaprevir and should be treated with new IFN-free therapies.

A multivariate analysis to evaluate the baseline markers that predict this IFN response after the lead-in phase, accessing previously untreated patients, showed that baseline markers of good response involved the following: IL28B C/C genotype, low baseline viral load, absence of cirrhosis, and lower body mass index (BMI). In previous treatment-failures, baseline predictors of good response after lead-in were IL28B C/C genotype and previous relapse to PR therapy. Statistically significant differences in SVR rates for patients who did not reach a 1 Log IU/mL decline after lead-in were observed such as the following: patients with genotype 1b *vs* 1a (47% *vs* 25%), METAVIR score F0/1/2 *vs* F3/4 (38% *vs* 17%), and baseline viral load ⩽ 800000 *vs* > 800000 (69% *vs* 31%). Gender, race (black *vs* nonblack), age, BMI and steatosis score were not associated with response in this subgroup of patients[44].

First generation DAA and IL28B

The IL28B C/C genotype is a strong predictor of IFN response with PR therapy, however, with first wave *DAA* IFN-based triple therapy IL28B C/C genotype is a good marker of early response in naïve or previous treatment experimented patients and viral kinetics is the strongest predictor of SVR.

Sprint-2 and Respond-2 were phase Ⅲ studies that evaluated the effectiveness of triple therapy with pegylated IFN, ribavirin and boceprevir in naïve and previous treated patients, respectively. Subanalysis of these studies assessed the impact of the IL28B polymorphism (rs12979860) as a predictor of therapy response[41,43,47,48]. The genotype C/C IL28B was the best predictor of treatment response at week 4 (after lead-in) and week 8. Considering the IL28B C/C and RVR (HCVRNA < 100 IU/mL at week 8 and 12), the duration of treatment with triple therapy was reduced in approximately 90% of previously treated and treatment-naïve patients. In the IL28B C/C genotype group in the Sprint-2 study, the SVR rates were higher in all three treatment studied arms (dual therapy, response-guided triple therapy with boceprevir and triple therapy with boceprevir fixed dose)[8]. However, in this cohort, the IL28B genotype C/C was a more important predictor of shortening treatment; 89% of patients cleared the virus at week 8 of treatment. IL28B C/C was a predictor of SVR in a limited model analysis with covariates (Respond-2: OR = 2.2, P = 0.025 and Sprint-2: OR = 4.5, P < 0.001), but when a model of logistic regression analysis was performed, the response after lead-in (week 4) was a stronger variable for predicting SVR than any other, including IL28B C/C, based on the baseline evaluation. In a combined Sprint-2 and Respond-2 studies analysis, early response to pegylated IFN and ribavirin, *i.e.,* response after week 4, was the best predictor of SVR in patients with unfavorable *IL28B* genotypes C/T and T/T. In the response-guided therapy, the duration of therapy was based on the detection of HCV-RNA at week 8 and patients who had undetectable HCV at this time point were eligible for shortening their therapy. The majority of C/C patients treated with boceprevir had undetectable HCV-RNA viral load by week 8 (89% in Sprint-2, and 76% in Respond-2), and consequently was eligible for abbreviated treatment. On the other hand, a fewer number of patients with the C/T and T/T *IL28B* genotypes had undetectable viral loads at week 8 (CT, 53% in Sprint-2 and 46% in Respond-2/TT, 42% in Sprint-2 and 63% in Respond-2). The SVR rate for patients in the boceprevir groups who became undetectable by week 8 was 81%-100%, regardless of the IL28B genotype[8,45].

Table 3 Cupic study evaluation of the risk/benefit of the treatment cirrhotic patients with telaprevir or boceprevir triple therapy considering the chances of death or severe complications and sustained virologic response 12 according to cutoffs of serum albumin level and platelet count *n* (%)

Factors	Platelet count > 100000/mm^3	Platelet count ⩽ 100000/mm^3
Serum albumin level > 35 g/L		
Patients with severe complications or death)	19 (6.2)	9 (12.2)
SVR12	168 (54.9)	27 (36.5)
Serum albumin level > 35 g/L		
Patients with severe complications or death	5 (16.1)	19 (51.4)
SVR12	9 (29.0)	10 (27.0)

Adapted from Hézode *et al*[50]. SVR12: Sustained virologic response at 12 wk after ending of treatment.

The phase Ⅲ study Advance analyzed triple therapy with pegylated IFN, ribavirin and telaprevir in treatment-naïve patients with genotype 1 chronic hepatitis C[49]. Retrospective analysis of the Advance study evaluated SVR rates based on *IL28B* genotypes in 42% (*n* = 454/1088) of patients from the study population, all Caucasians based on self-reported ancestry. In the group with IL28B C/C, 90% of subjects (*n* = 45/50) achieved SVR with triple therapy *vs* 64% SVR among those who received pegylated IFN and ribavirin in the control group. The genotypes C/T and T/T patients had SVR rates of 71% and 73%, respectively in the group treated with triple therapy compared with SVR rates of 23% and 25% in those treated with dual therapy. The C/C patients achieved higher rates of extended RVR (eRVR) characterized by HCVRNA viral load < 1000 IU/mL at week 4 and 12 of treatment; subjects with eRVR were eligible for shortened therapy. In this study, eRVR was the best predictor of SVR, although, notably, individuals with the IL28B C/C genotype also had high rates of SVR. The overall SVR rate in the group with eRVR was 91%, but among individuals who also had the IL28B C/C genotype, the SVR rate increased by 6% (97%); among those who did not achieve eRVR, the SVR rate was significantly lower (43%), but in those without eRVR and with genotype C/C IL28B, the SVR was 63%.

The phase Ⅲ study Realize compared the efficacy, safety and tolerability of telaprevir (with or without lead-in) in combination with pegylated IFN and ribavirin, with the control group treated with pegylated IFN and ribavirin in patients non responsive to prior treatment, including relapsers, as well as partial and null responders. In a retrospective analysis, the association between the IL28B genotype (rs12979860) and SVR was investigated in 527 (80%) patients included in the study; however, the IL28B C/C genotypes were not predictors of SVR among individuals treated with triple therapy. SVR rates were greater in telaprevir treated groups *vs* PR for all *IL28B* genotypes (CC: 79% *vs* 29%, CT: 60% *vs* 16%, TT: 61% *vs* 13%, respectively)[42].

Role of advanced liver fibrosis in first wave DAA treatment regimens

Although triple therapy combining the protease inhibitors (telaprevir or boceprevir), pegylated-IFN and ribavirin have increased the chances to eliminate HCV in many groups of patients, its efficacy remains suboptimal in treatment experienced cirrhosis patients. These patients are considered difficult-to-treat, and lower SVR rates than noncirrhotics are achieved; they have an enlarged risk of developing serious adverse events. Moreover, cirrhosis patients were underrepresented in first generation DAA clinical trials.

A real life study analyzed a total of 660 cirrhosis patients who were previous relapsers, partial responders and null responders to pegylated IFN and ribavirin treatment, including 299 treated with telaprevir and 212 with boceprevir. Patients were included in each group at the discretion of the physician[50]. The first endpoint (SVR 12) achieved among patients treated with telaprevir was 74.2% for relapsers, 40.0% for partial responders and 19.4% for null responders. Among individuals treated with boceprevir, 53.9% of relapsers, 38.3% of partial responders and none of null responders got SVR at week 12. A late virologic breakthrough during therapy was observed after discontinuing TVR in 16.4% of the cases, a relapse in 14.7%, and 4.7% patients failed for other reasons (7 were lost to follow-up, 4 died, and 3 were missing HCV-RNA level measurements). Among the 121 patients who failed boceprevir treatment, virologic breakthrough during therapy was observed in 9%, 17% relapsed and 1.9% patients failed for other reasons (2 deaths and 2 missing HCV-RNA level measurements).

Variables associated with SVR 12 among patients treated with telaprevir were HCV subtype 1b and RVR. In the group treated with boceprevir, HCV subtype 1b, 1 Log HCV-RNA decline after lead in (week 4), 3 Log HCV-RNA decline or undetectable viral load at week 8 were good predictors of SVR12. In multivariate analysis, factors associated with SVR12 included previous response to previous treatment, HCV subtype 1b and baseline platelet count greater than 100000/mm^3.

Serious adverse events occurred in 49.9% of cases, comprising hepatic decompensation, severe infections in 10.4%, and death in 2.2%. According to multivariate analysis, baseline serum albumin level less than 35 g/L and platelet counts of 100000/mm^3 or less predict serious side effects or death. Among patients with serum albumin levels < 35 g/L and platelet counts ⩽ 100.000/mm^3, the proportion of severe complications or death was 51.4%, and therefore, this treatment is not advisable for this subgroup of patients (Table 3)[50].

In the Cupic study, the long follow-up period (60 wk) of subjects treated with telaprevir or boceprevir revealed a large number of serious adverse events (SAE), such as severe infection or hepatic decompensation and death in 10.6% of patients. These SAE were attributed, in part, to

a higher mean age of the study population compared to phase 3 studies, as well as a more severe liver disease and portal hypertension. Therefore, treatment with first-generation DAA is not recommended for patients with advanced cirrhosis and severe portal hypertension[50].

First wave DAA and drug resistance

The emergence of drug-resistant variants is a concern with the utilization of antiviral drugs. A large amount of genetically different variants, or viral quasispecies may occur in a unique individual, considering that about 10^{12} HCV are produced daily with a mutation rate of approximately 1×10^{4} to 1×10^{-5} per nucleotides[51]. These resistant variants can be selected in antiviral treatment as the level of wild-type HCV decreases.

The most common resistance variants related to therapy failure are comparable for telaprevir and boceprevir. V36A/M, T54A/S, R155K/T and A156S/T are clinically expressive variants that are resistant to both drugs[52]. The V55A and A156V are variants associated with boceprevir only. In patients treated with telaprevir or boceprevir, diverse resistance patterns are identified for the HCV genotype 1 subtypes (1a and 1b). Genotype 1b is correlated with a low-resistant variant selection rate, the higher genetic barrier and superior response to triple therapy in comparison with genotype 1a[53]. The most probable reason for these differences are the lower genetic barriers to resistant variants at key-sites on the HCV NS3 protease in genotype 1a patients in comparison to patients with genotype 1b.

In phase 3 trials, patients who did not reach SVR with telaprevir and boceprevir triple therapy, resistant variants were identified in 86% and 55% of genotype 1a patients, respectively; 56% and 47%, of those with genotype 1b, respectively[54]. Genotype 1b-resistant variants sustained for a median of 1-2 mo comparatively with 8-11 mo for genotype 1a, in phase 3 clinical trials with telaprevir[54]. In phase 1b trials, ultradeep sequencing showed more variants in patients treated with telaprevir or boceprevir: R117H in patients treated with telaprevir, S174F in boceprevir-treated patients and A87T in both groups; they were only found in genotype 1b, and the effect of these variants on triple therapy is undetermined.

If patients do not get SVR with telaprevir-based therapy, telaprevir-resistant variants are often increased at the end of treatment. Population sequencing revealed that the telaprevir-resistant variants are typically not detectable at baseline (prevalence of patients $\leqslant$ 5%) and the majority of the variants present at the time of treatment failure are no longer detectable at the end of the study. The analysis performed in the Realize study using a deep-sequencing technique showed that before treatment, telaprevir-resistant variants (T54A, T54S, or R155K) were detected in 2% of patients and that these variants were not essentially detected at the time of treatment failure. Analysis of 49 patients, deep-sequencing technique, revealed the presence of variants V36A/L/M, T54S or R155K in 16 patients (33%) at the end of the study[55].

NEW WAVES OF DAA

Currently, the hepatitis C virus antiviral therapy challenge is the development of drugs and therapy regimens with markedly antiviral activity, high genetic barrier to resistance, few side effects and short duration. An improved understanding of the HCV genome lifecycle led to the discovery of many potential targets for antiviral therapy. The polyprotein processing and replication, viral entry and fusion, the RNA virus translation, assembly and release of host cells and numerous other factors are attractive targets for new and alternative antiviral therapies. Combinations of antiviral agents with different action mechanisms have brought about IFN-free treatment regimens, with expressive rates of sustained response, better tolerability and fewer side effects[56]. Some characteristics of the new DAA are summarized in Table 4.

Sofosbuvir

Sofosbuvir is a nucleotide analogue inhibitor of the HCV NS5B RNA-dependent RNA polymerase, with HCV pangenotypic action.

The phase 3 NEUTRINO trial evaluated sofosbuvir (400 mg/d) in combination with PEG-IFN and weight-based RBV (1000 mg to 1200 mg daily) for 12 wk[57]. In the NEUTRINO study, of the 456 patients with HCV genotype 1, 4, 5, or 6, 291 had HCV genotype 1 and 327 began treatment. A total of 17% of patients were black, 71% were non-CC *IL28B* genotype, and 17% had cirrhosis. The SVR rate at 12 wk after treatment was 90%. The variables associated with therapy response were cirrhosis, *IL28B* (rs12979860) genotype and ribavirin exposure. The SVR12 for patients with genotype 1 infection was 89.4% (SVR12 91.6% to HCV subtype 1a and 81.9% to HCV 1b); SVR12 was inferior in patients with cirrhosis (80%) than in those without cirrhosis (92%). Subjects with IL28B CC achieved a 97.9% SVR12 compared to 87.1% among non-CC *IL28B* genotypes[57].

In the FISSION study, 499 patients with HCV genotype 2 or 3 began treatment and 20.5% of patients in all groups had cirrhosis[57]. In this phase 3 trial, among patients receiving sofosbuvir-ribavirin, the predictive factors associated with SVR12 were HCV genotype, presence of cirrhosis, HCV-RNA viral load at baseline and ribavirin exposure. The response rates were lower among patients with HCV genotype 3 than among those with genotype 2 (55.7% *vs* 97.1%); in addition, SVR rates observed were worse for cirrhotic patients than for those without cirrhosis (46.9% *vs* 72.1%), and patients with HCVRNA $\geqslant$ 6 $\log_{10}$ UI/mL at baseline also had lower SVR rates at week 12 (61.6% *vs* 78%).

In both Neutrino and Fission trials, known variables such as older age, black race (self-reported), body mass index $\geqslant$ 30 kg/m^2 that are commonly associated with failure of previous IFN-based treatments were not

Table 4 Characteristics of the "New-wave" direct acting antivirals and the most important variables associated with sustained virological response

Drug	Characteristics	Resistance-associated	SVR predictive factors	OR[1]	*P*-value[1]	
Sofosbuvir	Nucleotide analogue HCV NS5B	HCV mutation S282T	Genotype 1			McHutchison *et al*[15]
	Polymerase inhibitor		Cirrhosis: no *vs* yes	3.93	0.0018	Neutrino study
	Against all HCV genotypes		IL28B: CC *vs* CT/TT	7.99	0.006	
			RBV exposure (mg/kg per day)	1.39	0.0005	
			Genotypes 2 and 3			
			HCV genotypes 2 *vs* 3	42.49	< 0.0001	Fission study
			Cirrhosis: no *vs* yes	2.94	0.005	
			HCV RNA baseline: < *vs* ⩾ 6 Log IU/mL	2.33	0.009	
			RBV exposure (mg/kg per day)	1.26	0.002	
Simeprevir	NS3/4A serine protease inhibitor	Q80K-HCV subtype 1a	HCV 1a: Q80K: no *vs* yes	0.19	1.7×10^{-5}	Jacobson *et al*[61]
	HCV genotype 1	R155K	F0-F2 *vs* F3-F4	2.09	0.029	Quest-1 study
		D168V - HCV subtype 1b	IL28B: CC *vs* CT/TT	5.11	1.3×10^{-4}	
			HCV RNA baseline: ⩽ *vs* > 800.000 IU/mL	3.13	0.028	
Daclatasvir	NS5A replication complex inhibitor	NS3 polymorphisms	HCV genotype 1a *vs* 1b	2.82	0.025	Hézode *et al*[63]
	Against all HCV genotypes	NS5A-A30K	HCV genotypes 2 *vs* 3	1.31	0.740	Sulkowski *et al*[46]
Ledipasvir	HCV NS5A replication complex inhibitor					Afdhal *et al*[67]
	HCV genotype 1					
ABT-450	NS3/4A protease inhibitor		Treatment *vs* placebo	7.19	4.3×20^{-11}	Feld *et al*[70]
Ritonavir	HCV NS5A replication complex inhibitor					
Ombitasvir	HCV NS5A inhibitor (Pangenotipic)		HCV genotype 1a			
Dasabuvir	HCV NS5B RNA non-nucleoside polymerase inhibitor		Ribavirin: with *vs* without	3.50	0.038	Ferenci *et al*[69]
	HCV genotype 1					

[1]Statistical analyses were performed using Fisher's test, two-tailed. An alpha error < 5% was considered. IL28B: Interleukin-28B; HCV: Hepatitis C virus; SVR: Sustained virologic response; RBV: Ribavirin.

associated with SVR based on the multivariate logistic regression.

Notably, 28 patients from NEUTRINO study and the 74 patients from FISSION study who received sofosbuvir relapsed after a virologic response at the end of treatment; however, the reason for this non-response is unknown. Testing for viral resistance did not find the S282T HCV mutation associated with Sofosbuvir.

Daily sofosbuvir (400 mg) and weight-based ribavirin plus weekly pegylated IFN for 12 wk is one of the recommended treatment regimens for IFN-eligible subjects with HCV genotype 1, 2 and 3 infection[58,59].

Cost-effectiveness exploratory analysis showed that in developed countries the strategy of treating all individuals with genotype 1 and 4 chronic hepatitis C with Peg-IFN alpha-2a, ribavirin and sofosbuvir (12 wk) as well as treating HCV genotype 2 and 3 patients with sofosbuvir PR (12 wk) would be cost-effective when compared to no treatment or to restricting therapy according to stage of fibrosis (⩾ F2, analyzed by non-invasive tests). This analysis has considered other treatment options and has showed that treating everyone would be cost-effective if the overall increase in treatment reached up to about £ 37500, but not over. If costs increased to greater than £ 37500 the strategy to restrict the treatment for patients with METAVIR ⩾ F2 would be the most cost effective. Unfortunately these results can't be extrapolated to developing countries, where local cost-effectiveness analyzes need to be evaluated[60].

Simeprevir

Simeprevir is a specific inhibitor of the HCV NS3/4A serine protease, which is used to treat HCV genotype 1 patients. The recommendations have comprised a treatment regimen of simeprevir plus PR for HCV infected patients, especially HCV genotype 1b. If the IFN-based treatment is not appropriate for patients, combination with sofosbuvir ± ribavirin should be considered.

In the Quest-1 trial, patients with HCV genotype 1 were treated with simeprevir once daily plus peg-IFN alpha 2a and ribavirin compared to PR treatment[61]. Therapy period was 24 wk or 48 wk in the simeprevir group agreeing with response-guided therapy. The SVR12 from the Simeprevir group was higher when compared to the PR group (80% *vs* 50%, respectively), without worsening the adverse events associated with peg-IFN. RVR and SVR12 was better in the simeprevir group compared to the PR group independent of

baseline HCV RNA, HCV subtype (1a without Q80K or 1b), METAVIR score (F0-F2, F3, or F4), *IL28B* genotype (CC, CT, or TT) and race. Cirrhotic patients achieved 58% SVR12 in the Simeprevir group compared with 29% in the PR group; F0-F2 had 83% and 60% SVR12 in simeprevir and PR regimens, respectively; F3, achieved 78% SVR12 when treated with simeprevir and 26% SVR12 with PR treatment. Black or African-American patients in the simeprevir group achieved 63% SVR12 compared with 25% in the PR group; 59% black or African-American patients treated with simeprevir had RVR and 94% of them achieved SVR12. In the Quest-2 trial, simeprevir was added to peg-IFN alpha 2a or peg-IFN alpha 2b PR, and the combination improved SVR in treatment-naive patients with HCV genotype 1 infection[62].

In the Cosmos study, combination treatment with simeprevir and sofosbuvir, in an IFN-free regimen was evaluated in 167 patients with chronic HCV genotype 1 infection who had previously not response to peg-IFN and ribavirin or who were untreated[57]. There were two groups: cohort 1 (enrolled patients with a null response to PEG-IFN/RBV with Metavir fibrosis stage 0 or 2) and Cohort 2 (including patients who were treatment-naïve or with a previous null response with Metavir fibrosis stage 3 or 4). SVR12 were reached within 154 (92%) patients, 90% in cohort 1 and 94% in-group 2.

The ATTAIN phase Ⅲ study compared two treatments regimens, simeprevir plus PR and telaprevir plus PR in HCV genotype 1 patients who were previously treated with PR therapy. The SVR in the simeprevir group was achieved in 69.7% in partial responders and in 43.6% of previous null responders to PR. In the teleprevir group, the SVR was 68.5% among partial responders and 46.6% among the null responders[47,59].

For patients with HCV genotype 1a infection, the Q80K polymorphism is a negative predictive variable for achieving SVR, and baseline resistance testing may be considered because the mutation clearly modifies the probability of SVR to simeprevir. The SVR12 was 90% in the HCV genotype 1 group; however, in subtype 1a with Q80K polymorphism at baseline, SVR12 was 52% whereas in the 1a group without Q80K, SVR12 was 85%[61]. Most likely, the Q80K polymorphism does not prevent treatment with simeprevir plus sofosbuvir since SVR rate remains high in patients with genotype 1a/Q80K infection treated with this regimen (SVR 12 in cohort 1 was 86%)[57].

Patients treated with simeprevir who did not achieve SVR12 progressed with increasing number of mutations up to the time of failure in 92% (35 of 38) of patients. In HCV genotype 1a infected patients, the mutations were mainly R155K and for patients infected with HCV genotype 1b, the mutations were mainly D168V[57].

Daclatasvir

Daclatasvir is a HCV NS5A replication complex inhibitor that has great antiviral activity and is effective in patients infected with HCV genotypes 1, 2, 3 and 4. Treatment may be combined with peg-IFN PR or sofosbuvir PR and therapy duration varies between 12 and 24 wk.

The phase Ⅱb COMMAND-1 study analyzed HCV genotype 1 untreated patients and showed SVR rates of 87% in subtype 1b and 58% in subtype 1a patients[63]. This trial evaluated the combination of sofosbuvir and daclatasvir in HCV genotypes 1, 2 and 3 infected patients[46]. The results showed that once-daily oral daclatasvir plus sofosbuvir was related with increased rates of SVR among patients infected with HCV genotypes 1, 2 or 3, comprising those with no response to previous therapy with telaprevir or boceprevir. Among individuals infected by genotype 1, 98% of previously naive patients and 98% who did not achieve SVR with first-generation protease inhibitors had SVR12.Among HCV genotype 2 patients, 92% had SVR as well as 89% of HCV genotype 3 subjects. The SVR rates at 12 wk after treatment were similar in subgroups in accordance with viral subtype (genotype 1a, 98% *vs* genotype 1b, 100%), *IL28B* genotype (CC, 93% *vs* CT/TT, 98%), race (white, 97% *vs* black, 96% and other race, 90%), ribavirin presence (ribavirin, 94% *vs* without ribavirin, 98%) and, finally, previous treatment failure with telaprevir or boceprevir (98%)[46].

The virologic response was high despite the presence or absence of baseline NS3 mutations that confer resistance to telaprevir or boceprevir. At baseline, resistance analysis detected an NS5A-A30K existing polymorphism related with daclatasvir resistance at baseline; nevertheless, all of the patients with preexisting daclatasvir resistance variants achieved SVR[46,63,64].

Ledipasvir

Ledispavir is a NS5A inhibitor that has been associated with high rates of SVR among patients with HCV genotype 1. Multicentric trials have showed that combination treatment with sofosbuvir and ledipasvir for 12 wk was effective in patients with HCV genotype 1 infection[65-67].

In naïve patients, the SVR12 rate among those who were treated with 12 wk of ledipasvir plus sofosbuvir was 99%; among patients treated with 12 wk of ledipasvir plus sofosbuvir PR, the SVR rate was 97%. Among individuals who received 24 wk of ledipasvir plus sofosbuvir the SVR12 was 98%, while subjects treated with 24 wk of ledipasvir plus sofosbuvir PR had an SVR of 99%[65]. In this trial, the SVR rates were similar among known subgroups traditionally associated with low chances of SVR such as cirrhotic patients in whom SVR12 ranged from 94% to 100%[65]. Patients with HCV genotype 1a had 97%-99% rates of SVR; among those with a non-CC IL28B allele, the SVR12 rate also ranged between 97%-99%, and among black patients, the SVR12 ranged from 91% to 100%[65].

Good SVR rates were also detected when previous non-responders to IFN-based therapies (including protease inhibitors previous non-responders) were treated with sofosbuvir plus ledipasvir regimens. A trial with 440 patients previously non responders to IFN

associated treatment, including 20% cirrhosis patients and 79% HCV genotype 1a infected individuals, the SVR12 rates in treatment groups were as follows: 94% response in patients receiving 12-wk-ledipasvir sofosbuvir; 96% among those who received 12 wk of ledipasvir-sofosbuvir and ribavirin; 99% among patients who received 24 wk of ledipasvir-sofosbuvir; and 99% in the group that received 24 wk of ribavirin and ledipasvir-sofosbuvir[67]. Treatment was well tolerated with rare cases of breakthrough or relapse.

ABT-450/r-ombitasvir and dasabuvir

A 12-wk treatment with the combination of ABT-450/r-ombitasvir, an NS5a inhibitor (a once-daily dose of 150 mg of ABT-450, 100 mg of ritonavir, and 25 mg of ombitasvir), dasabuvir, NS5B RNA non-nucleoside polymerase inhibitor (250 mg twice daily) and ribavirin according to body weight was tested in the HCV genotype 1 infected non-cirrhotic patients who achieved SVR12 rates of approximately 96%[68-71]. There was no difference in SVR12 among HCV genotype 1b or 1a. Among patients with HCV genotype 1b, the SVR rates were 99.5% including ribavirin in the regimen and 99.0% without ribavirin; on the other hand, among those with HCV genotype 1a, the addition of ribavirin appeared to increase the SVR rates (97.0% and 90.2%, SVR12 with and without ribavirin, respectively)[69]. Another trial analysed the efficacy of ABT-450/r-ombitasvir and dasabuvir ± ribavirin among HCV genotype 1b infected patients without cirrhosis and previously treated with peg-IFN and ribavirin. The SVR12 rate including ribavirin was 96.6% and without ribavirin, was 100%[71]. The SVR rates were 95.3% for previously relapsed patients, 100% among patients with previous partial response and 95.2% among those with a null response[71].

MK-5172 + MK-8742 ± RBV in HCV genotype 1

MK-5172 100 mg once daily plus MK-8742 50 mg once daily and RBV 1000-1200 mg divided twice daily is another treatment regimen, which is under evaluation for HCV genotype 1 patients. Therapy-naïve patients with HCV genotype 1 and cirrhosis were treated for 12 wk with MK-5172 + MK-8742 ± ribavirin and obtained SVR 4/8 rates of 90% with ribavirin containing regimen and 97% when ribavirin was not included. Groups in which patients were treated for 18 wk presented SVR rates of 97% with or without ribavirin. Previous HCV genotype 1 patients null responders to PR therapy when treated for 12 wk had a 94% SVR 4/8 rate in the regimen with ribavirin, and a 91% SVR 4/8 rate in the regimen without ribavirin. When treated for 18 wk, the SVR4/8 was 100% in the ribavirin-containing regimen group and 97% if ribavirin was not added to therapy[72].

LT

Treatment of HCV infection in the transplant scenario is indicated in two different situations: patients awaiting LT to prevent HCV infection of the graft; and patients with hepatitis C recurrence after LT in order to reduce the damage to the already infected graft.

Patients awaiting LT

Post-transplant recurrence of HCV is common in patients who have detectable HCV RNA at the time of transplantation. In patients waiting for LT, antiviral therapy may be indicated because it prevents graft infection if HCV RNA becomes undetectable at least 30 d prior to transplantation[59]. Current IFN-based treatments are not effective and safe in patients with advanced cirrhosis[73]. With the recent approval of sofosbuvir, simeprevir and daclatasvir in the United States and Europe, IFN-free regimens are being used in those cirrhotic patients with compensated liver disease awaiting LT. Guidelines have recommended antiviral therapy to Child-Pugh A patients in whom the indication for transplantation is HCC[59].

Sixty-one patients with hepatocellular carcinoma, HCV- any genotype, Child-Pugh score $\leqslant$ 7, on LT wait lists, were evaluated in an open-label phase 2 study, that aimed to avoid HCV recurrence after LT. Subjects received up to 48 wk sofosbuvir and ribavirin before LT. Among them, 46 received transplanted livers, 43 (93.5%) patients had HCV-RNA level less than 25 IU/mL at the time of transplantation; 30 of 43 subjects (70%) achieved a post-transplantation SVR at week 12. In this analysis, 10 (23%) had recurrent infection that was related inversely to the number of consecutive days of undetectable HCV RNA before transplantation. The authors concluded that sofosbuvir and ribavirin before LT can prevent post-transplant HCV recurrence[74].

In decompensated cirrhotic patients (Child-Pugh B or C) waiting for transplantation, antiviral therapy may be offered on an individual decision in experienced centers. Data about safety and efficacy data are still scarce and therefore there are no clear recommendations as well as there are insufficient data about time of treatment, post-LT relapse rate and safety. Of note, a few patients without HCC may be delisted they improve liver function and/or portal hypertension after achieving SVR.

Post-LT hepatitis C recurrence

Hepatitis C recurrence after transplantation is responsible for reduced post-transplant survival. Approximately 30% of patients with HCV develop severe recurrent acute hepatitis C after transplant which rapidly progress to liver cirrhosis; 5% to 7% have fibrosing cholestatic hepatitis and may rapidly progress to death[75,76]. Patients with HCV post-transplant recurrence should be considered for therapy. The treatment of recurrent HCV infection with combination of peg-IFN PR after LT is associated with low rates of SVR, ranging between 15%-35%, and with significant adverse effects[77]. The triple therapy adding boceprevir and telaprevir to PEG-IFN and ribavirin have improved the therapeutic efficacy in comparison with dual therapy, increasing in 30% the SVR rate, however, this is accompanied by an additional cost and high

toxicity with often serious adverse events and important drug interactions, especially with calcineurin inhibitors[78]. The new wave of DAA with fully oral schemes of simeprevir, sofosbuvir and/or daclatasvir has achieved significant SVR rates and better tolerability. Combination of sofosbuvir PR has been associated to better SVR rates as high as 70% and good tolerance[79].

All-oral sofosbuvir plus daclatasvir combination shows high virological efficacy in liver transplant recipients and appears not to interact with immunosuppressants[80]. Drug-drug interactions may be important in the post-transplant setting; until this moment, no clinically significant drug-drug interactions have been found between sofosbuvir, simeprevir or daclatasvir and cyclosporine and tacrolimus imunossupressants.

FINAL REMARKS

As the efficacy of new drug regimens used to treat chronic hepatitis C is improved, the influence of host and viral factors that may interfere with the chances of obtaining a SVR decreases.

Because IFN-based antiviral therapies were the main option, several studies searched for variables that are predictive of SVR in patients with chronic hepatitis C. Various viral factors, the host and the genetic variables, metabolic and immunological characteristics have influenced the response to IFN-based therapy. The most important viral factors that influence the response to IFN-based antiviral treatment appear to be HCV genotype and HCV RNA kinetics. Conversely, the strongest identified host baseline risk factor associated with SVR was IL28B polymorphisms, especially rs12979860 and rs8099917. Other important SVR risk factors at baseline were high viral load (> 600.000 UI/mL), older age, African ancestry, body weight, insulin resistance, steatosis, and advanced fibrosis stage. Viral kinetics is a strong predictor of SVR, particularly when the viral load is not detectable at week 4 (RVR).

With the arrival of first generation DAAs, some variables previously associated with SVR lost their value. Considering telaprevir and boceprevir, viral kinetics is the most important predictive factor of SVR. The IL28B was associated with greater chances to shorten therapy but not to achieve SVR.

With the new generation DAAs, it has been possible to identify treatment regimens that substantially improve SVR rates, including difficult to treat subgroups of patients such as patients with cirrhosis. Nonetheless, HCV subtype 1a, cirrhosis, some cases of HCV genotype 3 and "failure" of viral load decrease on-treatment may still indicate low rates of non-response with regimens that include sofosbubir, daclatasvir and ledipasvir.

It is expected that newer high genetic barrier drug treatment regimens will produce very high SVR rates with short therapy durations independent of the presence of the current known unfavorable host, viral and immunogenetic variables associated with response.

REFERENCES

1 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]

2 **Ly KN**, Xing J, Klevens RM, Jiles RB, Ward JW, Holmberg SD. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. *Ann Intern Med* 2012; **156**: 271-278 [PMID: 22351712 DOI: 10.7326/0003-4819-156-4-201202210-00004]

3 **Promrat K**, McDermott DH, Gonzalez CM, Kleiner DE, Koziol DE, Lessie M, Merrell M, Soza A, Heller T, Ghany M, Park Y, Alter HJ, Hoofnagle JH, Murphy PM, Liang TJ. Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. *Gastroenterology* 2003; **124**: 352-360 [PMID: 12557141 DOI: 10.1053/gast.2003.50061]

4 **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 DOI: 10.1111/jvh.12168]

5 **Seeff LB**, Hoofnagle JH. Appendix: The National Institutes of Health Consensus Development Conference Management of Hepatitis C 2002. *Clin Liver Dis* 2003; **7**: 261-287 [PMID: 12691470 DOI: 10.1016/S1089-3261(02)00078-8]

6 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]

7 **Tanaka Y**, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105-1109 [PMID: 19749757 DOI: 10.1038/ng.449]

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

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

10 **Shepherd J**, Brodin H, Cave C, Waugh N, Price A, Gabbay J. Pegylated interferon alpha-2a and -2b in combination with ribavirin in the treatment of chronic hepatitis C: a systematic review and economic evaluation. *Health Technol Assess* 2004; **8**: iii-iv, 1-125 [PMID: 15461877 DOI: 10.3310/hta8390]

11 **Afdhal NH**, McHutchison JG, Zeuzem S, Mangia A, Pawlotsky JM, Murray JS, Shianna KV, Tanaka Y, Thomas DL, Booth DR, Goldstein DB. Hepatitis C pharmacogenetics: state of the art in 2010. *Hepatology* 2011; **53**: 336-345 [PMID: 21254181 DOI: 10.1002/hep.24052]

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

13 **Hadziyannis SJ**, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C:

a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355 [PMID: 14996676 DOI: 10.7326/0003-4819-140-5-200403020-00010]

14 **Manns MP**, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. *Gut* 2006; **55**: 1350-1359 [PMID: 16905701 DOI: 10.1136/gut.2005.076646]

15 **McHutchison JG**, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER, Galati JS, Bacon BR, Davis MN, Mukhopadhyay P, Koury K, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; **361**: 580-593 [PMID: 19625712 DOI: 10.1056/NEJMoa0808010]

16 **Jacobson IM**, Brown RS, Freilich B, Afdhal N, Kwo PY, Santoro J, Becker S, Wakil AE, Pound D, Godofsky E, Strauss R, Bernstein D, Flamm S, Pauly MP, Mukhopadhyay P, Griffel LH, Brass CA. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. *Hepatology* 2007; **46**: 971-981 [PMID: 17894303 DOI: 10.1002/hep.21932]

17 **Flori N**, Funakoshi N, Duny Y, Valats JC, Bismuth M, Christophorou D, Daurès JP, Blanc P. Pegylated interferon-α2a and ribavirin versus pegylated interferon-α2b and ribavirin in chronic hepatitis C : a meta-analysis. *Drugs* 2013; **73**: 263-277 [PMID: 23436591 DOI: 10.1007/s40265-013-0027-1]

18 **Cavalcante LN**, Abe-Sandes K, Angelo AL, Machado TM, Lemaire DC, Mendes CM, Pinho JR, Malta F, Lyra LG, Lyra AC. IL28B polymorphisms are markers of therapy response and are influenced by genetic ancestry in chronic hepatitis C patients from an admixed population. *Liver Int* 2012; **32**: 476-486 [PMID: 22098416 DOI: 10.1111/j.1478-3231.2011.02653.x]

19 **Conjeevaram HS**, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, Brown RS, Belle SH, Hoofnagle JH, Kleiner DE, Howell CD. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology* 2006; **131**: 470-477 [PMID: 16890601 DOI: 10.1053/j.gastro.2006.06.008]

20 **Nguyen GC**, Thuluvath PJ. Racial disparity in liver disease: Biological, cultural, or socioeconomic factors. *Hepatology* 2008; **47**: 1058-1066 [PMID: 18302296 DOI: 10.1002/hep.22223]

21 **Urban TJ**, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD, Hong L, McKenzie A, Patel K, Shianna KV, McHutchison JG, Goldstein DB, Afdhal N. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. *Hepatology* 2010; **52**: 1888-1896 [PMID: 20931559 DOI: 10.1002/hep.23912]

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

23 **Thomas DL**, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798-801 [PMID: 19759533 DOI: 10.1038/nature08463]

24 **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]

25 **Poynard T**, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, Albrecht J. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002; **122**: 1303-1313 [PMID: 11984517]

26 **Diago M**, Castellano G, García-Samaniego J, Pérez C, Fernández I, Romero M, Iacono OL, García-Monzón C. Association of pretreatment serum interferon gamma inducible protein 10 levels with sustained virological response to peginterferon plus ribavirin therapy in genotype 1 infected patients with chronic hepatitis C. *Gut* 2006; **55**: 374-379 [PMID: 16150856 DOI: 10.1136/gut.2005.074062]

27 **Romero AI**, Lagging M, Westin J, Dhillon AP, Dustin LB, Pawlotsky JM, Neumann AU, Ferrari C, Missale G, Haagmans BL, Schalm SW, Zeuzem S, Negro F, Verheij-Hart E, Hellstrand K. Interferon (IFN)-gamma-inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection. *J Infect Dis* 2006; **194**: 895-903 [PMID: 16960776 DOI: 10.1086/507307]

28 **García-Álvarez M**, Pineda-Tenor D, Jiménez-Sousa MA, Fernández-Rodríguez A, Guzmán-Fulgencio M, Resino S. Relationship of vitamin D status with advanced liver fibrosis and response to hepatitis C virus therapy: a meta-analysis. *Hepatology* 2014; **60**: 1541-1550 [PMID: 24975775 DOI: 10.1002/hep.27281]

29 **Gal-Tanamy M**, Bachmetov L, Ravid A, Koren R, Erman A, Tur-Kaspa R, Zemel R. Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. *Hepatology* 2011; **54**: 1570-1579 [PMID: 21793032 DOI: 10.1002/hep.24575]

30 **Petta S**, Craxì A. Optimal therapy in hepatitis C virus genotypes 2 and 3 patients. *Liver Int* 2011; **31** Suppl 1: 36-44 [PMID: 21205136 DOI: 10.1111/j.1478-3231.2010.02382.x]

31 **Zhu Q**, Li N, Han Q, Zhang P, Yang C, Zeng X, Chen Y, Lv Y, Liu X, Liu Z. Statin therapy improves response to interferon alfa and ribavirin in chronic hepatitis C: a systematic review and meta-analysis. *Antiviral Res* 2013; **98**: 373-379 [PMID: 23603497 DOI: 10.1016/j.antiviral.2013.04.009]

32 **Abd-Eldaem AA**, Azmy MK, Ahmad YK, Badr GA, Houssein MA, El-Dahshan T. Role of statins in the treatment of chronic hepatitis C virus infection. *J Egypt Soc Parasitol* 2012; **42**: 535-540 [PMID: 23469629 DOI: 10.12816/0006339]

33 **Di Marco V**, Covolo L, Calvaruso V, Levrero M, Puoti M, Suter F, Gaeta GB, Ferrari C, Raimondo G, Fattovich G, Santantonio T, Alberti A, Bruno R, Mussini C, Mondelli M, Donato F, Craxì A. Who is more likely to respond to dual treatment with pegylated-interferon and ribavirin for chronic hepatitis C? A gender-oriented analysis. *J Viral Hepat* 2013; **20**: 790-800 [PMID: 24168258 DOI: 10.1111/jvh.12106]

34 **Salgado AL**, Carvalho Ld, Oliveira AC, Santos VN, Vieira JG, Parise ER. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. *Arq Gastroenterol* 2010; **47**: 165-169 [PMID: 20721461 DOI: 10.1590/S0004-28032010000200009]

35 **Codes L**, Asselah T, Cazals-Hatem D, Tubach F, Vidaud D, Paraná R, Bedossa P, Valla D, Marcellin P. Liver fibrosis in women with chronic hepatitis C: evidence for the negative role of the menopause and steatosis and the potential benefit of hormone replacement therapy. *Gut* 2007; **56**: 390-395 [PMID: 17005762 DOI: 10.1136/gut.2006.101931]

36 **Villa E**, Karampatou A, Cammà C, Di Leo A, Luongo M, Ferrari A, Petta S, Losi L, Taliani G, Trande P, Lei B, Graziosi A, Bernabucci V, Critelli R, Pazienza P, Rendina M, Antonelli A, Francavilla A. Early menopause is associated with lack of response to antiviral therapy in women with chronic hepatitis C. *Gastroenterology* 2011; **140**: 818-829 [PMID: 21167831 DOI: 10.1053/j.gastro.2010.12.027]

37 **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]

38 **Mangia A**, Bandiera F, Montalto G, Mottola L, Piazzolla V, Minerva N, Pellicelli A, Ricci GL, Cela M, Carretta V, Scotto G, Bacca D, Annicchiarico B, Romano M, Russello M, Barbarini G, Agostinacchio E, Andriulli A. Individualized treatment with combination of Peg-interferon alpha 2b and ribavirin in patients

infected with HCV genotype 3. *J Hepatol* 2010; **53**: 1000-1005 [PMID: 20843575 DOI: 10.1016/j.jhep.2010.04.042]

39 **Manns MP**, Markova AA, Calle Serrano B, Cornberg M. Phase III results of Boceprevir in treatment naïve patients with chronic hepatitis C genotype 1. *Liver Int* 2012; **32** Suppl 1: 27-31 [PMID: 22212568]

40 **McHutchison JG**, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J, Bsharat M, George S, Kauffman RS, Adda N, Di Bisceglie AM. Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010; **362**: 1292-1303 [PMID: 20375406 DOI: 10.1056/NEJMoa0908014]

41 **Zeuzem S**, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]

42 **Pol S**, Aerssens J, Zeuzem S, Andreone P, Lawitz EJ, Roberts S, Younossi Z, Foster GR, Focaccia R, Horban A, Pockros PJ, Van Heeswijk RP, De Meyer S, Luo D, Botfield M, Beumont M, Picchio G. Limited impact of IL28B genotype on response rates in telaprevir-treated patients with prior treatment failure. *J Hepatol* 2013; **58**: 883-889 [PMID: 23321318 DOI: 10.1016/j.jhep.2012.12.023]

43 **Choi IJ**. Upper endoscopy in international digestive endoscopy network 2012: towards upper end of quality. *Clin Endosc* 2012; **45**: 217-219 [PMID: 22977805 DOI: 10.5946/ce.2012.45.3.217]

44 **Poordad F**, Bronowicki JP, Gordon SC, Zeuzem S, Jacobson IM, Sulkowski MS, Poynard T, Morgan TR, Molony C, Pedicone LD, Sings HL, Burroughs MH, Sniukiene V, Boparai N, Goteti VS, Brass CA, Albrecht JK, Bacon BR. Factors that predict response of patients with hepatitis C virus infection to boceprevir. *Gastroenterology* 2012; **143**: 608-618.e1-5 [PMID: 22626609 DOI: 10.1053/j.gastro.2012.05.011]

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

46 **Sulkowski MS**, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]

47 **Pascale A**, Serfaty L. Triple therapy with first generation HCV protease inhibitors: lead-in or no lead-in phase? *J Hepatol* 2013; **58**: 391-394 [PMID: 23063419 DOI: 10.1016/j.jhep.2012.09.023]

48 **Zhu Y**, Chen S. Antiviral treatment of hepatitis C virus infection and factors affecting efficacy. *World J Gastroenterol* 2013; **19**: 8963-8973 [PMID: 24379621 DOI: 10.3748/wjg.v19.i47.8963]

49 **Shiffman ML**, Esteban R. Triple therapy for HCV genotype 1 infection: telaprevir or boceprevir? *Liver Int* 2012; **32** Suppl 1: 54-60 [PMID: 22212573 DOI: 10.1111/j.1478-3231.2011.02718.x]

50 **Hézode C**, Fontaine H, Dorival C, Zoulim F, Larrey D, Canva V, De Ledinghen V, Poynard T, Samuel D, Bourliere M, Alric L, Raabe JJ, Zarski JP, Marcellin P, Riachi G, Bernard PH, Loustaud-Ratti V, Chazouilleres O, Abergel A, Guyader D, Metivier S, Tran A, Di Martino V, Causse X, Dao T, Lucidarme D, Portal I, Cacoub P, Gournay J, Grando-Lemaire V, Hillon P, Attali P, Fontanges T, Rosa I, Petrov-Sanchez V, Barthe Y, Pawlotsky JM, Pol S, Carrat F, Bronowicki JP. Effectiveness of telaprevir or boceprevir in treatment-experienced patients with HCV genotype 1 infection and cirrhosis. *Gastroenterology* 2014; **147**: 132-142.e4 [PMID: 24704719 DOI: 10.1053/j.gastro.2014.03.051]

51 **Neumann AU**, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998; **282**: 103-107 [PMID: 9756471]

52 **Boceprevir**. AIDSinfo Drug Database. [Accessed 2015 Jan 31]. Available from: URL: http://aidsinfo.nih.gov/drugs/488/boceprevir/0/patient

53 **Wyles DL**, Gutierrez JA. Importance of HCV genotype 1 subtypes for drug resistance and response to therapy. *J Viral Hepat* 2014; **21**: 229-240 [PMID: 24597691 DOI: 10.1111/jvh.12230]

54 **Sullivan JC**, De Meyer S, Bartels DJ, Dierynck I, Zhang EZ, Spanks J, Tigges AM, Ghys A, Dorrian J, Adda N, Martin EC, Beumont M, Jacobson IM, Sherman KE, Zeuzem S, Picchio G, Kieffer TL. Evolution of treatment-emergent resistant variants in telaprevir phase 3 clinical trials. *Clin Infect Dis* 2013; **57**: 221-229 [PMID: 23575197 DOI: 10.1093/cid/cit226]

55 **Dierynck I**, Thys K, Ghys A, Sullivan JC, Kieffer TL, Aerssens J, Picchio G, De Meyer S. Deep-sequencing analysis of the gene encoding the hepatitis C virus nonstructural 3-4A protease confirms a low prevalence of telaprevir-resistant variants at baseline and the end of the REALIZE study. *J Infect Dis* 2014; **210**: 1871-1880 [PMID: 24943725 DOI: 10.1093/infdis/jiu340]

56 **Vermehren J**, Sarrazin C. New HCV therapies on the horizon. *Clin Microbiol Infect* 2011; **17**: 122-134 [PMID: 21087349 DOI: 10.1111/j.1469-0691.2010.03430.x]

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

58 **Infectious Diseases Society of America**. Recommendations for testing, managing, and treating hepatitis C. [Accessed 2015 Jan 31]. Available from: URL: http://www.hcvguidelines.org/

59 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C 2014. [Accessed 2015 Jan 31]. Available from: URL: http://www.easl.eu/_newsroom/latest-news/easl-recommendations-on-treatment-of-hepatitis-c-2014

60 **Tsochatzis EA**, Crossan C, Longworth L, Gurusamy K, Rodriguez-Peralvarez M, Mantzoukis K, O'Brien J, Thalassinos E, Papastergiou V, Noel-Storr A, Davidson B, Burroughs AK. Cost-effectiveness of noninvasive liver fibrosis tests for treatment decisions in patients with chronic hepatitis C. *Hepatology* 2014; **60**: 832-843 [PMID: 25043847 DOI: 10.1002/hep.27296]

61 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]

62 **Manns M**, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, Janczewska E, Villamil F, Scott J, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2014; **384**: 414-426 [PMID: 24907224 DOI: 10.1016/S0140-6736(14)60538-9]

63 **Hézode C**, Hirschfield GM, Ghesquiere W, Sievert W, Rodriguez-Torres M, Shafran SD, Thuluvath PJ, Tatum HA, Waked I, Esmat G, Lawitz EJ, Rustgi VK, Pol S, Weis N, Pockros PJ, Bourlière M, Serfaty L, Vierling JM, Fried MW, Weiland O, Brunetto MR, Everson GT, Zeuzem S, Kwo PY, Sulkowski M, Bräu N, Hernandez D, McPhee F, Wind-Rotolo M, Liu Z, Noviello S,

Hughes EA, Yin PD, Schnittman S. Daclatasvir plus peginterferon alfa and ribavirin for treatment-naive chronic hepatitis C genotype 1 or 4 infection: a randomised study. *Gut* 2015; **64**: 948-956 [PMID: 25080450 DOI: 10.1136/gutjnl-2014-307498]

64 **Pol S**, Ghalib RH, Rustgi VK, Martorell C, Everson GT, Tatum HA, Hézode C, Lim JK, Bronowicki JP, Abrams GA, Bräu N, Morris DW, Thuluvath PJ, Reindollar RW, Yin PD, Diva U, Hindes R, McPhee F, Hernandez D, Wind-Rotolo M, Hughes EA, Schnittman S. Daclatasvir for previously untreated chronic hepatitis C genotype-1 infection: a randomised, parallel-group, double-blind, placebo-controlled, dose-finding, phase 2a trial. *Lancet Infect Dis* 2012; **12**: 671-677 [PMID: 22714001 DOI: 10.1016/S1473-3099(12)70138-X]

65 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]

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

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

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

69 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]

70 **Feld JJ**, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]

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

72 **Lawitz E**, Hezode C, Gane E, Tam E, Lagging M, Balart L, Rossaro L, Ghalib R, Shaughnessy M, Hwang P, Wahl J, Robertson M, Haber B. Efficacy and safety of MK-5172 and MK-8742 ± ribavirin in hepatitis C genotype 1 infected patients with cirrhosis or previous null-response: the C-WORTHY study. Program and abstracts of the 49th Annual Meeting of the European Association for the Study of the Liver; April 9-13, 2014; London, England, 2014: Abstract O61

73 **Everson GT**, Terrault NA, Lok AS, Rodrigo del R, Brown RS, Saab S, Shiffman ML, Al-Osaimi AM, Kulik LM, Gillespie BW, Everhart JE. A randomized controlled trial of pretransplant antiviral therapy to prevent recurrence of hepatitis C after liver transplantation. *Hepatology* 2013; **57**: 1752-1762 [PMID: 22821361 DOI: 10.1002/hep.25976]

74 **Curry MP**, Forns X, Chung RT, Terrault NA, Brown R, Fenkel JM, Gordon F, O'Leary J, Kuo A, Schiano T, Everson G, Schiff E, Befeler A, Gane E, Saab S, McHutchison JG, Subramanian GM, Symonds WT, Denning J, McNair L, Arterburn S, Svarovskaia E, Moonka D, Afdhal N. Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology* 2015; **148**: 100-107.e1 [PMID: 25261839 DOI: 10.1053/j.gastro.2014.09.023]

75 **Gane EJ**. The natural history of recurrent hepatitis C and what influences this. *Liver Transpl* 2008; **14** Suppl 2: S36-S44 [PMID: 18825724 DOI: 10.1002/lt.21646]

76 **Narang TK**, Ahrens W, Russo MW. Post-liver transplant cholestatic hepatitis C: a systematic review of clinical and pathological findings and application of consensus criteria. *Liver Transpl* 2010; **16**: 1228-1235 [PMID: 21031537 DOI: 10.1002/lt.22175]

77 **Sharma P**, Marrero JA, Fontana RJ, Greenson JK, Conjeevaram H, Su GL, Askari F, Sullivan P, Lok AS. Sustained virologic response to therapy of recurrent hepatitis C after liver transplantation is related to early virologic response and dose adherence. *Liver Transpl* 2007; **13**: 1100-1108 [PMID: 17377914 DOI: 10.1002/lt.21121]

78 **Coilly A**, Roche B, Duclos-Vallée JC, Samuel D. Management of HCV transplant patients with triple therapy. *Liver Int* 2014; **34 Suppl 1**: 46-52 [PMID: 24373078 DOI: 10.1111/liv.12406]

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

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

P- Reviewer: Fouad YM, Rodriguez-Peralvarez M
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1632

World J Hepatol 2015 June 28; 7(12): 1632-1651
ISSN 1948-5182 (online)

REVIEW

Guide for diagnosis and treatment of hepatocellular carcinoma

Magdy Hamed Attwa, Shahira Aly El-Etreby

Magdy Hamed Attwa, Shahira Aly El-Etreby, Division of Hepatology and Gastroenterology, Specialized Medical Hospital, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt

Author contributions: Attwa MH designed, wrote and critically analysed the manuscript; El-Etreby SA performed the research and designed, wrote and revised the manuscript.

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

Correspondence to: Magdy Hamed Attwa, MD, Division of Hepatology and Gastroenterology, Specialized Medical Hospital, Faculty of Medicine, Mansoura University, Mansoura 35516, Dakahlia Governorate, Egypt. magdyhamed52@hotmail.com
Telephone: +2-12-22437771
Fax: +2-50-2230129

Received: August 30, 2014
Peer-review started: August 30, 2014
First decision: September 28, 2014
Revised: October 30, 2014
Accepted: May 26, 2015
Article in press: May 27, 2015
Published online: June 28, 2015

Abstract

Hepatocellular carcinoma (HCC) is ranked as the 5th common type of cancer worldwide and is considered as the 3rd common reason for cancer-related deaths. HCC often occurs on top of a cirrhotic liver. The prognosis is determined by several factors; tumour extension, alpha-fetoprotein (AFP) concentration, histologic subtype of the tumour, degree of liver dysfunction, and the patient's performance status. HCC prognosis is strongly correlated with diagnostic delay. To date, no ideal screening modality has been developed. Analysis of recent studies showed that AFP assessment lacks adequate sensitivity and specificity for effective surveillance and diagnosis. Many tumour markers have been tested in clinical trials without progressing to routine use in clinical practice. Thus, surveillance is still based on ultrasound (US) examination every 6 mo. Imaging studies for diagnosis of HCC can fall into one of two main categories: routine non-invasive studies such as US, computed tomography (CT), and magnetic resonance imaging, and more specialized invasive techniques including CT during hepatic arteriography and CT arterial portography in addition to the conventional hepatic angiography. This article provides an overview and spotlight on the different diagnostic modalities and treatment options of HCC.

Key words: Diagnosis of hepatocellular carcinoma; Surgical resection; Hepatocellular carcinoma; Liver transplantation; Radiofrequency ablation; Microwave ablation; Percutaneous ethanol or acetic acid ablation; Radio-embolisation; Systemic chemotherapy; Trans-arterial chemoembolisation

Core tip: This review aims to spotlight on the different diagnostic modalities, and treatment options of hepatocellular carcinoma (HCC). Despite lack of adequate sensitivity of ultrasound (US) examination and alpha-fetoprotein, both are still considered the cornerstone for surveillance for HCC. So, a plethora of clinical studies searching for a more ideal tool are running. One of these tools is the microRNAs which can be considered as a promising diagnostic as well as prognostic tool for HCC.

This review discusses the diagnostic utility of computed tomography and magnetic resonance imaging, as well as the enhanced US efficacy in diagnosis of HCC. Management of HCC depends on the tumour stage, liver function reserve, and patient performance status, and requires a multidisciplinary approach for optimal treatment. Liver transplantation and hepatic resection are the only curative options in early stage of disease. In addition, radiofrequency ablation is equivalent to surgical resection in well-selected patients. Radioembolization with use of resin or glass sphere appear promising. Novel molecular therapies are also discussed. For patients with advanced disease, sorafenib is the only approved therapy, but novel targeted agents and their combinations are emerging.

Attwa MH, El-Etreby SA. Guide for diagnosis and treatment of hepatocellular carcinoma. *World J Hepatol* 2015; 7(12): 1632-1651 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1632.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1632

INTRODUCTION

Hepatocellular carcinoma (HCC) is the 5th common type of cancer all over the world; after lung, prostate, colorectal, and stomach cancers. It evolves at a fairly constant rate of 3% per year, and is often preceded by liver cirrhosis[1]. In Egypt, incidence of HCC has increased over the last decade from 4% to 7.2%[2,3]. This remarkable increase may be explained by an increase in risk factors, such as the hepatitis C virus (HCV) infection that emerges over the same period of time[4]. Additionally, Egypt has a high prevalence of HCV affecting approximately 12% of the general population[5,6]. Cirrhosis represents an entity of diffuse hepatic disease that results from exposure to chronic injury with subsequent regeneration of liver cells and formation of abnormal structural nodules surrounded by fibrosis. It occurs secondary to chronic viral hepatitis, metabolic liver diseases (*e.g.,* hemochromatosis, α1-antitrypsin deficiency, Wilson's disease, non-alcoholic steatohepatitis), alcoholic liver disease, and autoimmune diseases (*e.g.,* primary sclerosing cholangitis, primary biliary cirrhosis, and autoimmune hepatitis)[7]. The prognosis of HCC is strongly correlated with diagnostic delay. To date, no ideal screening modality has been developed. Recent studies revealed poor sensitivity and specificity of alpha-fetoprotein (AFP) for proper surveillance and diagnosis. Thus, the basis for surveillance is still an abdominal ultrasound (US) every 6 mo despite inadequate sensitivity[8-10]. The barcelona-clinic liver cancer (BCLC) system has been certified for use by both European (EASL) and American (AASLD) associations for the Study of Liver Diseases[9,10]. The best treatment modality for liver cirrhosis as well as HCC is orthotopic liver transplantation (OLT)[11]. Different treatment options are available, including both surgical modalities (resection and liver transplantation) and radiological techniques [radiofrequency ablation (RFA), microwave ablation, percutaneous ethanol (or acetic acid) ablation and transarterial chemoembolisation (TACE)] in addition to systemic chemotherapy and molecularly targeted therapies.

DIAGNOSIS OF HCC

A multidisciplinary approach includes clinical, radiological, and laboratory modalities with or without liver biopsy (in certain cases) to establish the diagnosis of HCC.

CLINICAL PRESENTATION

In early stages, HCC usually runs a silent course making clinical diagnosis difficult. This may be due to the deep position of the liver underneath the lower ribs, making the liver difficult to feel; moreover, the tumour must reach a substantial size before it invades adjacent structures or organs. The liver has considerable functional reserves, so clinical manifestation as jaundice and other evidence of hepatic dysfunction do not appear until a large part of the organ has been replaced by tumour. Additionally, no pathognomonic symptoms or signs are attributable to HCC; tumours generally spread to distant sites late in the disease. As a result, the clinical picture is extremely variable and the patient may be completely asymptomatic with no physical signs other than of cirrhosis. Alternatively, the presentation may be florid, indicative that liver failure has occurred[12]. These complications are frequently associated with the tumour spread into the portal or hepatic veins or through arteriovenous shunt induced by the tumour[13].

Few patients may have weight loss, sense of poor appetite or early fullness of the stomach, or a visible mass in the upper abdominal part. These symptoms often signify the presence of an advanced hepatic lesion[13].

Pain

Pain is a common feature, which is dull aching in nature, of mild to moderate severity, continuous, felt as a non-specific, located in the epigastrium, and right upper quadrant or back. Rarely, pain of severe degree occurs and is due to perihepatitis or infiltration of the diaphragm. Acute abdomen may occur as a result of sudden intra-peritoneal bleeding when the tumour ruptures. The most important determinant of this complication is the superficial location of the tumour in the liver. Rupture of the tumour is either spontaneous, or follow mild blunt abdominal trauma[14]. This complication is associated with a severe drop in haematocrit and hypotension and diagnosis is made by peritoneal lavage and laparotomy. A liver mass and free intraperitoneal blood are typically seen on computed tomography (CT) scan[15]. This complication is a life-threatening, and control of bleeding is a goal. It may require emergent

angiography and embolization of the bleeding vessel or even surgery[16]. Delayed resection may be considered, although there is a high risk of peritoneal dissemination.

Other unusual clinical presentations include: (1) Obstructive jaundice owing to intrahepatic duct compression, infiltration of biliary tract or rarely, as a result of hemobilia. It occurs in less than 10% of patients from a high incidence population[17]; (2) Diarrhoea; (3) Bone aches or dyspnoea due to metastases; (4) Fever might develop in association with central tumour necrosis[18]; (5) Pyogenic liver abscess (very rare)[19]; (6) Paraneoplastic syndromes: Occasionally, patients with HCC presented by a paraneoplastic syndrome that can manifest as hypoglycaemia, thrombocytosis, erythrocytosis, hypercalcemia, or diarrhoea of watery nature. The presence of any of these manifestations, other than erythrocytosis, is generally associated with a poor prognosis[20]; (7) Hypoglycaemia: In advanced HCC, hypoglycaemia usually occurs as a reflection of increased metabolic demands of the tumour. Typically, the hypoglycaemia is mild degree and present without symptoms; however, severe reductions in the levels of plasma glucose can happen, resulting in state of lethargy and confusion. The types of hypoglycaemia have been described in HCC are two: Type A: hypoglycaemia that occurs in the late stages of the disease, the severity is a mild to moderate degree and associated with a poorly differentiated tumour; and Type B (less common): hypoglycaemia of severe degree that occurs in the early stages of the HCC and is associated with a well-differentiated slow growing tumour. The possible explanations of hypoglycaemia that occurs in HCC include high secretion of insulin-like growth factor Ⅱ by the tumour tissue (< 5% of patients) and impaired gluconeogenesis due to liver decompensation (glucose underproduction)[21-27]; (8) Erythrocytosis: In HCC, tumour secretion of erythropoietin raises in up to 23% of patients, this is probably the cause of erythrocytosis. However, elevations in packed cell volume or haemoglobin concentration are uncommon. Indeed, at the time of diagnosis most patients are anaemic, because of other effects of the tumour[28,29]; (9) Thrombocytosis: Underestimation of thrombocytosis might represent a difficult issue in HCC cases owing to the fact that most patients already have low platelet count caused by the underlying liver cirrhosis. Mean serum thrombopoietin level is significantly increased in HCC patients with thrombocytosis rather than those with normal or low platelet count. In addition, interventions like hepatic resection or TACE in HCC patients with thrombocytosis result in drop of both serum thrombopoietin level and platelet count which rise again in case of tumour recurrence[30,31]; (10) Hypercalcaemia: HCC cases might present with hypercalcaemia due to associated osteolytic metastases or secretion of parathyroid hormone-related protein[32,33]; (11) Watery diarrhoea: In one study, diarrhoea was statistically significantly more frequent among cirrhotic patients with HCC when compared to those without HCC (48% *vs* 9%, $P < 0.05$)[18]. It might be intractable and severe, leading to achlorhydria and hypokalaemia[34]. The actual mechanism of watery diarrhoea in HCC is not clearly understood. An increase in intestinal production of different peptides including vasoactive intestinal polypeptide, prostaglandin-like immunoreactivity peptides and gastrin might be a plausible explanation[34]; (12) Hyperthyroidism: may be due to increased thyroid-stimulating hormone production[35]; (13) Gynaecomastia: is painful and associated with increased level of oestrogen[36]; and (14) Systemic arterial hypertension in few patients with HCC[37].

Budd-Chiari syndrome

Budd-Chiari syndrome (BCS) is found in less than 1% of all HCC patients. Clinical features that occur in HCC patients complicated by BCS are diverse. These range from complete absence of symptoms and signs to a variable degree of abdominal or chest pain, dyspnoea, and even variceal haemorrhage. BCS diagnosis can be exclusively done by using imaging techniques only without the need to do percutaneous liver biopsy.

The diagnostic accuracy (sensitivity and specificity) of Doppler ultrasonography for the diagnosis of BCS is considered to be high ranging from 85% to 90%[38]. Bargalló *et al*[39] classified the ultrasonographic features of BCS into three categories: (1) Specific: Obstructed hepatic vein; (2) Suggestive: Collaterals between intrahepatic, portal or caval veins and caudate vein diameter more than 3 mm; and (3) Nonspecific: Hypertrophied caudate lobe, portal vein thrombosis, extrahepatic collaterals, regenerating nodules, non-homogeneous liver parenchyma, and ascites.

The most frequent US signs of BCS are altered hepatic (71.1%) and/or caval (28.9%) veins. Combination of these two signs together with caudate lobe hypertrophy has the highest positive predictive value (PPV) of 97.8% for the diagnosis of BCS[38].

CT scan allows for more comprehensive assessment of the patency of both hepatic and caval veins. It also allows for more accurate measurement of the degree of hypertrophy of caudate lobe. Magnetic resonance imaging (MRI) helps in the differentiation of chronic BCS from acute form as well as further delineation of vascular anatomy[39,40].

Cutaneous features

Numerous cutaneous manifestations have been reported in association with HCC; however, none of them are clue for the diagnosis of HCC[41]. These comprise; dermatomyositis, pemphigus foliaceus, pityriasis rotunda, Leser-Trélat sign, and porphyria cutanea tarda[42,43].

The physical findings in most patients with HCC are manifestations of decompensated cirrhosis[44]. Rarely, bruits over the liver are heard.

Laboratory examination is often nonspecific. The majority of HCCs patients have underlying hepatic disease, and subsequent cirrhosis, so hyperbilirubinaemia, hypoalbuminemia, and hypoprothrombinaemia are frequently encountered. Patients are often mildly

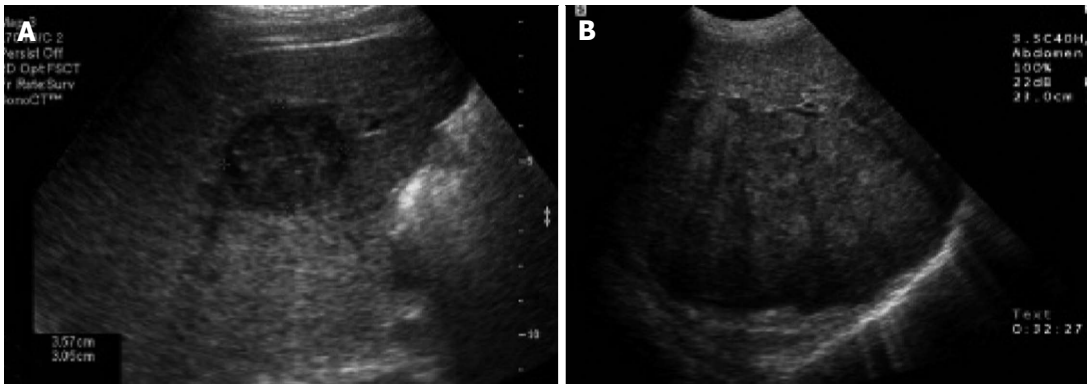

Figure 1 Abdominal ultrasound of the liver. A: Transverse sonogram shows a small, 3 cm, hypoechoic mass in the right lobe of the liver; B: Transverse sonogram shows a heterogeneous large mass in the right lobe of the liver[47].

Table 1 Sensitivity and specificity of different radiological modalities in hepatocellular carcinoma

	Sensitivity (%)	Specificity (%)
US	60	97
Colour Doppler US	92	100
MPCT	68	93
MRI	81	85
Angiography	82-93	73

MPCT: Multiphasic helical computed tomography; MRI: Magnetic resonance imaging; US: Ultrasound.

anaemic and may have thrombocytopenia. They may have electrolyte disturbances (*e.g.,* hyponatraemia, hypokalaemia, metabolic alkalosis) that are associated with defective water handling. Liver enzymes as serum aminotransferases, alkaline phosphatase, and gamma glutamyl transpeptidase are often abnormal with non-specific patterns[45].

RADIOLOGICAL DIAGNOSIS

Imaging studies for diagnosis of HCC can fall into one of two main categories: routine non-invasive studies such as US, CT, and MRI, and more specialized invasive techniques including CT during hepatic arteriography, iodised oil-CT, and CT arterial portography in addition to the conventional hepatic angiography[46]. Diagnostic accuracy assessed by the sensitivity and specificity of these different radiological modalities in HCC detection was shown in Table 1.

US

To date, the surveillance for hepatic focal lesions in high risk patients is based on US. Different appearances in US may be present; either hypoechoic, hyperechoic or target lesions. Unfortunately, all of these signs are non-specific. Suspicion for HCC is raised when any lesion is recognized in US, particularly if it is more than 1 cm in size in the background of liver cirrhosis[47] (Figure 1). Several studies assessed the diagnostic accuracy of US as a screening tool for early detection of HCC[48-51]. One systematic review concluded that the specificity was 97% (95%CI: 95%-98%) and sensitivity was 60% (95%CI: 44%-76%) compared with pathologic assessment of resected or explanted liver as a standard reference[52,53]. In a large prospective study enrolling non-cirrhotic hepatitis B carriers, the specificity, sensitivity, and PPV of US were 93%, 71%, and 15%, respectively[49]. The sensitivity increased up to 79%, when combined with AFP assessment. In other studies, sensitivity values have ranged from 78%[50] to much lower values of 40% to 50%, mostly when the diagnosis is established by examination of the resected liver after transplantation or autopsy specimens[54].

Abdominal US allows the recognition of tumours as small as 1 cm in size. Furthermore, the identification of metastases by US in a normal liver is much easier than the recognition of small HCC in a cirrhotic liver[46]. In advanced tumour stage, the patency of vascular structures and the existence of adenopathies at the hilum can be assessed by US[55]. In addition, small tumour nodules can be detected by intraoperative US during hepatic resection.

US offers many signs that raise the suspicion of malignant transformation, including the presence of intrahepatic venous thrombosis[38] (Figure 2), a mass protruding from the hepatic surface or dilated intrahepatic bile duct, even in the absence of a definite liver mass[56].

Colour Doppler US

When combining Doppler with US in patients with HCC, the detection rate of malignant portal vein thrombosis can be increased. Thus, the diagnostic accuracy can be improved, to achieve a sensitivity of 92% and a specificity of nearly 100%[47] (Figure 3). Portal thrombosis is ruled out, when US-Doppler shows no obstruction and evidence of normal permeability of the portal venous system[55].

Contrast-enhanced US

Ultrasonographic visualization and characterization of hepatic tumor vascularity can be improved by using contrast-enhanced US (CEUS) that utilizes on-

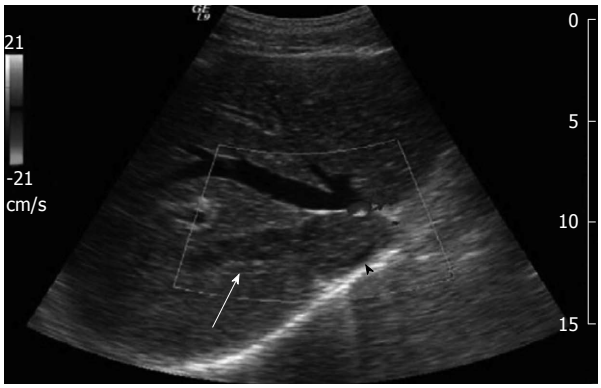

Figure 2 Doppler abdominal ultrasound shows the disappearance of Doppler flow in the intrahepatic segment of the inferior vena cava (arrowhead) and the right hepatic vein[38].

linear imaging modes[57]. CEUS can provide useful data about the characteristics of hepatic lesions that are not recognized with conventional US. CEUS has comparable diagnostic performance of CT and MRI in the characterization of hepatic focal lesions that are identified during US screening in patients with chronic liver disease or with a past history of prior malignancy[58]. CEUS is well tolerated, safe and may be the best choice when the CT or MRI are contraindicated[59]. However, a meta-analysis of 18 studies was unable to determine whether CEUS was adequate to exclude HCC lesions of size less than 30 mm[60].

Endoscopic US

Endoscopic US can establish a diagnosis of HCC especially if combined with fine needle aspiration (FNA) and has the probability to enhance the diagnostic accuracy of staging of HCC compared with CT and MRI[61]. The role of endoscopic US (EUS) in the assessment of patients with suspected HCC is still being investigated. Early experience in assessment of EUS-FNA diagnostic accuracy in detection of focal liver lesions, suggests that EUS-FNA is comparable to the results of CT-FNA. However, EUS-FNA is limited anatomically to only a portion of the hepatic parenchyma including the left hepatic lobe, hilum and proximal biliary tree. Meanwhile, extrahepatic biliary tree, gallbladder, and perihilar lymph nodes are all readily accessible[62].

Abdominal CT multiphase perfusion CT

Currently, multiphase perfusion CT is the technique of choice for diagnosis of HCC with an excellent performance in the early detection of hepatic focal lesions and staging. It comprises four subsequent phases; namely pre-contrast, hepatic arterial, portal venous and finally the delayed phases. This technique utilizes a single detector spiral scanner of a high-speed and images are taken out after injection of the contrast at a delay of 25, 70 and 300 s that correspond to the three phases of arterial, portal venous, and equilibrium, respectively. From the anatomical view, hepatic artery supplies the principal blood flow to the tumour tissues, owing to their hypervascular appearance throughout the hepatic arterial phase. Conversely, throughout the delayed phase due to early washout of contrast, HCCs appear hypodense. It was observed that images of delayed phase could assist in confirming the diagnosis of HCC in 14% of patients[56]. The gold standard imaging modality for evaluating the response after loco-regional intervention of HCC is Spiral CT[63]. In some centers, CT scan is also used as a principal screening modality for HCC in cirrhotic patients. Numerous studies have assessed test characteristics of CT for diagnosis of HCC. A systematic review estimated that the specificity was 93% (95%CI: 89%-96%) and the sensitivity was 68% (95%CI: 55%-80%) compared with pathologic tissue examination of an explanted or resected liver as the reference standard[52].

Multi-detector helical CT

Multi-detector helical CT (MDCT) has permitted us to collect early arterial phase images (18-28 s post contrast injection) as well as late (early parenchymal) arterial phase images (35-45 s post contrast injection). Although the lesions are demonstrated better in the "late" rather than the "early" arterial phase, the latter clarifies more optimally the vessels in patients who are considered for surgical resection[64]. The capability and sensitivity of MDCT in the identification of HCC lesions within the cirrhotic parenchyma of the liver is very high. This can be attributed to its elasticity and rapid speed of the device which lead to gaining images of high quality by using both thin sections and 3-D capabilities[65]. In the equilibrium phase (3-5 min after injection), the appearance of vascular tumors is hypodense relative to the surrounding liver tissue. In addition, focal hepatic lesions that measure less than 2 cm can be accurately identified in this phase due to washout of contrast rapidly from the tumor tissue than from the normal parenchyma of the liver[66,67] (Figure 4). It was observed that MDCT scan is valuable in early diagnosis of HCC and in monitoring the successful treatment of HCC lesions particularly the small ones. This is the most important issue especially during follow-up of patients with chronic viral hepatitis and/or cirrhosis[46]. Non-contrast CT scan has very low sensitivity for detecting small HCCs. If contrast cannot be given safely, US or MRI is the preferred diagnostic modality[62].

MRI

The advantage of MRI over CT is obtaining images of the liver of high-resolution without using ionizing radiation or nephrotoxic contrast agents. MRI has an equivalent diagnostic accuracy as helical CT in the early detection and diagnosis of HCC[68,69]. On T2-weighted MRI images, HCC gives a high intensity pattern, while on T1-weighted MRI images it gives a low intensity pattern[70] (Figure 5). MRI is superior to both US and CT in differentiating the nature of regenerative nodules from HCC nodules in the patient with cirrhosis[71]. A systematic review described that the sensitivity was 81% (95%CI: 70%-91%) and specificity was 85% (95%CI: 77%-93%) compared

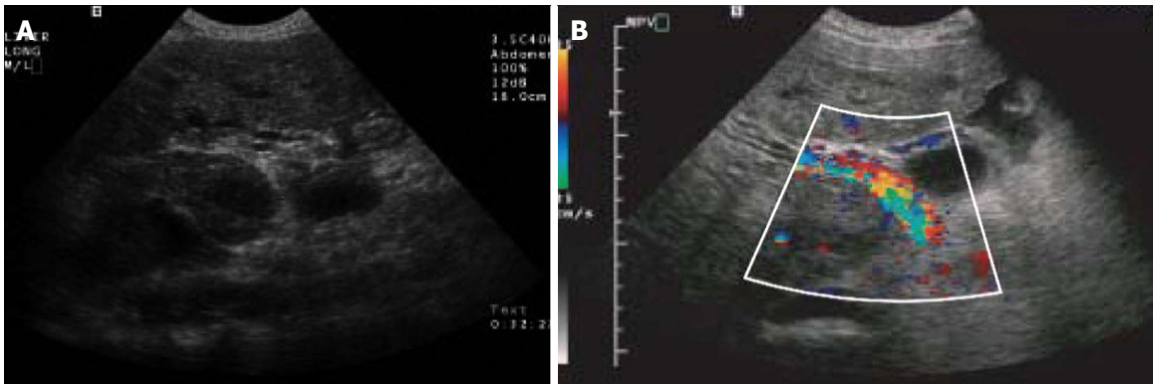

Figure 3 Colour Doppler ultrasound of the liver. A: Transverse sonogram shows portal vein thrombus; B: Transverse colour Doppler sonogram of the right upper quadrant shows heterogeneous flow within the tumour thrombus[47].

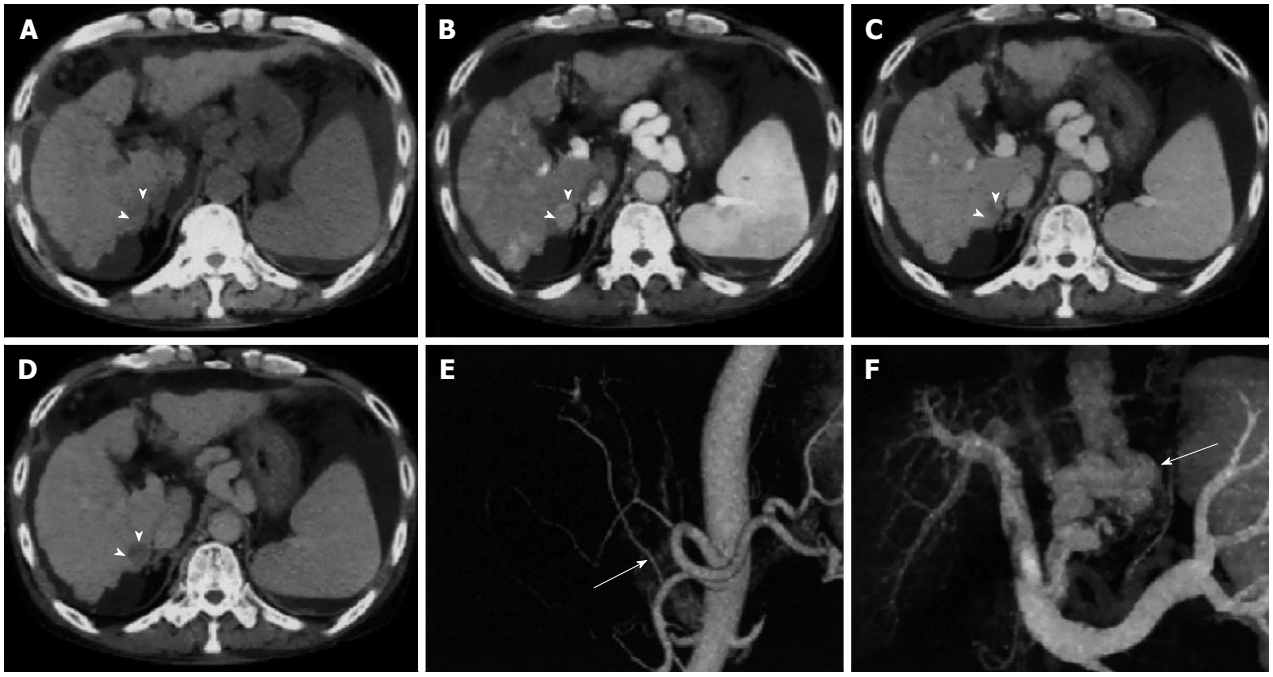

Figure 4 Multi-detector computed tomography of hepatocellular carcinoma. A: Pre-contrast; B: Late arterial phase; C: Portal venous phase; D: Equilibrium phase dynamic multi-detector computed tomography images. The hepatocellular carcinoma (arrowheads) is visualised as an enhanced nodule in the late arterial phase and as a hypo-attenuated nodule in the equilibrium phase; E: Three-dimensional computed tomography angiography of hepatic arteries shows that the right hepatic artery branches (arrow in E) from the superior mesenteric artery; F: The portal venous system with creation of varices (arrow)[66].

with pathologic tissue assessment of an explanted or resected liver tissue as the reference standard[52]. The sensitivity may be higher when used in conjunction with US. In one series of patients with known HCC, the combination of MRI and US detected 85 out of 87 HCCs[72]. The sensitivity also appears to be augmented when gadoxetic acid-enhanced and diffusion-weighted imaging are combined[73,74]. In a study of 130 patients with small ($\leqslant$ 2.0 cm) HCCs and 130 cirrhotic patients without HCC[73], the sensitivity of the combined approach was 91% to 93%, compared with 81% to 82% for gadoxetic acid-enhanced imaging and 78% to 80% for diffusion-weighted imaging alone. The specificity did not differ among the groups. In patients with advanced renal failure, gadolinium used for MRI can cause nephrogenic systemic fibrosis/nephrogenic fibrosing dermopathy, and thus alternatives should be used. As a novel technique, MRI angiography during a single breath-hold, allows for the achievement of a three-dimensional data set. This will include the 3 subsequent phases; arterial phase, portal venous phase and late venous phase[75]. In one series, comparing this procedure to triphasic CT found that it had higher sensitivity (76% *vs* 61%) for detection of HCC nodules $\geqslant$ 10 mm. Indeed, helical CT scanning is still the preferred technique used by most radiologists, because of both the high cost of MRI and the extended duration necessitated to achieve standard MRI images with good quality. MRI may be important in patients presented with renal impairment or those who had a hypersensitivity to CT contrast agents. MRI may also be valuable in cases in which CT results are unclear; this is predominantly true when the liver is severely nodular,

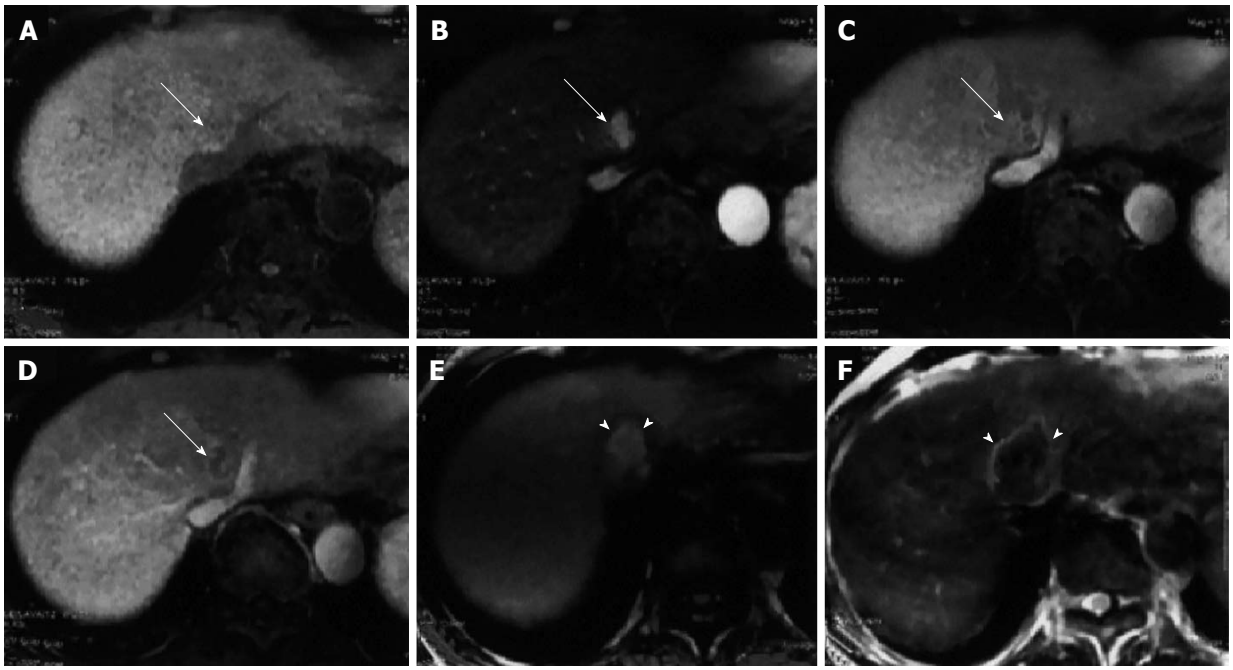

Figure 5 Dynamic magnetic resonance imaging of hepatocellular carcinoma. A: Precontrast; B: Arterial phase; C: Portal venous phase; D: Equilibrium phase; dynamic magnetic resonance imaging obtained with the LAVA sequence prior to therapeutic treatment. The hepatocellular carcinoma (HCC) (arrow) is seen adjacent to the hepatic vein as an enhanced nodule in the late arterial phase and as hypo-attenuated nodule in the portal venous and equilibrium phases; E: T1-weighted gradient echo image; F: Spin echo T2-weighted image two days after radiofrequency ablation therapy for HCC. The completely ablated area shows as hyperintense on T1-weighted images and as hypointense on T2–weighted images (arrowheads)[66].

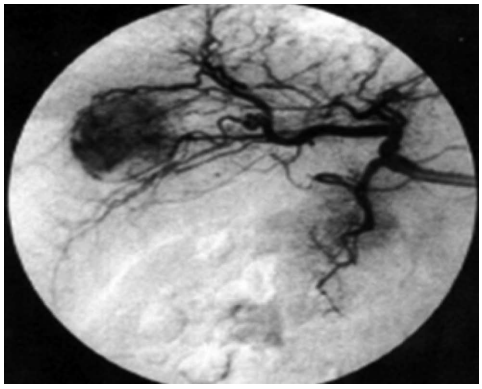

Figure 6 Hepatic angiography. Typical hyper-vascular pattern, corresponding to the findings of enhanced Doppler study, is clearly demonstrated by angiography[77].

because MRI can distinguish HCC focal lesions from dysplastic nodules[71-76]. MRI has higher performance compared to CT scanning in the characterization of focal fat lesion from HCC and vascular lesions (such as a haemangioma).

Angiography

Owing to the hypervascular characteristic of HCC, the tumor frequently has dilated, distorted, tortuous, and displaced arterial supply[77] (Figure 6). Strong tumour staining, presence of vascular lakes and venous pools are the most common features described in angiography[56]. It can enhance the detection and characterization of HCC if combined with CT or MRI scanning[78-82]. The technique includes injection of intra-arterial contrast dye (usually in the superior mesenteric, splenic, or hepatic artery) instantly before performing CT or MRI, followed by achieving images through both arterial and portal phases. The diagnostic performance of hepatic arteriography largely depends on the size of hepatic tumour and the pattern of vascularization[83]. The overall diagnostic accuracy of angiography in the diagnosis of smaller HCCs (< 5 cm) is up to 89%, with sensitivity of 82%-93%, and specificity of 73%. These estimated values were reduced, when the size of the tumor was smaller than 2 cm[55]. Recently, the less invasive and novel techniques discussed above have substituted conventional angiography for the diagnosis and early detection of HCC. So, angiography became reserved for therapeutic use during chemoembolization of tumors and for the control of bleeding from ruptured focal HCC[65]. In a randomized trial with 280 HCC patients eligible for RFA, performing CT hepatic arteriography and portography led to the identification of 75 nodules that were not detected by conventional CT scan, but this fact did not improve the recurrence-free survival rates[84].

LABORATORY DIAGNOSIS

Serum markers

The ideal tumour marker should possess the following properties: (1) high specificity and not detected in benign diseases and healthy subjects; (2) high sensitivity and detectable very early when few cancer cells are present; (3) organ specificity; (4) correlation with tumour stage

Table 2 Currently used and investigational serum tumour markers[101]

Markers	Character	Cut-off level	Sensitivity	Specificity	Comments
AFP	Oncofoetal glycoprotein	10-16 ng/dL	60%-80%	70%-90%	Poor marker alone
		20 ng/dL	39%-66%	76%-97%	
AFP-L3	AFP variant (subtype)	10%	39.9%	93.4%	Useful in combination
		15%	36.1%-96%	92%-99.5%	with other markers
GP73	Golgi-specific membrane protein	10 relative units	69%	86%	Promising marker
GPC3	Oncofoetal glycoprotein	2 ng/dL	51%	90%	Limited utility as a marker
DCP	Abnormal prothrombin	40 mAU/mL	48%-62%	81%-98%	Useful in combination
HS-GGT	Abnormal prothrombin	5.5 IU/mL	43.8%-74%	Not available	Non specific
AFU	Lysosomal enzyme	870 nmol/mL per hour	82%	71%	Lower specificity and poor marker

AFP: Alpha-fetoprotein; AFP-L3: Lens culinaris-reactive AFP; GP73: Golgi protein 73; GPC3: Glypican-3; DCP: Des-gamma-carboxyprothrombin; GGT: Gamma-glutamyl transferase; AFU: Alpha-L-fucosidase.

or tumour mass; (5) correlation with prognosis; and (6) reliable predictive value. Such a tumour marker could be used for diagnosis (in screening programs), prognosis, monitoring the effects of the therapy and as a target for localisation and therapy.

The most frequently used marker for diagnosis of HCC is the serum concentration level of AFP. Numerous serologic markers (such as des-gamma-carboxy-prothrombin) may be used; the diagnostic accuracy of which may be improved when they are combined with serum AFP. While in clinical practice, these markers are not routinely used, they represent a fruitful area of investigation (Table 2).

AFP is a glycoprotein of an oncofoetal origin. Its level is increased in patients with cirrhosis complicated by HCC. Primarily, it is a foetal-specific antigen produced in the liver of the foetus. After birth, its serum concentration falls rapidly and decreases through adult life. Elevation of serum AFP occurs normally during pregnancy, and an abnormal rise has been reported with gonadal tumours (both germ cell and non-germ cell)[85] and in a different variety of other malignancies, especially cancer stomach[86]. Several studies reported a rise of serum AFP in patients with chronic hepatic disease without HCC, and in patients with acute or chronic viral hepatitis[87,88]. In addition, patients with HCV-related cirrhosis may have a slightly higher concentration level of AFP. In one series, patients who had been treated with a combination of pegylated interferon plus ribavirin for HCV-related cirrhosis demonstrated significant decrease in AFP level after treatment[89]. Additionally, in small HCCs the serum concentrations of AFP are normal in up to 40%, as not all tumours secrete AFP[90].

A fibrolamellar carcinoma is a variant of HCC characterized by normal AFP levels in the majority of cases[91]. In one report, patients with cirrhosis and persistently elevated AFP values compared with those who have fluctuating or normal levels have an increased risk of developing HCC (29% *vs* 13% and 2.4%, respectively)[92].

Assessment of diagnostic performance of serum AFP as a screening tool in the early detection of HCC using certain parameters as sensitivity, specificity, and predictive values depends upon the value of cut-off chosen for confirming the diagnosis, the natural characteristics of the population under study, and the gold standard test used to establish the diagnosis of HCC. Test characteristics in different situations have described in several studies[49,93]. A large systematic review that enrolled five studies revealed the following prognostic values; sensitivity of 41% to 65%, specificity of 80% to 94%, negative likelihood ratio of 0.4 to 0.6 and a positive likelihood ratio of 3.1 to 6.8 when a cut-off value based upon > 20 mcg/L[89-94].

Although values of AFP above 400 ng/dL are considered diagnostic for HCC, such a high level of AFP present only in a small percentage of patients[95]. Patients with high AFP levels (above 400 ng/dL) tend to have larger tumour mass, diffuse or multilobar involvement, thrombosis of the portal vein and a lower survival rate[96].

The utility of AFP in differentiating HCC from benign liver diseases has also been noted to be limited with false negative rates and high false positive rates[97]. Although AFP is considered to be the gold standard as a serum bio-marker of HCC, its utility as a screening tool for HCC detection is of a questionable issue due to its poor performance. The role of variant forms of AFP has been investigated, to improve the diagnostic performance of AFP.

Three different AFP variants (AFP-L1, AFP-L2, and AFP-L3) have been investigated. Each variant has a different sugar chain with a differential affinity for lectins, such as *Lens culinaris* agglutinin. AFP-L3 (*Lens culinaris*-reactive AFP) is more specific and superior for HCC than is global AFP[98]. The United States Food and Drug Administration approved AFP-L3 as a screening marker for HCC. The AFP-L3 percentage is calculated as a ratio of AFP-L3 to whole AFP. Initial investigations demonstrated a specificity and sensitivity of 93.9% and 55.3%, respectively, when a cut-off value of 15% AFP-L3 was used[95].

More recent studies using the same cut-off value showed a sensitivity of 96% and specificity of 92%[89]. Another series, using cut-off values of AFP-L3 about 10% to 15% exhibited a lower sensitivity (30.9%-36.1%) but comparable specificity (93.4%-99.5%)[99]. However, the diagnostic accuracy of AFP-L3 *vs* whole AFP in

Table 3 MicroRNAs with potential prognostic impact in patients with hepatocellular carcinoma

MiRNAs	Molecular alteration	Clinical significance	Ref.
20 miRNAs	Signature	Venous metastasis, overall survival	[154]
19 mi NAs	Signature	Poor survival	[155]
MiR-19a, miR-886-5p, miR-126, miR-233, miR-24, and miR-147	Signature	Predictor of overall survival and recurrence-free survival after LT	[156]
MiR-26a	Down-regulation	Poor survival	[157]
MiR-122	Down-regulation	Gain of metastasis properties	[158,159]
MiR-122	Down-regulation	Early recurrence	[160]
Let-7 members	Down-regulation	Early recurrence	[161]
MiR-199a-3p	Down-regulation	Reduced time to recurrence	[162]
MiR-199b-5p	Down-regulation	Poor overall survival and progression-free survival rates	[163]
MiR-101	Down-regulation	Advanced tumour progression, poor prognosis	[164]
MiR-125a	Up-regulation	Better survival	[165]
MiR-92, miR-20, miR-18	Up-regulation	Poor differentiation	[166]
MiR-372	Up-regulation	Advanced TNM stage	[167]
MiR-221	Up-regulation	Multi-nodularity, reduced time to recurrence	[168]
MiR-221	Up-regulation	Gain of metastatic properties	[169]
MiR-221	Up-regulation	High tumour capsular infiltration	[170]
MiR-17-5p	Up-regulation	Multiple tumour nodules, vein invasion, shortened overall survival	[171]
MiR-155	Up-regulation	High recurrence and poor prognosis following OLT	[172]
MiR-203	Up-regulation	Good prognosis	[173]
MiR-18	Up-regulation	Poor prognosis	[174]

Data adapted from Negrini *et al*[175]. MiRNAs: MicroRNAs; TNM: Tumour-node-metastasis; LT: Liver transplantation; OLT: Orthotopic liver transplantation.

distinguishing patients with HCC from patients who have only cirrhosis and regenerating nodules, is still a matter of debate and has not been well investigated. Interestingly, several studies have reported that AFP-L3 may be a useful prognostic bio-marker for HCC, and higher percentage values are associated with increased tumour size, poorly differentiated HCC, vascular invasion, and metastasis[100]. Therefore, it appears that because of inconsistencies in its sensitivity and specificity data for predicting HCC occurrence, AFP-L3 is still unreliable, even though it is more precise and specific than whole AFP. Also, AFP-L3 may not be very useful for surveillance even though it may be a valuable prognostic bio-marker in patients with known HCC[101].

Des-gamma-carboxyprothrombin (also recognized as "prothrombin produced by vitamin K absence or antagonism Ⅱ") has also shown advances in the detection of HCC. In one series, 69 out of total 76 patients diagnosed as HCC, had a mean serum level concentration of this marker in the range of 900 mcg/L; much lower mean values were reported in chronic hepatitis patients, metastatic liver disease, and healthy individuals (10 and 42 mcg/L and undetectable, respectively). Tumours of less than 3 cm in size show less frequent elevations in des-gamma-carboxyprothrombin. In addition, there is no significant correlation between serum levels of abnormal prothrombin and AFP[102-107].

MicroRNAs

The plasma microRNA expression has also been investigated as a possible marker of HCC[108-110]. One study examined 934 participants in 4 groups; healthy, chronic HBV, cirrhosis, and HBV-related HCC[108]. Regardless of the stage of HCC, a microRNA panel that included miR-122, miR-192, miR-21, miR-223, miR-26a, and miR-801 accurately identified patients with HCC (AUC 0.89 with a specificity of 84% and a sensitivity of 82% for the validation set). This microRNA panel also accurately differentiated patients with HCC from healthy individuals and those with cirrhosis or chronic HBV. Additionally, microRNAs have a potential prognostic impact in patients with HCC (Table 3).

Other serum bio-markers of HCC that have been studied include: (1) Tumour-associated isoenzymes of gamma-glutamyl transpeptidase[111]; (2) Transforming growth factor-β-1 in urine[112]; (3) Serum circulating intercellular adhesion molecule-1 level[113]; (4) Serum alpha-L-fucosidase activity[114]; (5) Glypican-3 (a cell-surface heparin sulfate proteoglycan)[115]; (6) Dickkopf-1[116]; (7) Human carboxylesterase 1[117]; and (8) Plasma proteasome[118,119].

PERCUTANEOUS LIVER BIOPSY

It should only be considered when diagnostic imaging results are doubtful, for example, in patients with cirrhosis and nodules of hypovascular nature, and the result would directly have an impact on management[120,121]. Both the European (EASL) and the American (AASLD) Associations for the Study of Liver Diseases have proposed a role for liver biopsy in confirming the diagnosis and identification of HCC[9,10]. In addition, clinical criteria have been developed that can be designed for prioritising patients with any suspicion of HCC prior to liver transplantation without confirming the diagnosis with a biopsy[63]. Possible risks encountered of a biopsy include bleeding, and tumour spread through the track of the needle. The magnitude of the risk ranges from 1.6% to 5%[122-126]. A large meta-analysis of eight clinical studies estimated that the overall risk was 2.7%[127].

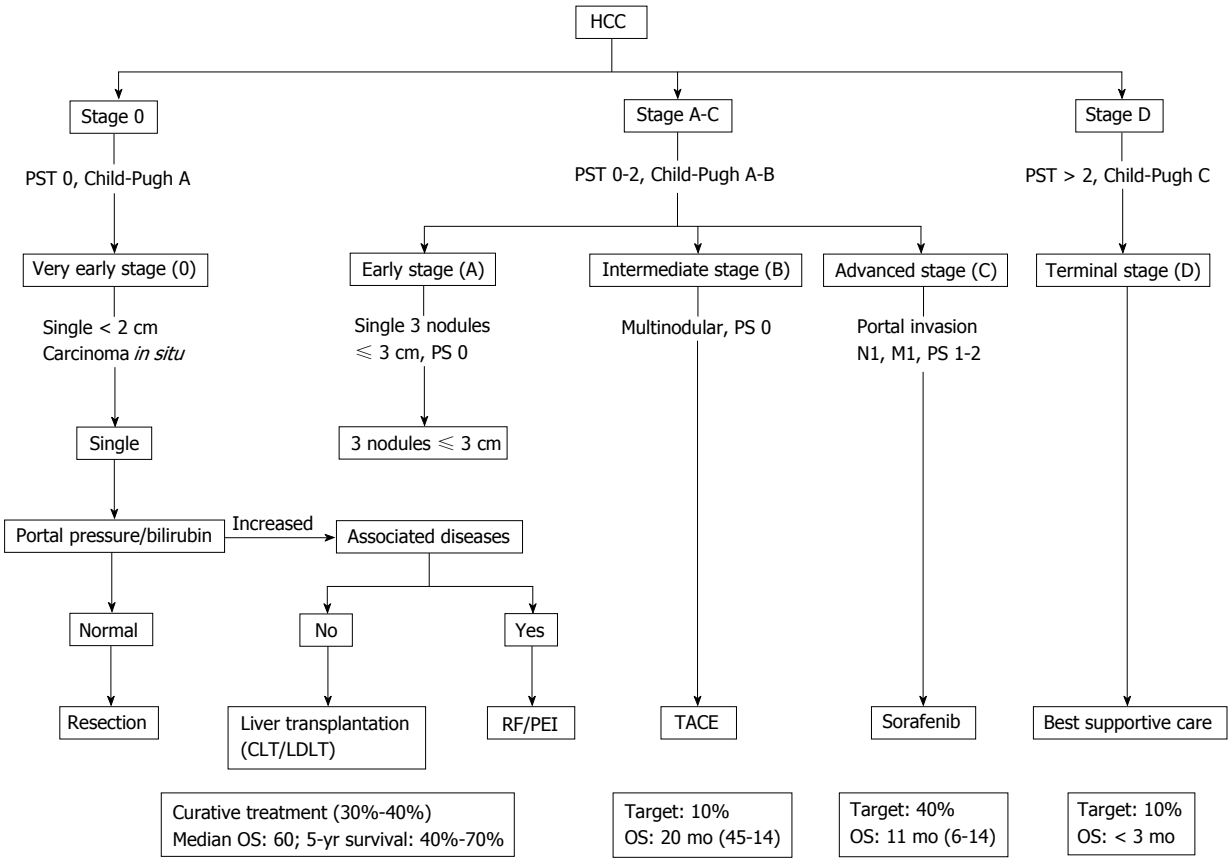

Figure 7 Updated Barcelona Clinic Liver Cancer staging system and treatment strategy, 2011[10]. The BCLC algorithm classifies HCC into five stages-based on the extent of disease, Child-Pugh score, and ECOG performance status-that enables prognostication and informs allocation of first-line treatment. BCLC: Barcelona Clinic Liver Cancer (group); HCC: Hepatocellular carcinoma; PST: Performance status test; TACE: Transarterial chemoembolization; RF: Radiofrequency; PEI: Percutaneous ethanol infusion; CLT: Cadaveric liver transplant; LDLT: Living donor liver transplant.

However, some reports have not observed any adverse impact of preoperative FNA, or an increased risk of needle tract seeding, on long-term outcome and survival in patients who were considered for elective surgical resection of HCC[120,128,129]. Although the potential risk of spreading tumour through the biopsy tract should always be considered, liver biopsy might be performed in certain circumstances as surgical resection and liver transplantation.

TREATMENT OF HCC

Surveillance programs have been conducted in the high-risk group patients; and led to an increasing number of early detection of HCC[130]. Nevertheless, HCC is usually diagnosed late along its course, and the median survival is approximately 6 to 20 mo after the establishment of the diagnosis[9,10]. Based upon pre-established prognostic criteria, the BCLC classification categorizes HCC patients into five stages (0, A, B, C and D)[10] (Figure 7). Different treatment modalities are available including: (1) Surgical resection; (2) Liver transplantation; (3) RFA; (4) Microwave ablation; (5) Percutaneous ethanol or acetic acid ablation; (6) TACE; (7) Radioembolization; (8) Cryoablation; (9) Radiation therapy (RT) and stereotactic radiotherapy; and (10) Systemic chemotherapy and molecularly targeted therapies.

SURGICAL RESECTION

In patients with adequate liver functional reserve, potentially curative partial hepatectomy is the optimal treatment for HCC. Solitary HCC confined to the liver is ideal for surgical resection, which shows no radiographic evidence of portal hypertension, no evidence of invasion of the hepatic vasculature, and preserved hepatic function. In carefully selected patients, Relapse-free Long-term survival rates, average 40% or better and five-year survival rates as high as 90% are reported.

The preoperative evaluation should focus on the probability of disease being confined to the liver, and whether the anatomic bonds of the intrahepatic tumour and the underlying liver function will permit resection. Although surgeons restrict suitability for resection of patients with tumours that are ⩽ 5 cm in diameter, there is no general basis regarding tumour size for determination of patients for resection. A comparable survival rate reported in patients with a solitary HCC without vascular invasion regardless of tumour size, although patients with smaller tumours tend to have a better outcome.

Hepatic reserve assessment is of paramount im-

portance in the selection of patients for resection. Perioperative mortality is twice as high in cirrhotic as in non-cirrhotic patients unless proper selection of patient is applied. Patients with Child-Pugh class (A) cirrhosis, who have a normal bilirubin and well-preserved hepatic function, can undergo operative surgical resection safely. Also, liver volumetry, and portal vein patency should be assessed before major surgical liver resection in those patients.

The utilization of intraoperative US allows accurate localisation and staging of tumours. Anatomical consideration is favoured by some gastrointestinal surgeons during surgical resection, where segment-wise hepatectomies is carried out whenever feasible. This depends on the fact that microscopic intrahepatic metastasis is expected to rise in the same original segment from which the tumour rise. Interestingly, even after apparently curative surgical resection, recurrent HCC develops in 80% of patients within five years because of latent metachronous multicentric carcinogenesis or intrahepatic metastasis[131].

LIVER TRANSPLANTATION

Practically all patients who are candidate for liver transplantation are unresectable because of the degree of underlying liver dysfunction rather than tumour extent. Liver transplantation can be considered as an appropriate treatment option for patients with end stage liver disease and earlier stage HCC. OLT is an appropriate option for patients with chronic liver disease (usually cirrhosis) who would not tolerate liver resection and according to Milan criteria have a single HCC focal mass with size $\leqslant$ 5 cm in diameter or up to three separate focal lesions, with any one of them is not larger than 3 cm, with no distant metastases or regional nodal and no evidence of gross invasion of vascular structure.

When these strict selection criteria used, excellent overall three- to four-year actuarial (75% to 85%) and recurrence-free survival rates (83% to 92%) could be achieved. Long-term survival is similar to or only a little worse in precisely designated patients experiencing OLT for HCC, than survival for patients performing OLT for non-malignant aetiologies. Patel *et al*[132] found that patients transplanted within Milan criteria and those transplanted outside of Milan criteria but within the University of California, San Francisco criteria (one tumor $\leqslant$ 6.5 cm, or $\leqslant$ 3 tumors with largest tumor diameter $\leqslant$ 4.5 cm and total tumor diameter $\leqslant$ 8 cm) had equivalent outcomes.

A major disadvantage with OLT (in addition to the need for lifelong immunosuppression with its attendant risks) is the long waiting time for donor organs. The requirement for livers continues to go up as the waiting list of possible recipients remains to be extended. The shortage of donor organs is a universal problem. With 12 mo duration waiting lists, many patients (up to 25%) are expected to be excluded from liver transplantation due to tumour progression. A scarcity of donor livers has led to the development of schema, whereby preference for donor organs is given to the most critically ill patients. In the United States, the selection depends on the "model for end-stage liver disease" score which is used in the prediction of median survival in cirrhotic patients putting into consideration that diagnosis of HCC will increase the priority score for donor organs[131,133].

RFA

As regards loco-regional intervention ablation therapy of HCC, RFA has been the most commonly evaluated option. Distinctive types of electrode are available including internally cooled electrodes and multi-tined expandable electrodes with or without perfusion for clinical RF ablation[134]. The raising of temperature with subsequent damage is dependent on both the degree of the temperature achieved within the tissue and the whole length of exposure to the heating process. Irreversible cellular damage produced by raising the temperature of tumour tissue to 50 ℃-55 ℃ for 4-6 min. In addition, tissue vaporises and carbonises at more than 100 ℃-110 ℃. A key factor that affects the achievement of successfully RF ablation is the capability to destroy all viable tumour tissues and probably a satisfactory tumour-free margin. Ideally, ablative margin at 360°, and 0.5-1 cm-thick should be created around the tumour. This cutoff would verify that microscopic invasions around the margin of a tumour have been eliminated[135].

While there is no precise tumour size beyond which RFA should not be applied, single tumour < 4 cm in diameter have the best outcome. As noted above, RFA has also been used as a "bridging" therapy[136].

Five randomised controlled trials (RCTs) have compared the efficacy of RFA *vs* percutaneous ethanol infusion (PEI) in the management of early-stage HCC. RF ablation showed superiority in the local control of the HCC than PEI due to its higher anti-cancer effects[137]. In addition, two large meta-analyses endorsed RF ablation as the standard percutaneous modality for HCC treatment[138].

A question arises here, whether RFA can replace the surgical resection as the first-line option for patients with small, single HCCs. A RCT comparing RFA ablation *vs* surgical resection in Child A patients with a solitary HCC lesion of diameter 5 cm or less, has failed to demonstrate any statistically significant differences in disease-free survival and overall survival rate between the two treatment groups[139].

Another limitation of RFA applicability in the treatment of HCC is the anatomical location of the HCC lesions; those who are close to the gastrointestinal system or near portahepatis or gall bladder are risky, with the potential for major complications[140]. Up to 30% of the small size tumours may not be appropriate for RFA due to their unfavourable position. Consequently, there are no clear data to support RFA as an alternative technique for surgical resection as a first-line in the management plan for patients with early-stage HCC[141].

MICROWAVE ABLATION

Microwave ablation is a medical term used for all electromagnetic methods that result in tumour damage by using different devices with various frequencies (equal to or more than 900 kHz). These microwaves move through the cells or other materials containing water leading to rotation of the different molecules. The rapid rotation of these molecules produces a consistent and evenly distributed hotness, which is continued until the radiation is completely stopped. A necrosis area in the form of round or column shape, created by microwave irradiation around the needle is formed, depending on the type of needle used and the amount of power generated[142].

One RCT compared the efficacy of both microwave ablation and RF ablation in the management of HCC. There is a propensity favouring RFA over microwave ablation despite taking into account the rate of both complications and local recurrences and that there was no statistically significant differences in the efficacy of the two procedures. It has to be noted that technology of microwave ablation has improved dramatically. Newer devices seem to have overcome the limitation encountered by the small size of the coagulation area produced by a single probe insertion. Microwave ablation offers an important advantage over RF ablation in that treatment outcome is not influenced by the site of the tumour[135].

PEI OR ACETIC ACID ABLATION

PEI is often indicated in patients who are not suitable for resection and with small HCCs due to their poor functional hepatic reserve. The ethanol produces coagulation necrosis of the tumour mass, through different mechanisms as dehydration of cells, denaturation of protein, and occlusion of small vessels. PEI is a well-established technique for the management of HCC nodular-type. Nodules of HCC have soft consistency within a firm cirrhotic liver. Consequently, the injected ethanol diffuses easily and selectively within these lesions. Multiple (4-6) sessions of PEI have been shown to be effective with complete response (CR) in approximately 70% of all lesions of small size[143].

An inherent advantages of PEI are low financial cost and low morbidity, but the higher local recurrence rate is a major limitation, that may occur in up to 33% in small lesions (less than 3 cm) and in up to 43% in larger lesions[144].

The CR is not usually achieved by ethanol injection, due to the uneven allocation of the ethanol within the lesion (particularly in the existence of intra-tumoural septa) and a restricted influence on extracapsular cancer cells spread. The current advance of dedicated multi-pronged PEI needles has been shown to overcome some of these limitations, leading to the achievement of 90% sustained rate of CR, when the size of the tumour is smaller than 3 cm and furthermore treated only with a single session ablation. Acetic acid injection as another method of chemical ablation has been utilized for the treatment of HCC. However, this procedure has been investigated by very few researchers worldwide[145].

TACE

TACE has been commonly used for treatment of unresectable type of HCC. The principle of TACE depends on deprivation of HCC lesions from their blood supply which is predominantly derived from the hepatic artery, whereas portal system supplies blood to surrounding liver tissue. TACE is preferable for multiple or large size focal HCC lesions and even in cases of impaired hepatic reserve. The tip of the catheter is introduced at the nearest and the achievable site of the feeding artery of HCC lesion. Under fluoroscopic monitoring, an emulsion of anti-cancer agents combined with lipidol followed by careful injection of gelatin sponge particles. The dose of chemotherapeutic agent and lipidol emulsion used in TACE is calculated and determined according to tumour size and extension of the lesions[131]. A more recent method of chemoembolisation involves the use of drug-eluting polyvinyl alcohol microspheres ("beads"), which seems to cause less toxicity with similar efficacy. Use of drug-eluting beads causes simultaneous or sequential occlusion of the feeding branch of hepatic artery until blood flow to the tumour tissue ceases, which may lead to greater efficacy of anti-cancer drug than chemotherapy alone.

TACE is the first-line strategy in the plan of down-staging of HCC tumours that exceed the criteria for transplantation[146]. During follow up, dynamic CT or MRI has been done every 3 to 4 mo and TACE was considered when local recurrence, second primary HCCs and/or intrahepatic metastasis were found. Limitations of TACE include invasion of liver capsule, extracapsular growth of the tumour, or invasion of the vessels with thrombosis. Rarely, complete ablation and necrosis of whole lesions is obtained, so the TACE should be considered for advanced HCC lesions that cannot be treated by resection or ablation.

Takayaso *et al*[146] noticed that TACE resulted in a five-year survival rate of 26%. A mortality rate of 0.5% was reported in 8510 patients with unresectable tumour of HCC. Patient survival can be predicted by assessment of hepatic reserve, characteristics of HCC (size, number of lesions, portal vein patency, and presence of tumour invasion) and values of alpha fetoprotein[147,148].

Adverse events associated with TACE were described in approximately 10% of treated patients; these events include abdominal pain, nausea, vomiting, bone marrow suppression, and ischemic cholecystitis. A post-embolisation syndrome has been observed in > 50% of patients treated with TACE and the patients usually presented with fever, abdominal pain, and intestinal obstruction of moderate degree. Treatment-related mortality is less than 5%[147].

Absolute contraindications to TACE include portal

vein thrombosis (absence of hepatopetal blood flow), encephalopathy, and biliary obstruction. Relative contraindications include increased level of bilirubin > 2 mg/dL, aspartate aminotransferase > 100 unit/L, lactate dehydrogenase > 425 unit/L, tumour burden occupying > 50% of the liver, cardiac or renal co-morbidities, ascites, significant thrombocytopaenia, or recent variceal hemorrhage[149].

TACE PLUS RFA

Combined TACE and RFA can be applied to overcome the limitations of RFA when used alone. Three meta-analyses have concluded that combined TACE together with RFA was associated with an improvement of overall survival rate than RFA alone. While the combination of TACE plus RFA may be better than RFA alone, there is no obvious indication that it is better than TACE alone[10].

CRYOABLATION

Cryoablation is the application of alternating freeze-thaw cycles through the use of a cryoprobe inserted directly into the tumour; this procedure is used intraoperatively more frequently in HCC patients with unresectable lesions. RFA has supplanted the use of percutaneous cryoablation[10].

RT AND STEREOTACTIC BODY RADIOTHERAPY

External-beam RT is an emerging utility in the management of liver cancer, although its place among other treatment modalities for patients with unresectable HCC, its role has yet to be clarified. HCC is considered one of the radiosensitive tumours, and the liver is an extremely radiosensitive organ. As a whole, the liver can only tolerate radiation doses of approximately 20 Gy, although newer techniques using three-dimensional conformal treatment planning or stereotactic focusing may permit the guided delivery of up to 100 Gy.

Stereotactic body radiotherapy (SBRT) is a technique in which a high-dose radiation fractions used as a single or in limited numbers are delivered and condensed to a small, accurately distinct target lesion by utilizing multiple, non-parallel beams of radiation. These beams focus and converge exactly on the precise target lesion, minimising adjacent healthy tissue from exposure to radiation. This precise targeting facilitate treatment of small- or medium-sized tumours in extracranial sites in either both types of single or limited number of dose fractions, but experience with SBRT is still limited[150-152].

RADIOEMBOLISATION

A variety of hepatic artery-directed treatment is radionuclide yttrium-90 therapy. Microspheres containing yttrium-90 of approximately 25 mm in diameter are introduced through a catheter precisely inserted into the hepatic artery at the lobar or segmental level, and discharge of local radiation with minimal radiation exposure to neighbouring healthy tissue[125].

High intensity focused US (HIFU) therapy has been evolved for the treatment of solid organs tumours. The principle of HIFU is based on an extracorporal US source that focuses to a predetermined target lesion inside the body. The US energy passes safely through overlying tissues to a predetermined specific target area. The energy deposition rapidly produces a rapid elevation of the temperature of this area, resulting in an irreversible death of cells with an obvious area of tissue necrosis. The reported disadvantages of HIFU therapy include the long duration of the procedure (3-4 h) and the rarely required rib resection when the tumour is situated behind the rib bone[131]. In addition to the high cost, certain anatomical constraints (*e.g.,* pass-through of the radioactive material to the lung in some patients with shunting) and a question of less effective tumour necrosis than is seen with TACE limit the utility of this treatment. It is under active study for patients with thrombosis of the portal vein and those with advanced disease, but still there is an appropriate liver reserve. No RCTs are currently available.

SYSTEMIC THERAPY

Molecularly targeted therapy (sorafenib)

One of the oral multi-tyrosine kinase inhibitor is sorafenib which is currently considered as the first drug that might improve survival in patients with advanced HCC. The multicentre European randomised SHARP trial in 2007, demonstrated monotherapy with sorafenib agent as a standard systemic treatment for advanced tumour of HCC.

In a large series, double-blinded placebo-controlled phase Ⅲ, the estimated median overall survival estimated in months was found to increase from 7.9 in the placebo arm to 10.7 in the sorafenib arm (HR = 0.69; 95%CI: 0.55-0.87; P = 0.00058), which results in a 31% decrease in the relative risk of death.

A preliminary data from a randomised phase Ⅱ trial comparing the results of sorafenib plus doxorubicin *vs* doxorubicin alone suggested an advantage for combined therapy; however, whether this combination is superior to sorafenib alone will necessitate a randomised trial in which the sorafenib is the control arm.

Cytotoxic chemotherapy

Chemotherapy has not been used consistently in the treatment of advanced HCC for a several reasons: (1) the relatively chemotherapy-refractory nature of HCC tumour. This is partially due to the high rate of drug resistance gene expression, including mutations in p53, glutathione-S-transferase, P-glycoprotein, and the heat shock proteins; (2) systemic chemotherapy is typically

Table 4 Ongoing randomised phase Ⅱ-Ⅲ trials aimed at changing the standard of care in hepatocellular carcinoma management during the period 2012-2013[110]

Indication	Randomised studies
Adjuvant	Sorafenib *vs* placebo
Intermediate HCC	Chemoembolisation ± sorafenib
	Chemoembolisation ± brivanib
	Chemoembolisation ± everolimus
Advanced HCC	
First line	Sorafenib ± erlotinib
	Sorafenib *vs* brivanib
	Sorafenib *vs* sunitinib
	Sorafenib *vs* linifanib
	Sorafenib ± yttrium-90
	Sorafenib ± doxorubicin
Second line	Brivanib *vs* placebo
	Everolimus *vs* placebo
	Ramucirmab *vs* placebo

HCC: Hepatocellular carcinoma.

not properly acknowledged and endured by patients owing to the presence of significant underlying poor hepatic reserve. The overall survival of the patients is most often estimated neither by tumour aggressiveness nor the impact of a systemic treatment, but by the degree of impairment of hepatic reserve; and (3) clinical trials of systemic chemotherapy in patients with advanced stage of HCC have been conducted in different variety of populations.

Despite these issues, emerging data suggest a modest degree of antitumour efficacy for several cytotoxic agents and/or combined drug regimens; a chemotherapy trial may be warranted in many individuals, particularly if they have normal underlying liver. Reactivation of viral hepatitis may happen in patients with HCC who are experiencing intense systemic chemotherapy; it is therefore important to continue their antiviral medications.

Using a combination therapy of intra-arterial infusion of 5-Fluorouracil (5-FU) and subcutaneous interferon in 116 HCC patients with portal vein invasion has shown promising results[153].

The treatment cycle comprises of 4 wk; 5000000 u (5 MU) IFU was administered three times weekly, given intramuscularly at days 1, 3, and 5 from each week, with a total dose of 60 MU per cycle. 5-FU (500 mg/d) was injected into the hepatic artery through a portable infusion pump on days 1-5 of the first and second weeks through the intra-arterial catheter (5 gm per cycle) over a period of five hours each time. A CR achieved in 19 (16%) patients and a partial response obtained in the other 42 (36%). Only, nausea and appetite loss were noticed as adverse events. The overall survival rates reported at 12 and 14 mo among those patients were 34% and 18%, respectively, and those among patients who achieved CR were 81% and 59%, respectively[131].

Other molecular targets under clinical development are shown in Table 4.

CONCLUSION

At present, the surveillance of HCC is based on US examination every 6 mo because AFP lacks the satisfactory sensitivity and specificity necessary for successful surveillance and diagnosis. Numerous clinical trials searching for a more ideal tool are running. One of these tools is the microRNAs which can be considered as a promising diagnostic as well as prognostic tool for HCC. Treatment of HCC depends on assessment of the tumour stage using BCLC or other scoring systems, preserved hepatic function, and performance status of the patients. Thus, a multidisciplinary approach is required for an optimal treatment of HCC. Indeed, hepatic surgical resection and liver transplantation are the only curative treatment options in the early stages of disease. RFA is equivalent to surgical resection in highly-selected patients with an early stage of HCC. Radioembolisation using resin or glass spheres appears to be a promising tool. Molecular studies of HCC have recognized peculiar activation of different signalling pathways, which signify key targets for emerging molecular therapies. The only approved therapy in patients with advanced disease is sorafenib, but novel and emerging targeted agents and their combinations are promising and being used in several clinical trials.

REFERENCES

1 **El-Serag HB**. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]

2 **Rahman El-Zayadi A**, Abaza H, Shawky S, Mohamed MK, Selim OE, Badran HM. Prevalence and epidemiological features of hepatocellular carcinoma in Egypt-a single center experience. *Hepatol Res* 2001; **19**: 170-179 [PMID: 11164741 DOI: 10.1016/S1386-6346(00)00105-4]

3 **el-Zayadi AR**, Badran HM, Barakat EM, Attia Mel-D, Shawky S, Mohamed MK, Selim O, Saeid A. Hepatocellular carcinoma in Egypt: a single center study over a decade. *World J Gastroenterol* 2005; **11**: 5193-5198 [PMID: 16127751]

4 **el-Serag HB**. Epidemiology of hepatocellular carcinoma. *Clin Liver Dis* 2001; **5**: 87-107, vi [PMID: 11218921 DOI: 10.1016/S1089-3261(05)70155-0]

5 **Arafa N**, El Hoseiny M, Rekacewicz C, Bakr I, El-Kafrawy S, El Daly M, Aoun S, Marzouk D, Mohamed MK, Fontanet A. Changing pattern of hepatitis C virus spread in rural areas of Egypt. *J Hepatol* 2005; **43**: 418-424 [PMID: 16019104 DOI: 10.1016/j.jhep.2005.03.021]

6 **Strickland GT**, Elhefni H, Salman T, Waked I, Abdel-Hamid M, Mikhail NN, Esmat G, Fix A. Role of hepatitis C infection in chronic liver disease in Egypt. *Am J Trop Med Hyg* 2002; **67**: 436-442 [PMID: 12452500]

7 **Moradpour D**, Blum HE. Pathogenesis of hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 2005; **17**: 477-483 [PMID: 15827436 DOI: 10.1097/00042737-200505000-00002]

8 **Singal A**, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, Marrero JA. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009; **30**: 37-47 [PMID: 19392863 DOI: 10.1111/j.1365-2036.2009.04014.x]

9 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

10 **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]
11 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262]
12 **Stevens WR**, Johnson CD, Stephens DH, Batts KP. CT findings in hepatocellular carcinoma: correlation of tumor characteristics with causative factors, tumor size, and histologic tumor grade. *Radiology* 1994; **191**: 531-537 [PMID: 8153335 DOI: 10.1148/radiology.191.2.8153335]
13 **Sugano S**, Miyoshi K, Suzuki T, Kawafune T, Kubota M. Intrahepatic arteriovenous shunting due to hepatocellular carcinoma and cirrhosis, and its change by transcatheter arterial embolization. *Am J Gastroenterol* 1994; **89**: 184-188 [PMID: 8304300]
14 **Kew MC**, Hodkinson J. Rupture of hepatocellular carcinoma as a result of blunt abdominal trauma. *Am J Gastroenterol* 1991; **86**: 1083-1085 [PMID: 1650131]
15 **Choi BG**, Park SH, Byun JY, Jung SE, Choi KH, Han JY. The findings of ruptured hepatocellular carcinoma on helical CT. *Br J Radiol* 2001; **74**: 142-146 [PMID: 11718385 DOI: 10.1259/bjr.74.878.740142]
16 **Chearanai O**, Plengvanit U, Asavanich C, Damrongsak D, Sindhvananda K, Boonyapisit S. Spontaneous rupture of primary hepatoma: report of 63 cases with particular reference to the pathogenesis and rationale treatment by hepatic artery ligation. *Cancer* 1983; **51**: 1532-1536 [PMID: 6297703]
17 **Kew MC**, Dos Santos HA, Sherlock S. Diagnosis of primary cancer of the liver. *Br Med J* 1971; **4**: 408-411 [PMID: 5124443 DOI: 10.1136/bmj.4.5784.408]
18 **Bruix J**, Castells A, Calvet X, Feu F, Bru C, Solé M, Bruguera M, Rodés J. Diarrhea as a presenting symptom of hepatocellular carcinoma. *Dig Dis Sci* 1990; **35**: 681-685 [PMID: 2160881 DOI: 10.1007/BF01540166]
19 **Lin YT**, Liu CJ, Chen TJ, Chen TL, Yeh YC, Wu HS, Tseng CP, Wang FD, Tzeng CH, Fung CP. Pyogenic liver abscess as the initial manifestation of underlying hepatocellular carcinoma. *Am J Med* 2011; **124**: 1158-1164 [PMID: 22114829 DOI: 10.1016/j.amjmed.2011.08.012]
20 **Luo JC**, Hwang SJ, Wu JC, Lai CR, Li CP, Chang FY, Chiang JH, Lui WY, Chu CW, Lee SD. Clinical characteristics and prognosis of hepatocellular carcinoma patients with paraneoplastic syndromes. *Hepatogastroenterology* 2002; **49**: 1315-1319 [PMID: 12239934]
21 **Eastman RC**, Carson RE, Orloff DG, Cochran CS, Perdue JF, Rechler MM, Lanau F, Roberts CT, Shapiro J, Roth J. Glucose utilization in a patient with hepatoma and hypoglycemia. Assessment by a positron emission tomography. *J Clin Invest* 1992; **89**: 1958-1963 [PMID: 1318326 DOI: 10.1172/JCI115803]
22 **Yeung RT**. Hypoglycaemia in hepatocellular carcinoma: a review. *Hong Kong Med J* 1997; **3**: 297-301 [PMID: 11847375]
23 **Tietge UJ**, Schöfl C, Ocran KW, Wagner S, Böker KH, Brabant G, Zapf J, Manns MP. Hepatoma with severe non-islet cell tumor hypoglycemia. *Am J Gastroenterol* 1998; **93**: 997-1000 [PMID: 9647037 DOI: 10.1111/j.1572-0241.1998.00297.x]
24 **Thipaporn T**, Bubpha P, Varaphon V. Hepatocellular carcinoma with persistent hypoglycemia: successful treatment with corticosteroid and frequent high carbohydrate intake. *J Med Assoc Thai* 2005; **88**: 1941-1946 [PMID: 16518997]
25 **Nikeghbalian S**, Bananzadeh A, Yarmohammadi H. Hypoglycemia, the first presenting sign of hepatocellular carcinoma. *Saudi Med J* 2006; **27**: 387-388 [PMID: 16532104]
26 **Jayaprasad N**, Anees T, Bijin T, Madhusoodanan S. Severe hypoglycemia due to poorly differentiated hepatocellular carcinoma. *J Assoc Physicians India* 2006; **54**: 413-415 [PMID: 16909744]
27 **Sorlini M**, Benini F, Cravarezza P, Romanelli G. Hypoglycemia, an atypical early sign of hepatocellular carcinoma. *J Gastrointest Cancer* 2010; **41**: 209-211 [PMID: 20204540 DOI: 10.1007/s12029-010-9137-0]
28 **Kew MC**, Fisher JW. Serum erythropoietin concentrations in patients with hepatocellular carcinoma. *Cancer* 1986; **58**: 2485-2488 [PMID: 2429757]
29 **Sakisaka S**, Watanabe M, Tateishi H, Harada M, Shakado S, Mimura Y, Gondo K, Yoshitake M, Noguchi K, Hino T. Erythropoietin production in hepatocellular carcinoma cells associated with polycythemia: immunohistochemical evidence. *Hepatology* 1993; **18**: 1357-1362 [PMID: 7694895 DOI: 10.1002/hep.1840180612]
30 **Hwang SJ**, Luo JC, Li CP, Chu CW, Wu JC, Lai CR, Chiang JH, Chau GY, Lui WY, Lee CC, Chang FY, Lee SD. Thrombocytosis: a paraneoplastic syndrome in patients with hepatocellular carcinoma. *World J Gastroenterol* 2004; **10**: 2472-2477 [PMID: 15300887]
31 **Carr BI**, Guerra V. Thrombocytosis and hepatocellular carcinoma. *Dig Dis Sci* 2013; **58**: 1790-1796 [PMID: 23314854 DOI: 10.1007/s10620-012-2527-3]
32 **Knill-Jones RP**, Buckle RM, Parsons V, Calne RY, Williams R. Hypercalcemia and increased parathyroid-hormone activity in a primary hepatoma. Studies before and after hepatic transplantation. *N Engl J Med* 1970; **282**: 704-708 [PMID: 4313545 DOI: 10.1056/NEJM197003262821302]
33 **Yen TC**, Hwang SJ, Wang CC, Lee SD, Yeh SH. Hypercalcemia and parathyroid hormone-related protein in hepatocellular carcinoma. *Liver* 1993; **13**: 311-315 [PMID: 8295494 DOI: 10.1111/j.1600-0676.1993.tb00651.x]
34 **Steiner E**, Velt P, Gutierrez O, Schwartz S, Chey W. Hepatocellular carcinoma presenting with intractable diarrhea. A radiologic-pathologic correlation. *Arch Surg* 1986; **121**: 849-851 [PMID: 3013124 DOI: 10.1001/archsurg.1986.01400070119025]
35 **Helzberg JH**, McPhee MS, Zarling EJ, Lukert BP. Hepatocellular carcinoma: an unusual course with hyperthyroidism and inappropriate thyroid-stimulating hormone production. *Gastroenterology* 1985; **88**: 181-184 [PMID: 2981080 DOI: 10.1016/S0016-5085(85)80152-9]
36 **Summerskill WH**, Adson MA. Gynecomastia as a sign of hepatoma. Report of a case. *Am J Dig Dis* 1962; **7**: 250-254 [PMID: 13918386 DOI: 10.1007/BF02231817]
37 **Kew MC**, Leckie BJ, Greeff MC. Arterial hypertension as a paraneoplastic phenomenon in hepatocellular carcinoma. *Arch Intern Med* 1989; **149**: 2111-2113 [PMID: 2549897 DOI: 10.1001/archinte.149.9.2111]
38 **Kao WY**, Hung HH, Lu HC, Lin HC, Wu JC, Lee SD, Su CW. Hepatocellular carcinoma with presentation of budd-Chiari syndrome. *J Chin Med Assoc* 2010; **73**: 93-96 [PMID: 20171589 DOI: 10.1016/S1726-4901(10)70008-3]
39 **Bargalló X**, Gilabert R, Nicolau C, García-Pagán JC, Ayuso JR, Brú C. Sonography of Budd-Chiari syndrome. *AJR Am J Roentgenol* 2006; **187**: W33-W41 [PMID: 16794137 DOI: 10.2214/AJR.04.0918]
40 **Bălăceanu LA**, Diaconu CC, Aron G. Budd-Chiari syndrome as an initial presentation of hepatocellular carcinoma: a case report. *Med Ultrason* 2014; **16**: 172-174 [PMID: 24791850 DOI: 10.11152/mu.2013.2066.162.lab1]
41 **Gregory B**, Ho VC. Cutaneous manifestations of gastrointestinal disorders. Part II. *J Am Acad Dermatol* 1992; **26**: 371-383 [PMID: 1307219 DOI: 10.1016/0190-9622(92)70019-C]
42 **Berkowitz I**, Hodkinson HJ, Kew MC, DiBisceglie AM. Pityriasis rotunda as a cutaneous marker of hepatocellular carcinoma: a comparison with its prevalence in other diseases. *Br J Dermatol* 1989; **120**: 545-549 [PMID: 2543437 DOI: 10.1111/j.1365-2133.1989.tb01329.x]
43 **Lim HW**, Mascaro JM. The porphyrias and hepatocellular carcinoma. *Dermatol Clin* 1995; **13**: 135-142 [PMID: 7712639]
44 **Kew MC**. Tumors of the liver. Hepatology: A textbook of liver disease. Philadelphia: WB Saunders Company, 1996: 1513
45 **Lai CL**, Ng RP, Lok AS. The diagnostic value of the ratio of serum gamma-glutamyl transpeptidase to alkaline phosphatase in alcoholic liver disease. *Scand J Gastroenterol* 1982; **17**: 41-47 [PMID: 6127789 DOI: 10.3109/00365528209181042]
46 **Gomaa AI**, Khan SA, Leen EL, Waked I, Taylor-Robinson SD. Diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 2009; **15**: 1301-1314 [PMID: 19294759 DOI: 10.3748/wjg.15.1301]
47 **Bhosale P**, Szklaruk J, Silverman PM. Current staging of

hepatocellular carcinoma: imaging implications. *Cancer Imaging* 2006; **6**: 83-94 [PMID: 16829469 DOI: 10.1102/1470-7330.2006.0014]

48 **Fasani P**, Sangiovanni A, De Fazio C, Borzio M, Bruno S, Ronchi G, Del Ninno E, Colombo M. High prevalence of multinodular hepatocellular carcinoma in patients with cirrhosis attributable to multiple risk factors. *Hepatology* 1999; **29**: 1704-1707 [PMID: 10347111 DOI: 10.1002/hep.510290604]

49 **Sherman M**, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995; **22**: 432-438 [PMID: 7543434 DOI: 10.1002/hep.1840220210]

50 **Pateron D**, Ganne N, Trinchet JC, Aurousseau MH, Mal F, Meicler C, Coderc E, Reboullet P, Beaugrand M. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. *J Hepatol* 1994; **20**: 65-71 [PMID: 7515408 DOI: 10.1016/S0168-8278(05)80468-4]

51 **Larcos G**, Sorokopud H, Berry G, Farrell GC. Sonographic screening for hepatocellular carcinoma in patients with chronic hepatitis or cirrhosis: an evaluation. *AJR Am J Roentgenol* 1998; **171**: 433-435 [PMID: 9694470 DOI: 10.2214/ajr.171.2.9694470]

52 **Colli A**, Fraquelli M, Casazza G, Massironi S, Colucci A, Conte D, Duca P. Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. *Am J Gastroenterol* 2006; **101**: 513-523 [PMID: 16542288 DOI: 10.1111/j.1572-0241.2006.00467.x]

53 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

54 **Dodd GD**, Miller WJ, Baron RL, Skolnick ML, Campbell WL. Detection of malignant tumors in end-stage cirrhotic livers: efficacy of sonography as a screening technique. *AJR Am J Roentgenol* 1992; **159**: 727-733 [PMID: 1326883 DOI: 10.2214/ajr.159.4.1326883]

55 **França AV**, Elias Junior J, Lima BL, Martinelli AL, Carrilho FJ. Diagnosis, staging and treatment of hepatocellular carcinoma. *Braz J Med Biol Res* 2004; **37**: 1689-1705 [PMID: 15517086 DOI: 10.1590/S0100-879X2004001100015]

56 **Yu SC**, Yeung DT, So NM. Imaging features of hepatocellular carcinoma. *Clin Radiol* 2004; **59**: 145-156 [PMID: 14746783 DOI: 10.1016/S0009-9260(03)00316-7]

57 **Albrecht T**, Blomley M, Bolondi L, Claudon M, Correas JM, Cosgrove D, Greiner L, Jäger K, Jong ND, Leen E, Lencioni R, Lindsell D, Martegani A, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines for the use of contrast agents in ultrasound. January 2004. *Ultraschall Med* 2004; **25**: 249-256 [PMID: 15300497 DOI: 10.1055/s-2004-813245]

58 **Quaia E**. Microbubble ultrasound contrast agents: an update. *Eur Radiol* 2007; **17**: 1995-2008 [PMID: 17351779 DOI: 10.1007/s00330-007-0623-0]

59 **Pompili M**, Riccardi L, Semeraro S, Orefice R, Elia F, Barbaro B, Covino M, Grieco A, Gasbarrini G, Rapaccini GL. Contrast-enhanced ultrasound assessment of arterial vascularization of small nodules arising in the cirrhotic liver. *Dig Liver Dis* 2008; **40**: 206-215 [PMID: 18054850 DOI: 10.1016/j.dld.2007.10.006]

60 **Westwood M**, Joore M, Grutters J, Redekop K, Armstrong N, Lee K, Gloy V, Raatz H, Misso K, Severens J, Kleijnen J. Contrast-enhanced ultrasound using SonoVue® (sulphur hexafluoride microbubbles) compared with contrast-enhanced computed tomography and contrast-enhanced magnetic resonance imaging for the characterisation of focal liver lesions and detection of liver metastases: a systematic review and cost-effectiveness analysis. *Health Technol Assess* 2013; **17**: 1-243 [PMID: 23611316 DOI: 10.3310/hta17160]

61 **Singh P**, Erickson RA, Mukhopadhyay P, Gopal S, Kiss A, Khan A, Ulf Westblom T. EUS for detection of the hepatocellular carcinoma: results of a prospective study. *Gastrointest Endosc* 2007; **66**: 265-273 [PMID: 17543307 DOI: 10.1016/j.gie.2006.10.053]

62 **Crowe DR**, Eloubeidi MA, Chhieng DC, Jhala NC, Jhala D, Eltoum IA. Fine-needle aspiration biopsy of hepatic lesions: computerized tomographic-guided versus endoscopic ultrasound-guided FNA. *Cancer* 2006; **108**: 180-185 [PMID: 16634071 DOI: 10.1002/cncr.21912]

63 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]

64 **Baron RL**, Brancatelli G. Computed tomographic imaging of hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S133-S143 [PMID: 15508077 DOI: 10.1053/j.gastro.2004.09.027]

65 **Bialecki ES**, Di Bisceglie AM. Diagnosis of hepatocellular carcinoma. *HPB* (Oxford) 2005; **7**: 26-34 [PMID: 18333158 DOI: 10.1080/13651820410024049]

66 **Onishi H**, Murakami T, Nakamura H. Diagnosis of hepatocellular carcinoma: multidetector-row computed tomography and magnetic resonance imaging. In: Hayat MA, editor. Methods of cancer diagnosis, therapy and prognosis, Volume 5. Netherlands: Springer, 2009: 221-235 [DOI: 10.1007/978-1-4020-9804-8_16]

67 **Saar B**, Kellner-Weldon F. Radiological diagnosis of hepatocellular carcinoma. *Liver Int* 2008; **28**: 189-199 [PMID: 18251978 DOI: 10.1111/j.1478-3231.2007.01655.x]

68 **Szklaruk J**, Silverman PM, Charnsangavej C. Imaging in the diagnosis, staging, treatment, and surveillance of hepatocellular carcinoma. *AJR Am J Roentgenol* 2003; **180**: 441-454 [PMID: 12540450 DOI: 10.2214/ajr.180.2.1800441]

69 **Yu NC**, Chaudhari V, Raman SS, Lassman C, Tong MJ, Busuttil RW, Lu DS. CT and MRI improve detection of hepatocellular carcinoma, compared with ultrasound alone, in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2011; **9**: 161-167 [PMID: 20920597 DOI: 10.1016/j.cgh.2010.09.017]

70 **Ishiguchi T**, Shimamoto K, Fukatsu H, Yamakawa K, Ishigaki T. Radiologic diagnosis of hepatocellular carcinoma. *Semin Surg Oncol* 1996; **12**: 164-169 [PMID: 8727605]

71 **Libbrecht L**, Bielen D, Verslype C, Vanbeckevoort D, Pirenne J, Nevens F, Desmet V, Roskams T. Focal lesions in cirrhotic explant livers: pathological evaluation and accuracy of pretransplantation imaging examinations. *Liver Transpl* 2002; **8**: 749-761 [PMID: 12200773 DOI: 10.1053/jlts.2002.34922]

72 **Kunishi Y**, Numata K, Morimoto M, Okada M, Kaneko T, Maeda S, Tanaka K. Efficacy of fusion imaging combining sonography and hepatobiliary phase MRI with Gd-EOB-DTPA to detect small hepatocellular carcinoma. *AJR Am J Roentgenol* 2012; **198**: 106-114 [PMID: 22194485 DOI: 10.2214/AJR.10.6039]

73 **Park MJ**, Kim YK, Lee MW, Lee WJ, Kim YS, Kim SH, Choi D, Rhim H. Small hepatocellular carcinomas: improved sensitivity by combining gadoxetic acid-enhanced and diffusion-weighted MR imaging patterns. *Radiology* 2012; **264**: 761-770 [PMID: 22843769 DOI: 10.1148/radiol.12112517]

74 **Granito A**, Galassi M, Piscaglia F, Romanini L, Lucidi V, Renzulli M, Borghi A, Grazioli L, Golfieri R, Bolondi L. Impact of gadoxetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance on the non-invasive diagnosis of small hepatocellular carcinoma: a prospective study. *Aliment Pharmacol Ther* 2013; **37**: 355-363 [PMID: 23199022 DOI: 10.1111/apt.12166]

75 **Burrel M**, Llovet JM, Ayuso C, Iglesias C, Sala M, Miquel R, Caralt T, Ayuso JR, Solé M, Sanchez M, Brú C, Bruix J. MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explant correlation. *Hepatology* 2003; **38**: 1034-1042 [PMID: 14512891 DOI: 10.1002/hep.1840380430]

76 **Lencioni R**, Mascalchi M, Caramella D, Bartolozzi C. Small hepatocellular carcinoma: differentiation from adenomatous hyperplasia with color Doppler US and dynamic Gd-DTPA-enhanced MR imaging. *Abdom Imaging* 1996; **21**: 41-48 [PMID: 8672971 DOI: 10.1007/s002619900007]

77 **Nanda NC**, Schlief R. Advances in echo imaging using contrast enhancement. Dordrecht: Kluwer Academic Publishers, 1993

78 **Heiken JP**, Weyman PJ, Lee JK, Balfe DM, Picus D, Brunt EM, Flye MW. Detection of focal hepatic masses: prospective evaluation with CT, delayed CT, CT during arterial portography, and MR imaging. *Radiology* 1989; **171**: 47-51 [PMID: 2538862 DOI: 10.1148/radiology.171.1.2538862]

79 **Yu JS**, Kim KW, Lee JT, Yoo HS. MR imaging during arterial portography for assessment of hepatocellular carcinoma: comparison with CT during arterial portography. *AJR Am J Roentgenol* 1998; **170**: 1501-1506 [PMID: 9609162 DOI: 10.2214/ajr.170.6.9609162]

80 **Kanematsu M**, Hoshi H, Murakami T, Inaba Y, Kim T, Yamada T, Kato M, Yokoyama R, Nakamura H. Detection of hepatocellular carcinoma in patients with cirrhosis: MR imaging versus angiographically assisted helical CT. *AJR Am J Roentgenol* 1997; **169**: 1507-1515 [PMID: 9393154 DOI: 10.2214/ajr.169.6.9393154]

81 **Murakami T**, Oi H, Hori M, Kim T, Takahashi S, Tomoda K, Narumi Y, Nakamura H. Helical CT during arterial portography and hepatic arteriography for detecting hypervascular hepatocellular carcinoma. *AJR Am J Roentgenol* 1997; **169**: 131-135 [PMID: 9207512 DOI: 10.2214/ajr.169.1.9207512]

82 **Kanematsu M**, Hoshi H, Imaeda T, Murakami T, Inaba Y, Yokoyama R, Nakamura H. Detection and characterization of hepatic tumors: value of combined helical CT hepatic arteriography and CT during arterial portography. *AJR Am J Roentgenol* 1997; **168**: 1193-1198 [PMID: 9129410 DOI: 10.2214/ajr.168.5.9129410]

83 **Ikeda K**, Saitoh S, Koida I, Tsubota A, Arase Y, Chayama K, Kumada H. Imaging diagnosis of small hepatocellular carcinoma. *Hepatology* 1994; **20**: 82-87 [PMID: 8020908 DOI: 10.1002/hep.1840200113]

84 **Ohki T**, Tateishi R, Akahane M, Mikami S, Sato M, Uchino K, Arano T, Enooku K, Kondo Y, Yamashiki N, Goto T, Shiina S, Yoshida H, Matsuyama Y, Omata M, Ohtomo K, Koike K. CT with hepatic arterioportography as a pretreatment examination for hepatocellular carcinoma patients: a randomized controlled trial. *Am J Gastroenterol* 2013; **108**: 1305-1313 [PMID: 23629602 DOI: 10.1038/ajg.2013.109]

85 **El-Bahrawy M**. Alpha-fetoprotein-producing non-germ cell tumours of the female genital tract. *Eur J Cancer* 2010; **46**: 1317-1322 [PMID: 20185298 DOI: 10.1016/j.ejca.2010.01.028]

86 **Liu X**, Cheng Y, Sheng W, Lu H, Xu Y, Long Z, Zhu H, Wang Y. Clinicopathologic features and prognostic factors in alpha-fetoprotein-producing gastric cancers: analysis of 104 cases. *J Surg Oncol* 2010; **102**: 249-255 [PMID: 20740583 DOI: 10.1002/jso.21624]

87 **Collier J**, Sherman M. Screening for hepatocellular carcinoma. *Hepatology* 1998; **27**: 273-278 [PMID: 9425947 DOI: 10.1002/hep.510270140]

88 **Sterling RK**, Wright EC, Morgan TR, Seeff LB, Hoefs JC, Di Bisceglie AM, Dienstag JL, Lok AS. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. *Am J Gastroenterol* 2012; **107**: 64-74 [PMID: 21931376 DOI: 10.1038/ajg.2011.312]

89 **Di Bisceglie AM**, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, Wright EC, Everson GT, Lindsay KL, Lok AS, Lee WM, Morgan TR, Ghany MG, Gretch DR. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. *J Hepatol* 2005; **43**: 434-441 [PMID: 16136646 DOI: 10.1016/j.jhep.2005.03.019]

90 **Chen DS**, Sung JL, Sheu JC, Lai MY, How SW, Hsu HC, Lee CS, Wei TC. Serum alpha-fetoprotein in the early stage of human hepatocellular carcinoma. *Gastroenterology* 1984; **86**: 1404-1409 [PMID: 6201411]

91 **Soreide O**, Czerniak A, Bradpiece H, Bloom S, Blumgart L. Characteristics of fibrolamellar hepatocellular carcinoma. A study of nine cases and a review of the literature. *Am J Surg* 1986; **151**: 518-523 [PMID: 2421594 DOI: 10.1016/0002-9610(86)90117-0]

92 **Colombo M**, de Franchis R, Del Ninno E, Sangiovanni A, De Fazio C, Tommasini M, Donato MF, Piva A, Di Carlo V, Dioguardi N. Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med* 1991; **325**: 675-680 [PMID: 1651452 DOI: 10.1056/NEJM199109053251002]

93 **Trevisani F**, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; **34**: 570-575 [PMID: 11394657 DOI: 10.1016/S0168-8278(00)00053-2]

94 **Gupta S**, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; **139**: 46-50 [PMID: 12834318 DOI: 10.7326/0003-4819-139-1-200307010-00012]

95 **Nguyen MH**, Garcia RT, Simpson PW, Wright TL, Keeffe EB. Racial differences in effectiveness of alpha-fetoprotein for diagnosis of hepatocellular carcinoma in hepatitis C virus cirrhosis. *Hepatology* 2002; **36**: 410-417 [PMID: 12143050 DOI: 10.1053/jhep.2002.34744]

96 **Fujioka M**, Nakashima Y, Nakashima O, Kojiro M. Immuno-histologic study on the expressions of alpha-fetoprotein and protein induced by vitamin K absence or antagonist II in surgically resected small hepatocellular carcinoma. *Hepatology* 2001; **34**: 1128-1134 [PMID: 11732002 DOI: 10.1053/jhep.2001.29202]

97 **Bae JS**, Park SJ, Park KB, Paik SY, Ryu JK, Choi CK, Hwang TJ. Acute exacerbation of hepatitis in liver cirrhosis with very high levels of alpha-fetoprotein but no occurrence of hepatocellular carcinoma. *Korean J Intern Med* 2005; **20**: 80-85 [PMID: 15906959 DOI: 10.3904/kjim.2005.20.1.80]

98 **Johnson PJ**, Poon TC, Hjelm NM, Ho CS, Blake C, Ho SK. Structures of disease-specific serum alpha-fetoprotein isoforms. *Br J Cancer* 2000; **83**: 1330-1337 [PMID: 11044358 DOI: 10.1054/bjoc.2000.1441]

99 **Oka H**, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, Osaki Y, Seki T, Kudo M, Tanaka M. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein. *J Gastroenterol Hepatol* 2001; **16**: 1378-1383 [PMID: 11851836 DOI: 10.1046/j.1440-1746.2001.02643.x]

100 **Khien VV**, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV, Hop TV, Tuan NA, Don LV, Taketa K, Satomura S. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. *Int J Biol Markers* 2001; **16**: 105-111 [PMID: 11471892]

101 Mohanty S, Jensen D. Tumor markers and molecular biology. In: Al-Knawy B, Reddy KR, Bolondi L, editors. Hepatocellular carcinoma: a practical approach. New York: Informa Healthcare, 2009: 63-74 [DOI: 10.3109/9780203092880-6]

102 **Liebman HA**, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, Coleman MS, Furie B. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984; **310**: 1427-1431 [PMID: 6201741 DOI: 10.1056/NEJM198405313102204]

103 **Nomura F**, Ishijima M, Horikoshi A, Nakai T, Ohnishi K. Determination of serum des-gamma-carboxy prothrombin levels in patients with small-sized hepatocellular carcinoma: comparison of the conventional enzyme immunoassay and two modified methods. *Am J Gastroenterol* 1996; **91**: 1380-1383 [PMID: 8677999]

104 **Aoyagi Y**, Oguro M, Yanagi M, Mita Y, Suda T, Suzuki Y, Hata K, Ichii K, Asakura H. Clinical significance of simultaneous determinations of alpha-fetoprotein and des-gamma-carboxy prothrombin in monitoring recurrence in patients with hepatocellular carcinoma. *Cancer* 1996; **77**: 1781-1786 [PMID: 8646674]

105 **Weitz IC**, Liebman HA. Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. *Hepatology* 1993; **18**: 990-997 [PMID: 8406374 DOI: 10.1002/hep.1840180434]

106 **Marrero JA**, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, Lok AS. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology* 2003; **37**: 1114-1121 [PMID: 12717392 DOI: 10.1053/jhep.2003.50195]

107 **Nakamura S**, Nouso K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, Toshikuni N, Tanaka H, Miyake Y, Matsumoto E, Shiratori Y. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *Am J Gastroenterol* 2006; **101**: 2038-2043 [PMID: 16848811 DOI: 10.1111/j.1572-0241.2006.00681.x]

108 **Borel F**, Konstantinova P, Jansen PL. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. *J Hepatol* 2012; **56**: 1371-1383 [PMID: 22314424 DOI: 10.1016/j.jhep.2011.11.026]

109 **Zhou J**, Yu L, Gao X, Hu J, Wang J, Dai Z, Wang JF, Zhang Z, Lu S, Huang X, Wang Z, Qiu S, Wang X, Yang G, Sun H, Tang Z, Wu Y, Zhu H, Fan J. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 4781-4788 [PMID: 22105822 DOI: 10.1200/JCO.2011.38.2697]

110 **Li L**, Guo Z, Wang J, Mao Y, Gao Q. Serum miR-18a: a potential marker for hepatitis B virus-related hepatocellular carcinoma screening. *Dig Dis Sci* 2012; **57**: 2910-2916 [PMID: 22865399 DOI: 10.1007/s10620-012-2317-y]

111 **Kew MC**, Wolf P, Whittaker D, Rowe P. Tumour-associated isoenzymes of gamma-glutamyl transferase in the serum of patients with hepatocellular carcinoma. *Br J Cancer* 1984; **50**: 451-455 [PMID: 6207851 DOI: 10.1038/bjc.1984.200]

112 **Tsai JF**, Jeng JE, Chuang LY, Yang ML, Ho MS, Chang WY, Hsieh MY, Lin ZY, Tsai JH. Clinical evaluation of urinary transforming growth factor-beta1 and serum alpha-fetoprotein as tumour markers of hepatocellular carcinoma. *Br J Cancer* 1997; **75**: 1460-1466 [PMID: 9166938 DOI: 10.1038/bjc.1997.250]

113 **Hamazaki K**, Gochi A, Shimamura H, Kaihara A, Maruo Y, Doi Y, Orita K, Lygidakis NJ. Serum levels of circulating intercellular adhesion molecule 1 in hepatocellular carcinoma. *Hepatogastroenterology* 1996; **43**: 229-234 [PMID: 8682469]

114 **Takahashi H**, Saibara T, Iwamura S, Tomita A, Maeda T, Onishi S, Yamamoto Y, Enzan H. Serum alpha-L-fucosidase activity and tumor size in hepatocellular carcinoma. *Hepatology* 1994; **19**: 1414-1417 [PMID: 7514563 DOI: 10.1002/hep.1840190615]

115 **International Consensus Group for Hepatocellular Neoplasia**; The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009; **49**: 658-664 [PMID: 19177576 DOI: 10.1002/hep.22709]

116 **Shen Q**, Fan J, Yang XR, Tan Y, Zhao W, Xu Y, Wang N, Niu Y, Wu Z, Zhou J, Qiu SJ, Shi YH, Yu B, Tang N, Chu W, Wang M, Wu J, Zhang Z, Yang S, Gu J, Wang H, Qin W. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. *Lancet Oncol* 2012; **13**: 817-826 [PMID: 22738799 DOI: 10.1016/S1470-2045(12)70233-4]

117 **Na K**, Jeong SK, Lee MJ, Cho SY, Kim SA, Lee MJ, Song SY, Kim H, Kim KS, Lee HW, Paik YK. Human liver carboxylesterase 1 outperforms alpha-fetoprotein as biomarker to discriminate hepatocellular carcinoma from other liver diseases in Korean patients. *Int J Cancer* 2013; **133**: 408-415 [PMID: 23319432 DOI: 10.1002/ijc.28020]

118 **Henry L**, Lavabre-Bertrand T, Vercambre L, Ramos J, Carillo S, Guiraud I, Pouderoux P, Bismuth M, Valats JC, Demattei C, Duny Y, Chaze I, Funakoshi N, Bureau JP, Daurès JP, Blanc P. Plasma proteasome level is a reliable early marker of malignant transformation of liver cirrhosis. *Gut* 2009; **58**: 833-838 [PMID: 19201777 DOI: 10.1136/gut.2008.157016]

119 **Hamed M**, El-Etreby S, Bahgat M, Attia M, Abdaziz S, Salem D, Kamr W. Is plasma proteasome level a reliable marker of hepatocellular carcinoma in hepatitis c virus related cirrhosis? *Hepatology* 2012; **56** Suppl 2: S281 [DOI: 10.1016/S0168-8278(12)60724-7]

120 **Bialecki ES**, Ezenekwe AM, Brunt EM, Collins BT, Ponder TB, Bieneman BK, Di Bisceglie AM. Comparison of liver biopsy and noninvasive methods for diagnosis of hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2006; **4**: 361-368 [PMID: 16527701 DOI: 10.1016/S1542-3565(05)00977-8]

121 **Crippin JS**. Biopsy of suspicious liver nodules: does it change management? *Clin Gastroenterol Hepatol* 2006; **4**: 296-298 [PMID: 16527691 DOI: 10.1016/j.cgh.2005.12.024]

122 **John TG**, Garden OJ. Needle track seeding of primary and secondary liver carcinoma after percutaneous liver biopsy. *HPB Surg* 1993; **6**: 199-203; discussion 203-204 [PMID: 8387809 DOI: 10.1155/1993/39539]

123 **Durand F**, Regimbeau JM, Belghiti J, Sauvanet A, Vilgrain V, Terris B, Moutardier V, Farges O, Valla D. Assessment of the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. *J Hepatol* 2001; **35**: 254-258 [PMID: 11580148 DOI: 10.1016/S0168-8278(01)00108-8]

124 **Huang GT**, Sheu JC, Yang PM, Lee HS, Wang TH, Chen DS. Ultrasound-guided cutting biopsy for the diagnosis of hepatocellular carcinoma--a study based on 420 patients. *J Hepatol* 1996; **25**: 334-338 [PMID: 8895013 DOI: 10.1016/S0168-8278(96)80120-6]

125 **Kim SH**, Lim HK, Lee WJ, Cho JM, Jang HJ. Needle-tract implantation in hepatocellular carcinoma: frequency and CT findings after biopsy with a 19.5-gauge automated biopsy gun. *Abdom Imaging* 2000; **25**: 246-250 [PMID: 10823443 DOI: 10.1007/s002610000025]

126 **Ohlsson B**, Nilsson J, Stenram U, Akerman M, Tranberg KG. Percutaneous fine-needle aspiration cytology in the diagnosis and management of liver tumours. *Br J Surg* 2002; **89**: 757-762 [PMID: 12027987 DOI: 10.1046/j.1365-2168.2002.02111.x]

127 **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]

128 **Ng KK**, Poon RT, Lo CM, Liu CL, Lam CM, Ng IO, Fan ST. Impact of preoperative fine-needle aspiration cytologic examination on clinical outcome in patients with hepatocellular carcinoma in a tertiary referral center. *Arch Surg* 2004; **139**: 193-200 [PMID: 14769580 DOI: 10.1001/archsurg.139.2.193]

129 **Liu YW**, Chen CL, Chen YS, Wang CC, Wang SH, Lin CC. Needle tract implantation of hepatocellular carcinoma after fine needle biopsy. *Dig Dis Sci* 2007; **52**: 228-231 [PMID: 17151805 DOI: 10.1007/s10620-006-9354-3]

130 **Bruix J**, Llovet JM. Major achievements in hepatocellular carcinoma. *Lancet* 2009; **373**: 614-616 [PMID: 19231618 DOI: 10.1016/S0140-6736(09)60381-0]

131 **Masuzaki R**, Omata M. HCC screening and surveillance. In: Al-Knawy B, Reddy KR, Bolondi L, editors. Hepatocellular carcinoma: a practical approach. London: Informa Healthcare, 2009: 26-35 [DOI: 10.3109/9780203092880-4]

132 **Patel SS**, Arrington AK, McKenzie S, Mailey B, Ding M, Lee W, Artinyan A, Nissen N, Colquhoun SD, Kim J. Milan Criteria and UCSF Criteria: A Preliminary Comparative Study of Liver Transplantation Outcomes in the United States. *Int J Hepatol* 2012; **2012**: 253517 [PMID: 22957260 DOI: 10.1155/2012/253517]

133 **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]

134 **Lencioni R**, Crocetti L. Radiofrequency ablation of liver cancer. *Tech Vasc Interv Radiol* 2007; **10**: 38-46 [PMID: 17980317 DOI: 10.1053/j.tvir.2007.08.006]

135 **Lencioni R**, Crocetti L, De Simone P, Filipponi F. Loco-regional interventional treatment of hepatocellular carcinoma: techniques, outcomes, and future prospects. *Transpl Int* 2010; **23**: 698-703 [PMID: 20492618 DOI: 10.1111/j.1432-2277.2010.01109.x]

136 **Lu DS**, Yu NC, Raman SS, Limanond P, Lassman C, Murray K, Tong MJ, Amado RG, Busuttil RW. Radiofrequency ablation of hepatocellular carcinoma: treatment success as defined by histologic examination of the explanted liver. *Radiology* 2005; **234**: 954-960 [PMID: 15681691 DOI: 10.1148/radiol.2343040153]

137 **Brunello F**, Veltri A, Carucci P, Pagano E, Ciccone G, Moretto P, Sacchetto P, Gandini G, Rizzetto M. Radiofrequency ablation

versus ethanol injection for early hepatocellular carcinoma: A randomized controlled trial. *Scand J Gastroenterol* 2008; **43**: 727-735 [PMID: 18569991 DOI: 10.1080/00365520701885481]

138 **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]

139 **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 DOI: 10.1097/01.sla.0000201480.65519.b8]

140 **Lencioni R**, Crocetti L. Image-guided thermal ablation of hepatocellular carcinoma. *Crit Rev Oncol Hematol* 2008; **66**: 200-207 [PMID: 18304832 DOI: 10.1016/j.critrevonc.2008.01.003]

141 **Ebara M**, Okabe S, Kita K, Sugiura N, Fukuda H, Yoshikawa M, Kondo F, Saisho H. Percutaneous ethanol injection for small hepatocellular carcinoma: therapeutic efficacy based on 20-year observation. *J Hepatol* 2005; **43**: 458-464 [PMID: 16005538 DOI: 10.1016/j.jhep.2005.03.033]

142 **Shibata T**, Iimuro Y, Yamamoto Y, Maetani Y, Ametani F, Itoh K, Konishi J. Small hepatocellular carcinoma: comparison of radio-frequency ablation and percutaneous microwave coagulation therapy. *Radiology* 2002; **223**: 331-337 [PMID: 11997534 DOI: 10.1148/radiol.2232010775]

143 **Lencioni R**, Pinto F, Armillotta N, Bassi AM, Moretti M, Di Giulio M, Marchi S, Uliana M, Della Capanna S, Lencioni M, Bartolozzi C. Long-term results of percutaneous ethanol injection therapy for hepatocellular carcinoma in cirrhosis: a European experience. *Eur Radiol* 1997; **7**: 514-519 [PMID: 9204330 DOI: 10.1007/s003300050194]

144 **Khan KN**, Yatsuhashi H, Yamasaki K, Yamasaki M, Inoue O, Koga M, Yano M. Prospective analysis of risk factors for early intrahepatic recurrence of hepatocellular carcinoma following ethanol injection. *J Hepatol* 2000; **32**: 269-278 [PMID: 10707867 DOI: 10.1016/S0168-8278(00)80072-0]

145 **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]

146 **Takayasu K**, Shima Y, Muramatsu Y, Moriyama N, Yamada T, Makuuchi M, Hasegawa H, Hirohashi S. Hepatocellular carcinoma: treatment with intraarterial iodized oil with and without chemotherapeutic agents. *Radiology* 1987; **163**: 345-351 [PMID: 3031724]

147 **El-Serag HB**, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1752-1763 [PMID: 18471552 DOI: 10.1053/j.gastro.2008.02.090]

148 **Guan YS**, Ahmad Al-Shatouri M, He Q, Liu WM. Hepatocellular carcinoma: carcinogenesis, establishment, progression, and therapies. *Biomed Res Int* 2014; **2014**: 706142 [PMID: 25028663 DOI: 10.1155/2014/239706]

149 **Rammohan A**, Sathyanesan J, Ramaswami S, Lakshmanan A, Senthil-Kumar P, Srinivasan UP, Ramasamy R, Ravichandran P. Embolization of liver tumors: Past, present and future. *World J Radiol* 2012; **4**: 405-412 [PMID: 23024842 DOI: 10.4329/wjr.v4.i9.405]

150 **Park W**, Lim DH, Paik SW, Koh KC, Choi MS, Park CK, Yoo BC, Lee JE, Kang MK, Park YJ, Nam HR, Ahn YC, Huh SJ. Local radiotherapy for patients with unresectable hepatocellular carcinoma. *Int J Radiat Oncol Biol Phys* 2005; **61**: 1143-1150 [PMID: 15752895 DOI: 10.1016/j.ijrobp.2004.08.028]

151 **Seong J**, Park HC, Han KH, Chon CY. Clinical results and prognostic factors in radiotherapy for unresectable hepatocellular carcinoma: a retrospective study of 158 patients. *Int J Radiat Oncol Biol Phys* 2003; **55**: 329-336 [PMID: 12527045 DOI: 10.1016/S0360-3016(02)03929-9]

152 **Seo YS**, Kim JN, Keum B, Park S, Kwon YD, Kim YS, Jeen YT, Chun HJ, Kim CY, Kim CD, Ryu HS, Um SH. Radiotherapy for 65 patients with advanced unresectable hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 2394-2400 [PMID: 18416468 DOI: 10.3748/wjg.14.2394]

153 **Obi S**, Yoshida H, Toune R, Unuma T, Kanda M, Sato S, Tateishi R, Teratani T, Shiina S, Omata M. Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. *Cancer* 2006; **106**: 1990-1997 [PMID: 16565970 DOI: 10.1002/cncr.21832]

154 **Budhu A**, Jia HL, Forgues M, Liu CG, Goldstein D, Lam A, Zanetti KA, Ye QH, Qin LX, Croce CM, Tang ZY, Wang XW. Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology* 2008; **47**: 897-907 [PMID: 18176954 DOI: 10.1002/hep.22160]

155 **Jiang J**, Gusev Y, Aderca I, Mettler TA, Nagorney DM, Brackett DJ, Roberts LR, Schmittgen TD. Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. *Clin Cancer Res* 2008; **14**: 419-427 [PMID: 18223217 DOI: 10.1158/1078-0432.CCR-07-0523]

156 **Han ZB**, Zhong L, Teng MJ, Fan JW, Tang HM, Wu JY, Chen HY, Wang ZW, Qiu GQ, Peng ZH. Identification of recurrence-related microRNAs in hepatocellular carcinoma following liver transplantation. *Mol Oncol* 2012; **6**: 445-457 [PMID: 22552153 DOI: 10.1016/j.molonc.2012.04.001]

157 **Ji J**, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, Ambs S, Chen Y, Meltzer PS, Croce CM, Qin LX, Man K, Lo CM, Lee J, Ng IO, Fan J, Tang ZY, Sun HC, Wang XW. MicroRNA expression, survival, and response to interferon in liver cancer. *N Engl J Med* 2009; **361**: 1437-1447 [PMID: 19812400 DOI: 10.1056/NEJMoa0901282]

158 **Tsai WC**, Hsu PW, Lai TC, Chau GY, Lin CW, Chen CM, Lin CD, Liao YL, Wang JL, Chau YP, Hsu MT, Hsiao M, Huang HD, Tsou AP. MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology* 2009; **49**: 1571-1582 [PMID: 19296470 DOI: 10.1002/hep.22806]

159 **Coulouarn C**, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009; **28**: 3526-3536 [PMID: 19617899 DOI: 10.1038/onc.2009.211]

160 **Fornari F**, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, Calin GA, Grazi GL, Croce CM, Tavolari S, Chieco P, Negrini M, Bolondi L. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2009; **69**: 5761-5767 [PMID: 19584283 DOI: 10.1158/0008-5472.CAN-08-4797]

161 **Viswanathan SR**, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, O'Sullivan M, Lu J, Phillips LA, Lockhart VL, Shah SP, Tanwar PS, Mermel CH, Beroukhim R, Azam M, Teixeira J, Meyerson M, Hughes TP, Llovet JM, Radich J, Mullighan CG, Golub TR, Sorensen PH, Daley GQ. Lin28 promotes transformation and is associated with advanced human malignancies. *Nat Genet* 2009; **41**: 843-848 [PMID: 19483683 DOI: 10.1038/ng.392]

162 **Fornari F**, Milazzo M, Chieco P, Negrini M, Calin GA, Grazi GL, Pollutri D, Croce CM, Bolondi L, Gramantieri L. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2010; **70**: 5184-5193 [PMID: 20501828 DOI: 10.1158/0008-5472.CAN-10-0145]

163 **Wang C**, Song B, Song W, Liu J, Sun A, Wu D, Yu H, Lian J, Chen L, Han J. Underexpressed microRNA-199b-5p targets hypoxia-inducible factor-1α in hepatocellular carcinoma and predicts prognosis of hepatocellular carcinoma patients. *J Gastroenterol Hepatol* 2011; **26**: 1630-1637 [PMID: 21557766 DOI: 10.1111/j.1440-1746.2011.06758.x]

164 **Zhang Y**, Guo X, Xiong L, Kong X, Xu Y, Liu C, Zou L, Li Z, Zhao J, Lin N. MicroRNA-101 suppresses SOX9-dependent tumorigenicity and promotes favorable prognosis of human hepatocellular carcinoma. *FEBS Lett* 2012; **586**: 4362-4370 [PMID: 23178713 DOI: 10.1016/j.febslet.2012.10.053]

165 **Li W**, Xie L, He X, Li J, Tu K, Wei L, Wu J, Guo Y, Ma X, Zhang

P, Pan Z, Hu X, Zhao Y, Xie H, Jiang G, Chen T, Wang J, Zheng S, Cheng J, Wan D, Yang S, Li Y, Gu J. Diagnostic and prognostic implications of microRNAs in human hepatocellular carcinoma. *Int J Cancer* 2008; **123**: 1616-1622 [PMID: 18649363 DOI: 10.1002/ijc.23693]

166 **Murakami Y**, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, Shimotohno K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene* 2006; **25**: 2537-2545 [PMID: 16331254 DOI: 10.1038/sj.onc.1209283]

167 **Gu H**, Guo X, Zou L, Zhu H, Zhang J. Upregulation of microRNA-372 associates with tumor progression and prognosis in hepatocellular carcinoma. *Mol Cell Biochem* 2013; **375**: 23-30 [PMID: 23291979]

168 **Gramantieri L**, Fornari F, Ferracin M, Veronese A, Sabbioni S, Calin GA, Grazi GL, Croce CM, Bolondi L, Negrini M. MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. *Clin Cancer Res* 2009; **15**: 5073-5081 [PMID: 19671867 DOI: 10.1158/1078-0432.CCR-09-0092]

169 **Fu X**, Wang Q, Chen J, Huang X, Chen X, Cao L, Tan H, Li W, Zhang L, Bi J, Su Q, Chen L. Clinical significance of miR-221 and its inverse correlation with p27Kip¹ in hepatocellular carcinoma. *Mol Biol Rep* 2011; **38**: 3029-3035 [PMID: 20146005 DOI: 10.1007/s11033-010-9969-5]

170 **Rong M**, Chen G, Dang Y. Increased miR-221 expression in hepatocellular carcinoma tissues and its role in enhancing cell growth and inhibiting apoptosis in vitro. *BMC Cancer* 2013; **13**: 21 [PMID: 23320393 DOI: 10.1186/1471-2407-13-21]

171 **Chen L**, Jiang M, Yuan W, Tang H. miR-17-5p as a novel prognostic marker for hepatocellular carcinoma. *J Invest Surg* 2012; **25**: 156-161 [PMID: 22583011 DOI: 10.3109/08941939.2011.618523]

172 **Han ZB**, Chen HY, Fan JW, Wu JY, Tang HM, Peng ZH. Up-regulation of microRNA-155 promotes cancer cell invasion and predicts poor survival of hepatocellular carcinoma following liver transplantation. *J Cancer Res Clin Oncol* 2012; **138**: 153-161 [PMID: 22071603 DOI: 10.1007/s00432-011-1076-z]

173 **Chen HY**, Han ZB, Fan JW, Xia J, Wu JY, Qiu GQ, Tang HM, Peng ZH. miR-203 expression predicts outcome after liver transplantation for hepatocellular carcinoma in cirrhotic liver. *Med Oncol* 2012; **29**: 1859-1865 [PMID: 21786180 DOI: 10.1007/s12032-011-0031-9]

174 **Murakami Y**, Tamori A, Itami S, Tanahashi T, Toyoda H, Tanaka M, Wu W, Brojigin N, Kaneoka Y, Maeda A, Kumada T, Kawada N, Kubo S, Kuroda M. The expression level of miR-18b in hepatocellular carcinoma is associated with the grade of malignancy and prognosis. *BMC Cancer* 2013; **13**: 99 [PMID: 23496901 DOI: 10.1186/1471-2407-13-99]

175 **Negrini M**, Gramantieri L, Sabbioni S, Croce CM. microRNA involvement in hepatocellular carcinoma. *Anticancer Agents Med Chem* 2011; **11**: 500-521 [PMID: 21554203 DOI: 10.2174/187152011796011037]

P- Reviewer: Sazci A, Takaki A, Wong GLH **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1652

World J Hepatol 2015 June 28; 7(12): 1652-1659
ISSN 1948-5182 (online)

MINIREVIEWS

Systematic review: Preventive and therapeutic applications of metformin in liver disease

Aparna Bhat, Giada Sebastiani, Mamatha Bhat

Aparna Bhat, Giada Sebastiani, Mamatha Bhat, Division of Gastroenterology and Hepatology, McGill University Health Centre, Montreal, Quebec H3A 1A1, Canada

Author contributions: Bhat A, Sebastiani G and Bhat M contributed to this paper.

Conflict-of-interest: The authors declare that they have no conflicts of interest to disclose related to this article.

Correspondence to: Mamatha Bhat, MD, Division of Gastroenterology and Hepatology, McGill University Health Centre, 687 Pine Avenue West, Montreal, Quebec H3A 1A1, Canada. mamatha.bhat@mail.mcgill.ca
Telephone: +1-514-8431616
Fax: +1-514-8431421

Received: March 13, 2015
Peer-review started: March 16, 2015
First decision: April 27, 2015
Revised: May 5, 2015
Accepted: June 4, 2015
Article in press: June 8, 2015
Published online: June 28, 2015

Abstract

Metformin, a biguanide derivative, is the most commonly prescribed medication in the treatment of type 2 diabetes mellitus. More recently, the use of metformin has shown potential as a preventive and therapeutic agent for a broad spectrum of conditions, including liver disease and hepatic malignancies. In this systematic review, we critically analyze the literature behind the potential use of metformin across the spectrum of liver disease and malignancies. The PubMed and Ovid MEDLINE databases were searched from 2000 to March 2015, using a combination of relevant text words and MeSH terms: metformin and mammalian target of rapamycin, hepatitis B virus (HBV), hepatitis B virus (HCV), non-alcoholic fatty liver disease (NAFLD), hepatocellular carcinoma (HCC) or cholangiocarcinoma. The search results were evaluated for pertinence to the issue of metformin in liver disease as well as for quality of study design. Metformin has a number of biochemical effects that would suggest a benefit in treating chronic liver diseases, particularly in the context of insulin resistance and inflammation. However, the literature thus far does not support any independent therapeutic role in NAFLD or HCV. Nonetheless, there is Level Ⅲ evidence for a chemopreventive role in patients with diabetes and chronic liver disease, with decreased incidence of HCC and cholangiocarcinoma. The use of metformin seems to be safe in patients with cirrhosis, and provides a survival benefit. Once hepatic malignancies are already established, metformin does not offer any therapeutic potential. In conclusion, there is insufficient evidence to recommend use of metformin in the adjunctive treatment of chronic liver diseases, including NAFLD and HCV. However, there is good evidence for a chemopreventive role against HCC among patients with diabetes and chronic liver disease, and metformin should be continued in patients even with cirrhosis to provide this benefit.

Key words: Non-alcoholic fatty liver disease; Metformin; Chemoprevention; Hepatocellular carcinoma; Hepatitis C

Core tip: There has recently been a growing literature on the use of metformin as a potential preventive and therapeutic agent for various chronic liver diseases and

hepatic malignancies. We therefore decided to review the efficacy of metformin across the spectrum of liver disease and malignancies. Based on our systematic review, there is insufficient evidence to recommend use of metformin in the adjunctive treatment of non-alcoholic fatty liver disease and hepatitis C. However, there is good evidence for a chemopreventive role against hepatocellular carcinoma among patients with diabetes and chronic liver disease, and metformin should be continued in patients even with cirrhosis to provide this benefit.

Bhat A, Sebastiani G, Bhat M. Systematic review: Preventive and therapeutic applications of metformin in liver disease. *World J Hepatol* 2015; 7(12): 1652-1659 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1652.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1652

INTRODUCTION

Metformin as an oral hypoglycemic medication has been typically prescribed for type 2 diabetes and insulin resistance in polycystic ovarian disease. Its hypoglycemic action occurs by virtue of its ability to inhibit both gluconeogenesis and glycogenolysis in hepatocytes[1] (Figure 1). It also indirectly downregulates circulating insulin and insulin growth factor-1, by virtue of decreasing serum glucose[2-4]. Additionally, metformin binds reversibly to complex I of the mitochondrial electron transport chain in hepatocytes, giving rise to cellular stress[1,5]. This inhibition leads to an increase in adenosine monophosphate (AMP) generation, with a concomitant decrease in adenosine triphosphate (ATP) production. Increasing levels of AMP bind to AMP kinase (AMPK), a key regulator of cellular metabolism in both normal and cancer cells, resulting in a conformational change and activation[1,6,7]. Cellular stress, such as nutrient deprivation and hypoxic conditions, also increase AMP levels[7]. Once activated by AMP, the conformational change facilitates liver kinase B1 (LKB1) phosphorylation of AMPK, which in turn phosphorylates Tsc1/2. This leads to negative regulation of the mammalian target of rapamycin (mTOR) pathway[8]. Metformin improves insulin resistance through activation of AMPK, which subsequently blocks hepatic glucose release and promotes glucose uptake in skeletal muscle[9]. It also likely uses various mechanisms to restore insulin sensitivity by limiting lipid storage through the inhibition of free fatty acid formation; AMPK suppression of acetyl-CoA carboxylase 1 (ACC1), ACC2, and HMG-CoA reductase decreases fatty acid synthase expression and activates malonyl-CoA carboxylase[3,10,11]. Moreover, AMPK suppresses fatty acid synthesis through the inhibition of transcription factor SREBP-1c. This transcription factor is induced by glucose and insulin excess, and is therefore inappropriately elevated in patients with non-alcoholic fatty liver disease (NAFLD)[12,13]. Interestingly, metformin has also been shown to modulate adipokine synthesis (cytokines that have action on adipose tissue). Adipokines such as tumor necrosis factor-α and interleukin-6 can directly stimulate AMPK, thereby preventing hepatic fat accumulation through an increase in β-oxidation of free fatty acids (FFAs) and/or by decreasing *de novo* synthesis[14]. Zhang *et al*[15] have shown that metformin exerts antagonistic effects on catecholamine-induced lipolysis *via* decrease in cAMP production.

Recent years have seen investigation of its use in a variety of conditions, such as NAFLD, in addition to potential use as a chemopreventive and chemotherapeutic agent. The exploration into the use of metformin in chemoprevention began in 2005, with Evans *et al*[16] demonstrating that metformin significantly reduced the risk of cancer development in diabetic patients. The findings of this large cohort study demonstrated a 23% reduction in overall cancer incidence among diabetic patients treated with metformin as compared to those treated with sulfonylurea derivatives[4]. The discovery gave rise to a number of studies focusing on the ability of metformin to lower the risk of cancer in long-term users of the medication. A prospective cohort study by Libby *et al*[17] demonstrated that 7.3% of patients taking metformin had a diagnosis of cancer, as compared to 11.6% of a control population. After adjusting for confounding variables, patients on metformin still had a significantly decreased risk of cancer, with a hazard ratio of 0.63 (95%CI: 0.53-0.75). A second prospective cohort study by Bowker *et al*[18] similarly showed that the use of metformin reduced cancer-related mortality as compared to sulfonylureas or insulin in diabetic patients, with an adjusted hazard ratio of 0.80 (95%CI: 0.65-0.98). These studies fuelled further investigation into chemopreventive use in hepatic malignancies, as well as applications to target insulin resistance in chronic liver disease. The biochemical basis of these effects, along with the literature behind potential applications in hepatology, are systematically detailed in this review.

LITERATURE STUDY

We systematically searched PubMed and Ovid MEDLINE databases from 2000 to March 2015, using a combination of metformin with the following relevant text words and MeSH terms: hepatitis B virus (HBV), HCV, NAFLD, hepatocellular carcinoma (HCC) or cholangiocarcinoma. The search results were evaluated for pertinence to the issue of metformin in liver disease as well as for quality of study design, and we used material written in English. The reference lists from all identified studies were searched for further relevant papers. Review articles were used as a reference, but not as primary sources of information.

NAFLD

Although the pathogenesis of NAFLD is not clearly understood, it is known that insulin resistance assumes a pivotal role. Importantly, NAFLD is associated with

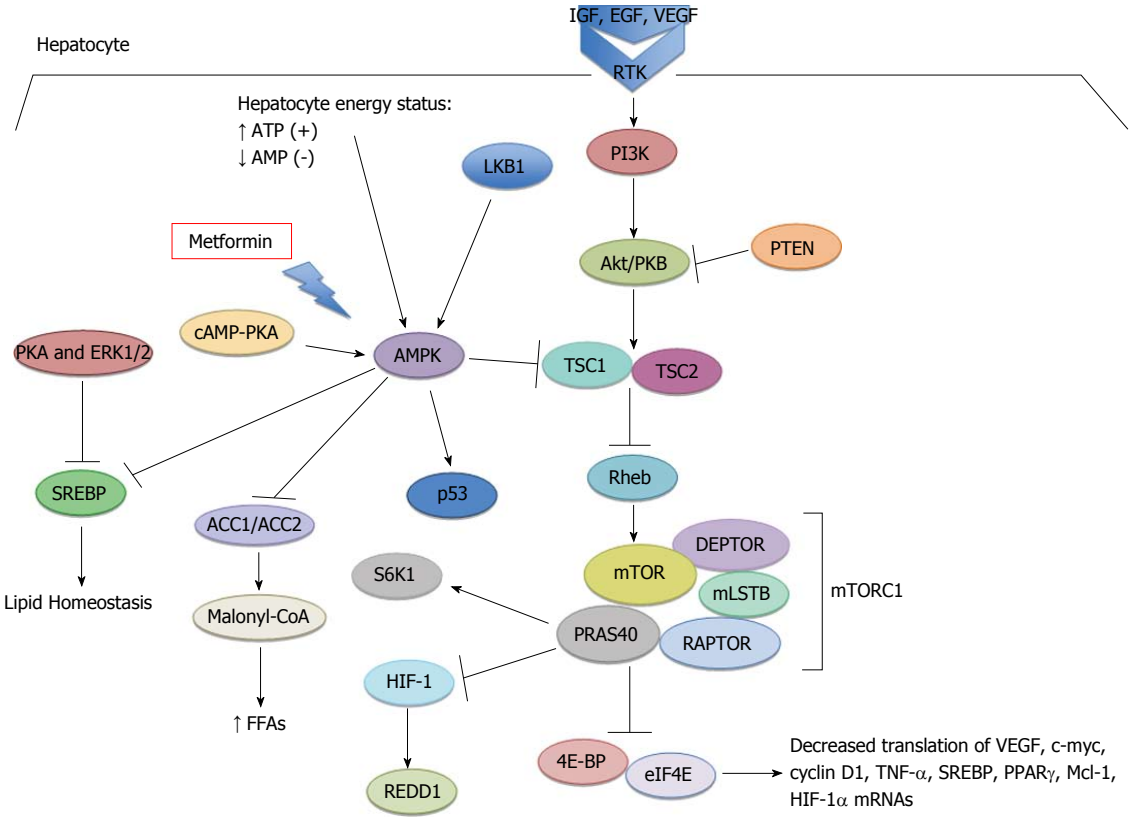

Figure 1 Effects of metformin on hepatocyte energy status and the mammalian target of rapamycin pathway, in turn affecting metabolism and inflammation. 4E-BP: 4 eukaryotic-binding protein; ACC: Acetyl-CoA carboxylase; PKB: Protein kinase B; AMPK: Adenosine monophosphate kinase; cAMP: Cyclic adenosine monophosphate; DEPTOR: DEP domain-containing mTOR interacting protein; EGF: Epidermal growth factor; eIF4E: Eukaryotic translation initiation factor 4E; FFAs: Free fatty acids; HIF-1α: Hypoxia-inducible factor-1 alpha; IGF: Insulin growth factor; LKB1: Liver kinase B1; mLSTB: Mammalian lethal with SEC13 protein B; mTOR: Mammalian target of rapamycin; PI3K: Phosphoinositol 3 kinase; PKA: Protein kinase A; PPARγ: Peroxisome proliferator-activated receptor gamma; PRAS40: Proline-rich Akt substrate of 40 kDa; RAPTOR: Regulatory-associated protein of mTOR; Rheb: Ras homolog enriched in brain; RTK: Receptor tyrosine kinase; SREBP: Sterol regulatory element-binding proteins; TSC: Tumor suppressor complex; VEGF: Vascular endothelial growth factor.

an increased risk of type 2 diabetes mellitus and cardiovascular disease[19]. In insulin-resistant patients, the increased influx of FFAs into the liver due to peripheral lipolysis leads to hepatic steatosis. Insulin resistance is said to increase hepatic lipogenesis through activation of the lipogenic transcription factor sterol-regulator element binding protein-1 (SREBP1). The state of hyperinsulinemia upregulates glycogenolysis, leading to increased fatty acid synthesis in the hepatocytes[20]. The "two-hit" hypothesis of non-alcoholic steatohepatitis (NASH) pathophysiology proposes a synergistic effect of "first-hit" obesity and diet, followed by the "second-hit" of inflammation and cellular injury. The "second-hit" is thought to contribute to the insulin resistance by releasing cytokines and free fatty acids, and increasing intracellular oxidative stress[20,21].

Current management of patients with NAFLD principally involves weight loss through diet and exercise[13]. Vitamin E is recommended as first line pharmacotherapy in non-diabetic adults with biopsy-proven NASH but not in diabetic patients due to lack of *ad hoc* data[22]. Hence, there is no pharmacotherapy as yet for diabetic NASH patients. There have been several pharmacotherapeutic attempts to target the insulin resistance thought to be the underlying pathophysiologic mechanism of NAFLD[23]. In a 6-mo, prospective, randomized study (level Ⅱ) that compared low-dose metformin (1 g/d) with diet to diet alone, both groups achieved a significant reduction in the proportion of patients with ultrasonographic evidence of hepatic steatosis[24]. Although metabolic parameters were significantly better in the metformin group, this study illustrated that diet in itself could ameliorate hepatic steatosis. Improvement in liver histology of NAFLD patients on metformin treatment has been documented (level Ⅳ - open-label, single arm)[25-27]. In a pilot study, 26 NASH patients were treated with 48 wk of metformin 2000 mg a day[26]. This resulted in a histological response and improvement in alanine aminotransferase levels in only 30% of the patients, and correlated with weight loss rather than with improvement in insulin sensitivity. Histological improvement was limited to hepatocellular injury and parenchymal inflammation. Along with histological improvement, there was a significant decrease in the aminotransferase levels observed[26]. Another uncontrolled trial of metformin (850-1000 mg/d) with N-acetylcysteine (1.2 g/d) for 12 mo demonstrated significant improvement in steatosis and fibrosis, although lobular inflammation and hepatocellular ballooning remained unchanged. Aminotransferase levels also were not significantly different following the treatment

course[27]. In three randomized trials (level Ⅱ), metformin treatment had very little effect on liver histology, but it did ameliorate the liver aminotransferases and insulin resistance[28-30]. A 12-mo, randomized, placebo-controlled trial of diet, exercise and metformin *vs* diet and exercise only in 19 non-diabetic patients with insulin resistance and NASH failed to show any improvement in histology and liver enzyme levels[28]. It was rather weight loss in itself through diet and exercise that correlated with improved liver histology, aminotransferases and insulin resistance. Another randomized trial (not placebo-controlled) of metformin (850 mg twice daily) and dietary treatment *vs* dietary treatment alone in 36 patients showed histological improvement in both groups[29]. The metformin group did nonetheless have significantly decreased insulin resistance and aminotransferases. An additional randomized, placebo-controlled trial of metformin in 48 patients with biopsy-proven NAFLD failed to show any histological improvement as compared to placebo[30].

In the pediatric context as well, metformin has failed to show any histological benefit. In a small observational pilot study of 57 overweight or obese children between ages of 9 to 18 years with biopsy-proven NAFLD or NASH for 24 mo, metformin was no more efficacious than lifestyle modifications in improving serum transaminases, hepatic steatosis or liver histology in patients (level Ⅳ - open label trial)[31]. A meta-analysis by Mazza *et al*[13] concluded that the addition of metformin may still be an attractive option to patients who have prediabetes or diabetes, due to clear evidence of improvement in insulin resistance associated with NAFLD. However, metformin has not demonstrated concrete improvement in liver histology in randomized, controlled studies, and therefore cannot be recommended for treatment of NASH[22].

METFORMIN IN HBV

There have been conflicting reports as to whether chronic HBV infection is correlated with insulin resistance[32,33]. Insulin resistance in patients with HBV is more likely concordant with their individual metabolic profiles. There are therefore no clinical studies of metformin on patients with HBV infection. Nonetheless, an *in vitro* study demonstrated that metformin transcriptionally downregulated hepatitis B surface antigen expression and HBV replication when used on a human hepatoma cell line. Additionally, it was found to act synergistically with antiviral effects of Lamivudine and interferon (IFN) alpha-2b[34]. Based on *in vitro* studies, metformin may have potential benefit for patients with HBV, however clinical trials are needed to further explore this therapy.

METFORMIN IN HCV

It is well established that HCV infection can induce a state of insulin resistance, ultimately leading to hepatic steatosis. It is hypothesized that HCV utilizes host cell glucose or lipid metabolism in order to complete its own life cycle, giving rise to the high prevalence of diabetes mellitus in patients with chronic HCV[35]. Evidence indicates that there is an association between patient metabolic profiles and the severity of hepatic fibrosis in HCV patients[36]. One of the key metabolic factors is insulin resistance, known to aggravate hepatic steatosis, which promotes liver fibrosis progression and increases the risk of HCC. It is also associated with high HCV viral load and poor virologic response to interferon treatment[37]. As oral hypoglycemic agents are the treatment of choice for insulin resistance, Romero-Gómez *et al*[37] hypothesized that metformin would aid in improved responses to peg-IFN (PEG-IFN) alfa-2a plus ribavirin (RBV) treatment in patients with naïve genotype 1 CHC patients. Although addition of metformin to peginterferon and RBV improved insulin sensitivity in this randomized, placebo-controlled trial of 123 patients, the results failed to show a significant difference in sustained virological response (SVR) between treatment and control groups[37]. Though the aforementioned results were not as promising as anticipated, additional trials were conducted to study whether metformin could help improve HCV treatment outcomes by correcting insulin resistance. A randomized, double-blind controlled trial of metformin *vs* placebo in addition to PEG-IFN and RBV treatment showed that SVR was no different between the 2 groups (75% *vs* 79%)[38]. In a small, randomized controlled trial by Hsu *et al*[35], various oral hypoglycemic agents, including metformin, were combined with the standard IFN-based therapy in patients with HCV genotype 1 and insulin resistance. Although the study was too small to derive definite conclusions, the data suggested that the addition of an oral hypoglycemic agent to PEG-IFN alfa-2a plus RBV achieved a better SVR (level Ⅱ - randomized, not placebo-controlled). Although new oral interferon-free regimens are rapidly changing the therapeutic landscape of HCV treatment, these findings suggest metformin may play a role in improving HCV treatment response specifically in the subgroup of patients with insulin resistance. Furthermore, metformin has an impact on the prognosis of HCV-induced liver cirrhosis, as shown by a reduction in the incidence of HCC and liver-related death or transplantation (level Ⅱ)[39].

METFORMIN IN HEPATIC MALIGNANCIES

The PI3K/Akt/mTOR pathway is often activated in malignancies, and phosphorylates downstream signaling effector molecules involved in cell cycle progression, protein synthesis, cell growth, and angiogenesis[4]. Metformin is known to inhibit the mTOR pathway at least partly through an LKB1-AMPK-dependent mechanism, as illustrated by the lack of its effects in LKB1-deficient mice[7]. The effects of metformin on cell survival and metabolism can be explained both by this LKB1-AMPK-dependent mechanism as well as its insulin-lowering effects. Insulin has mitogenic and pro-survival effects on cells, and tumor cells often express insulin receptors at higher levels, making them highly sensitive to insulin

stimulation[40]. Ultimately, metformin results in inhibition of the mTOR pathway and downstream effectors. These downstream effectors include eIF4E, which is normally bound to the 4E-BPs (eIF4E-binding proteins). When phosphorylated by mTORC1, the 4E-BPs detach from eIF4E, which is then free to complex with other proteins to initiate translation. The translation of mRNAs coding for proteins involved in processes pertinent to tumorigenesis, such as the cell cycle, angiogenesis, and apoptosis, is particularly favored[41]. Other effectors of mTORC1 are cyclin D1 (cell cycle regulation), p70S6K => phospho-S6 (ribosome biogenesis), and SREBP (lipid synthesis), which all contribute to fuelling tumorigenesis.

Specific effects of metformin on the hallmarks of cancer as defined by Weinberg and Hanahan have been elucidated[42]. In tumor development, the growth of the cell mass quickly exceeds its supply of nutrients and oxygen. Rapidly growing tumors encounter hypoxic conditions that hinder their ability to grow. However, cancer cells are able to circumvent these metabolic limitations. Despite conditions of cellular stress, there may be insufficient activity of AMPK, enabling the mTOR pathway-activated and uncontrolled cell growth. This makes metformin an attractive chemopreventive agent as it is an AMPK activator[8]. Angiogenesis is essential in the growth and invasive properties of tumor cells. Studies have shown that metformin negatively regulates hypoxia-inducible factor-1α, tumor necrosis factor-α, plasminogen activator inhibitor-1, and von Willebrand Factor, which decreases the levels of vascular endothelial growth factor (VEGF) and ultimately angiogenesis[8].

Metformin plays a role in induction of apoptosis through either p53-dependent or independent mechanisms. The tumor suppressor p53 is involved in DNA damage repair and cell cycle regulation. Ultimately, the activation of p53 induces apoptosis of cells under low nutrient conditions[2,8]. Tumor suppressor p53 regulates synthesis of cytochrome c oxidase 2 (SCO2) activity, allowing the cell to efficiently couple mitochondrial oxidative phosphorylation. Hence p53-defective cells, as is seen in over 50% of tumors, have a decrease in SCO2 activity and a deregulation of cell metabolism in a hypoxic environment. The inability of these cells to conserve energy when exposed to metformin-induced energetic crisis ultimately leads to apoptosis[1,5]. Metformin blocks the cell cycle partly through decreased levels of cyclin D1 expression, with a dose-dependent inhibition of cell proliferation[8]. Hence, metformin inhibits all of these processes that are key to tumorigenesis.

HCC is one of the leading causes of cancer-related deaths[43] and its incidence is on the rise in North America particularly due to the increasing burden of HCV cirrhosis[44]. Most HCC tumors are diagnosed at an advanced stage, when curative therapy is no longer an option. The only chemotherapy with survival benefit, though minimal, is the Ras-Raf kinase inhibitor sorafenib. The mTOR pathway is upregulated in up to 50% of HCCs. The mTOR pathway has also been found to play an essential role specifically in hepatocarcinogenesis arising in the context of NASH[45].

In vitro data have shown metformin to be a potent inhibitor of HepG2 and Huh7 liver cancer cell proliferation[7,46]. An apoptotic effect has also been noted through increased expression of cleaved caspase-3 and a significantly increased percentage of early apoptotic cells[44]. Cell cycle arrest in G0/G1 phase in several HCC cell lines has also been found *in vitro*, correlating with a strong decrease in expression of G1 cyclins, specifically cyclin D1, cyclin E and cyclin-dependent kinase 4[47]. *In vivo* studies have shown that metformin also exerts effects on apoptosis, cell cycle, and proliferation, likely through the mTOR pathway. In an *in vivo* study on HepG2 cells xenografted into nude mice, Xiong *et al*[44] demonstrated that metformin treatment at 200-mg/kg per day dose led to a 40.8% reduction in tumor volume. In this study as well, metformin was shown to suppress the protein synthesis machinery downstream of mTOR, inhibit cell proliferation and induce apoptosis. In a diethylnitrosamine-induced HCC mouse model, metformin was shown to down-regulate lipogenesis through decreased expression of lipogenic enzymes[48]. In addition, the restoration of these lipogenic enzymes through ectopic expression rescued the metformin-mediated growth inhibition. These findings provide an interesting application of metformin in patients with HCC in the context of disorders where there is upregulation of lipid synthesis such as NAFLD[48].

Population data have suggested a role for metformin as a chemopreventive agent against HCC among patients with diabetes[49-56] and chronic liver disease[57,58]. In a meta-analysis of 8 observational studies, including 22650 cases of HCC in 334307 patients with type 2 diabetes, it was found that metformin given to diabetic patients resulted in a 50% risk reduction in HCC incidence (OR = 0.50, 95%CI: 0.34-0.73)[53,54]. A similar meta-analysis of 7 studies (3 cohort, 4 case-control, with 562 HCC cases out of 16549 patients) reported an even further reduced risk of HCC in diabetic patients on metformin *vs* those using other hypoglycemic agents (OR = 0.24, 95%CI: 0.13-0.46)[55]. Although these data are striking, it should be kept in mind that the studies used in these meta-analysis were observational, and higher quality randomized trials would be optimal to consolidate whether metformin has a chemopreventive benefit.

In already-diagnosed HCC, the clinical literature is sparse. A retrospective clinical study determined that in patients with already-established HCC[59], though duration of exposure to metformin prior to diagnosis was not available. Combination therapy of metformin with radiofrequency ablation (RFA) has also been attempted prospectively. In a prospective case cohort study, diabetic patients with early stage HCC were treated with RFA concurrently with metformin[60]. The use of metformin as a chemotherapeutic adjunct in these patients was observed to have a lower mortality rate as compared to the untreated diabetic patients with early stage HCC. Furthermore, patients with early stage

HCC receiving sulfonylureas and insulin exposures did not achieve the same effects as metformin (level Ⅲ - prospective case cohort study)[60].

Physicians are often hesitant to use metformin in cirrhotic patients, given previous reports of lactic acidosis. A recent study has disproven this concern, revealing that cirrhotic patients may safely take metformin[61]. Additionally, use of metformin significantly extended survival in cirrhotic patients, with continuation decreasing risk of death by 57%. Hence, using metformin as a chemopreventive agent against HCC is a reasonable option in patients with chronic liver disease. However, it is unclear at this time whether metformin is beneficial as an adjunct in the treatment of HCC.

METFORMIN IN CHOLANGIOCARCINOMA

Cholangiocarcinoma is associated with a dismal prognosis, serving as an impetus to study its molecular basis and thereby develop therapeutic avenues. McKay *et al*[62] performed an array of comparative genomic hybridization to discover any potential therapeutic targets. The DNA extracted from 32 cholangiocarcinoma tumors was discovered to have copy number gains in several genes along the mTOR pathway[62]. Frequent gain of function mutations in genes related to the mTOR pathway, including mTOR, VEGF receptor, platelet-derived growth factor, and epidermal growth factor receptor, were identified making this a novel target for treatment options[41,62]. In a case-control study, it was established that type 2 diabetes mellitus independently predicts risk of intrahepatic cholangiocarcinoma (ICC), along with primary sclerosing cholangitis, cirrhosis, and smoking[63]. Diabetic patients with metformin had a significantly decreased odds ratio for ICC as compared to diabetics not on metformin (OR = 0.4; 95%CI: 0.2-0.9; P = 0.04). An *in vitro* study demonstrated a dose and time-dependent antiproliferative action of metformin through apoptosis induction and cell cycle arrest *via* the AMPK-mTORC1 pathway in ICC cell lines[64]. Additionally, metformin was found to enhance the sensitivity of ICC cells to sorafenib, 5-fluorouracil and As_2O_3. Hence, one might anticipate at least a chemopreventive role for metformin in ICC, although it would be interesting to determine whether such a role exists in prevention of perihilar and extrahepatic cholangiocarcinoma.

CONCLUSION

In conclusion, metformin is a widely used hypoglycemic agent with benefits beyond the management of diabetes. Given its effects on insulin resistance, there are data suggestive of benefit in HCV and NAFLD. However, at this time, there is insufficient evidence to recommend use of metformin in the adjunctive treatment of these chronic liver diseases. Nonetheless, by virtue of its inhibitory effect on the mTOR pathway, there is good evidence for a chemopreventive role against HCC among patients with diabetes and chronic liver disease, and metformin should be continued in patients even with cirrhosis to provide this benefit.

REFERENCES

1 **Buzzai M**, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, Viollet B, Thompson CB. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* 2007; **67**: 6745-6752 [PMID: 17638885 DOI: 10.1158/0008-5472.CAN-06-4447]

2 **Li D**. Metformin as an antitumor agent in cancer prevention and treatment. *J Diabetes* 2011; **3**: 320-327 [PMID: 21631893 DOI: 10.1111/j.1753-0407.2011.00119.x]

3 **Kohjima M**, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Fujino T, Yada M, Yada R, Harada N, Enjoji M, Takayanagi R, Nakamuta M. SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease. *Int J Mol Med* 2008; **21**: 507-511 [PMID: 18360697 DOI: 10.3892/ijmm.21.4.507]

4 **Pawałowska M**, Markowska A. The influence of metformin in the etiology of selected cancers. *Contemp Oncol* (Pozn) 2012; **16**: 223-229 [PMID: 23788884 DOI: 10.5114/wo.2012.29289]

5 **Pollak M**. Potential applications for biguanides in oncology. *J Clin Invest* 2013; **123**: 3693-3700 [PMID: 23999444 DOI: 10.1172/JCI67232]

6 **Rosilio C**, Ben-Sahra I, Bost F, Peyron JF. Metformin: a metabolic disruptor and anti-diabetic drug to target human leukemia. *Cancer Lett* 2014; **346**: 188-196 [PMID: 24462823 DOI: 10.1016/j.canlet.2014.01.006]

7 **Shackelford DB**, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer* 2009; **9**: 563-575 [PMID: 19629071 DOI: 10.1038/nrc2676]

8 **Jalving M**, Gietema JA, Lefrandt JD, de Jong S, Reyners AK, Gans RO, de Vries EG. Metformin: taking away the candy for cancer? *Eur J Cancer* 2010; **46**: 2369-2380 [PMID: 20656475 DOI: 10.1016/j.ejca.2010.06.012]

9 **Aljada A**, Mousa SA. Metformin and neoplasia: implications and indications. *Pharmacol Ther* 2012; **133**: 108-115 [PMID: 21924289 DOI: 10.1016/j.pharmthera.2011.09.004]

10 **Fullerton MD**, Galic S, Marcinko K, Sikkema S, Pulinilkunnil T, Chen ZP, O'Neill HM, Ford RJ, Palanivel R, O'Brien M, Hardie DG, Macaulay SL, Schertzer JD, Dyck JR, van Denderen BJ, Kemp BE, Steinberg GR. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nat Med* 2013; **19**: 1649-1654 [PMID: 24185692 DOI: 10.1038/nm.3372]

11 **Shaw RJ**. Metformin trims fats to restore insulin sensitivity. *Nat Med* 2013; **19**: 1570-1572 [PMID: 24309653 DOI: 10.1038/nm.3414]

12 **Li Y**, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, Park O, Luo Z, Lefai E, Shyy JY, Gao B, Wierzbicki M, Verbeuren TJ, Shaw RJ, Cohen RA, Zang M. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab* 2011; **13**: 376-388 [PMID: 21459323 DOI: 10.1016/j.cmet.2011.03.009]

13 **Mazza A**, Fruci B, Garinis GA, Giuliano S, Malaguarnera R, Belfiore A. The role of metformin in the management of NAFLD. *Exp Diabetes Res* 2012; **2012**: 716404 [PMID: 22194737 DOI: 10.1155/2012/716404]

14 **Huypens P**, Quartier E, Pipeleers D, Van de Casteele M. Metformin reduces adiponectin protein expression and release in 3T3-L1 adipocytes involving activation of AMP activated protein kinase. *Eur J Pharmacol* 2005; **518**: 90-95 [PMID: 16039647 DOI: 10.1016/j.ejphar.2005.06.016]

15 **Zhang S**, Liu X, Brickman WJ, Christoffel KK, Zimmerman D, Tsai HJ, Wang G, Wang B, Li Z, Tang G, Liu X, Yang J, Xu X, Wang X. Association of plasma leptin concentrations with

adiposity measurements in rural Chinese adolescents. *J Clin Endocrinol Metab* 2009; **94**: 3497-3504 [PMID: 19584182 DOI: 10.1210/jc.2009-1060]
16 **Evans JM**, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ* 2005; **330**: 1304-1305 [PMID: 15849206 DOI: 10.1136/bmj.38415.708634.F7]
17 **Libby G**, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 2009; **32**: 1620-1625 [PMID: 19564453 DOI: 10.2337/dc08-2175]
18 **Bowker SL**, Yasui Y, Veugelers P, Johnson JA. Glucose-lowering agents and cancer mortality rates in type 2 diabetes: assessing effects of time-varying exposure. *Diabetologia* 2010; **53**: 1631-1637 [PMID: 20407744 DOI: 10.1007/s00125-010-1750-8]
19 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]
20 **James OF**, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. *J Hepatol* 1998; **29**: 495-501 [PMID: 9765002 DOI: 10.1016/S0168-8278(98)80073-1]
21 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]
22 **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]
23 **Doycheva I**, Loomba R. Effect of metformin on ballooning degeneration in nonalcoholic steatohepatitis (NASH): when to use metformin in nonalcoholic fatty liver disease (NAFLD). *Adv Ther* 2014; **31**: 30-43 [PMID: 24385405 DOI: 10.1007/s12325-013-0084-6]
24 **Garinis GA**, Fruci B, Mazza A, De Siena M, Abenavoli S, Gulletta E, Ventura V, Greco M, Abenavoli L, Belfiore A. Metformin versus dietary treatment in nonalcoholic hepatic steatosis: a randomized study. *Int J Obes* (Lond) 2010; **34**: 1255-1264 [PMID: 20179669 DOI: 10.1038/ijo.2010.40]
25 **Nair S**, Diehl AM, Wiseman M, Farr GH, Perrillo RP. Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. *Aliment Pharmacol Ther* 2004; **20**: 23-28 [PMID: 15225167 DOI: 10.1111/j.1365-2036.2004.02025.x]
26 **Loomba R**, Lutchman G, Kleiner DE, Ricks M, Feld JJ, Borg BB, Modi A, Nagabhyru P, Sumner AE, Liang TJ, Hoofnagle JH. Clinical trial: pilot study of metformin for the treatment of non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2009; **29**: 172-182 [PMID: 18945255 DOI: 10.1111/j.1365-2036.2008.03869.x]
27 **de Oliveira CP**, Stefano JT, de Siqueira ER, Silva LS, de Campos Mazo DF, Lima VM, Furuya CK, Mello ES, Souza FG, Rabello F, Santos TE, Nogueira MA, Caldwell SH, Alves VA, Carrilho FJ. Combination of N-acetylcysteine and metformin improves histological steatosis and fibrosis in patients with non-alcoholic steatohepatitis. *Hepatol Res* 2008; **38**: 159-165 [PMID: 18197877 DOI: 10.1111/j.1872-034X.2007.00215.x]
28 **Shields WW**, Thompson KE, Grice GA, Harrison SA, Coyle WJ. The Effect of Metformin and Standard Therapy versus Standard Therapy alone in Nondiabetic Patients with Insulin Resistance and Nonalcoholic Steatohepatitis (NASH): A Pilot Trial. *Therap Adv Gastroenterol* 2009; **2**: 157-163 [PMID: 21180541 DOI: 10.1177/1756283X09105462]
29 **Uygun A**, Kadayifci A, Isik AT, Ozgurtas T, Deveci S, Tuzun A, Yesilova Z, Gulsen M, Dagalp K. Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2004; **19**: 537-544 [PMID: 14987322 DOI: 10.1111/j.1365-2036.2004.01888.x]
30 **Haukeland JW**, Konopski Z, Eggesbø HB, von Volkmann HL, Raschpichler G, Bjøro K, Haaland T, Løberg EM, Birkeland K. Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Scand J Gastroenterol* 2009; **44**: 853-860 [PMID: 19811343 DOI: 10.1080/00365520902845268]
31 **Nobili V**, Manco M, Ciampalini P, Alisi A, Devito R, Bugianesi E, Marcellini M, Marchesini G. Metformin use in children with nonalcoholic fatty liver disease: an open-label, 24-month, observational pilot study. *Clin Ther* 2008; **30**: 1168-1176 [PMID: 18640473 DOI: 10.1016/j.clinthera.2008.06.012]
32 **Kumar M**, Choudhury A, Manglik N, Hissar S, Rastogi A, Sakhuja P, Sarin SK. Insulin resistance in chronic hepatitis B virus infection. *Am J Gastroenterol* 2009; **104**: 76-82 [PMID: 19098853 DOI: 10.1038/ajg.2008.9]
33 **Lee JG**, Lee S, Kim YJ, Cho BM, Park JS, Kim HH, Cheong J, Jeong DW, Lee YH, Cho YH, Bae MJ, Choi EJ. Association of chronic viral hepatitis B with insulin resistance. *World J Gastroenterol* 2012; **18**: 6120-6126 [PMID: 23155341 DOI: 10.3748/wjg.v18.i42.6120]
34 **Xun YH**, Zhang YJ, Pan QC, Mao RC, Qin YL, Liu HY, Zhang YM, Yu YS, Tang ZH, Lu MJ, Zang GQ, Zhang JM. Metformin inhibits hepatitis B virus protein production and replication in human hepatoma cells. *J Viral Hepat* 2014; **21**: 597-603 [PMID: 24164660 DOI: 10.1111/jvh.12187]
35 **Hsu CS**, Hsu SJ, Lin HH, Tseng TC, Wang CC, Chen DS, Kao JH. A pilot study of add-on oral hypoglycemic agents in treatment-naïve genotype-1 chronic hepatitis C patients receiving peginterferon alfa-2b plus ribavirin. *J Formos Med Assoc* 2014; **113**: 716-721 [PMID: 24974131 DOI: 10.1016/j.jfma.2014.05.007]
36 **Hwang SJ**, Lee SD. Hepatic steatosis and hepatitis C: Still unhappy bedfellows? *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 96-101 [PMID: 21199519 DOI: 10.1111/j.1440-1746.2010.06542.x]
37 **Romero-Gómez M**, Diago M, Andrade RJ, Calleja JL, Salmerón J, Fernández-Rodríguez CM, Solà R, García-Samaniego J, Herrerías JM, De la Mata M, Moreno-Otero R, Nuñez O, Olveira A, Durán S, Planas R. Treatment of insulin resistance with metformin in naïve genotype 1 chronic hepatitis C patients receiving peginterferon alfa-2a plus ribavirin. *Hepatology* 2009; **50**: 1702-1708 [PMID: 19845037 DOI: 10.1002/hep.23206]
38 **Sharifi AH**, Mohammadi M, Fakharzadeh E, Zamini H, Zaer-Rezaee H, Jabbari H, Merat S. Efficacy of adding metformin to pegylated interferon and ribavirin in treatment naïve patients with chronic hepatitis C: a randomized double-blind controlled trial. *Middle East J Dig Dis* 2014; **6**: 13-17 [PMID: 24829699]
39 **Nkontchou G**, Cosson E, Aout M, Mahmoudi A, Bourcier V, Charif I, Ganne-Carrie N, Grando-Lemaire V, Vicaut E, Trinchet JC, Beaugrand M. Impact of metformin on the prognosis of cirrhosis induced by viral hepatitis C in diabetic patients. *J Clin Endocrinol Metab* 2011; **96**: 2601-2608 [PMID: 21752887 DOI: 10.1210/jc.2010-2415]
40 **Dowling RJ**, Goodwin PJ, Stambolic V. Understanding the benefit of metformin use in cancer treatment. *BMC Medicine* 2011; **9**: 33 [DOI: 10.1186/1741-7015-9-33]
41 **Bhat M**, Sonenberg N, Gores GJ. The mTOR pathway in hepatic malignancies. *Hepatology* 2013; **58**: 810-818 [PMID: 23408390 DOI: 10.1002/hep.26323]
42 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70 [PMID: 10647931 DOI: 10.1016/S0092-8674(00)81683-9]
43 **Cheng J**, Huang T, Li Y, Guo Y, Zhu Y, Wang Q, Tan X, Chen W, Zhang Y, Cheng W, Yamamoto T, Jing X, Huang J. AMP-activated protein kinase suppresses the in vitro and in vivo proliferation of hepatocellular carcinoma. *PLoS One* 2014; **9**: e93256 [PMID: 24709998 DOI: 10.1371/journal.pone.0093256]
44 **Xiong Y**, Lu QJ, Zhao J, Wu GY. Metformin inhibits growth of hepatocellular carcinoma cells by inducing apoptosis via mitochondrion-mediated pathway. *Asian Pac J Cancer Prev* 2012; **13**: 3275-3279 [PMID: 22994747]
45 **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]

46 **Qu Z**, Zhang Y, Liao M, Chen Y, Zhao J, Pan Y. In vitro and in vivo antitumoral action of metformin on hepatocellular carcinoma. *Hepatol Res* 2012; **42**: 922-933 [PMID: 22524458 DOI: 10.1111/j.1872-034X.2012.01007.x]

47 **Miyoshi H**, Kato K, Iwama H, Maeda E, Sakamoto T, Fujita K, Toyota Y, Tani J, Nomura T, Mimura S, Kobayashi M, Morishita A, Kobara H, Mori H, Yoneyama H, Deguchi A, Himoto T, Kurokohchi K, Okano K, Suzuki Y, Murao K, Masaki T. Effect of the anti-diabetic drug metformin in hepatocellular carcinoma in vitro and in vivo. *Int J Oncol* 2014; **45**: 322-332 [PMID: 24806290]

48 **Bhalla K**, Hwang BJ, Dewi RE, Twaddel W, Goloubeva OG, Wong KK, Saxena NK, Biswal S, Girnun GD. Metformin prevents liver tumorigenesis by inhibiting pathways driving hepatic lipogenesis. *Cancer Prev Res* (Phila) 2012; **5**: 544-552 [PMID: 22467080 DOI: 10.1158/1940-6207.CAPR-11-0228]

49 **Chen HP**, Shieh JJ, Chang CC, Chen TT, Lin JT, Wu MS, Lin JH, Wu CY. Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies. *Gut* 2013; **62**: 606-615 [PMID: 22773548 DOI: 10.1136/gutjnl-2011-301708]

50 **Donadon V**, Balbi M, Ghersetti M, Grazioli S, Perciaccante A, Della Valentina G, Gardenal R, Dal Mas M, Casarin P, Zanette G, Miranda C. Antidiabetic therapy and increased risk of hepatocellular carcinoma in chronic liver disease. *World J Gastroenterol* 2009; **15**: 2506-2511 [PMID: 19469001 DOI: 10.3748/wjg.15.2506]

51 **Lee MS**, Hsu CC, Wahlqvist ML, Tsai HN, Chang YH, Huang YC. Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. *BMC Cancer* 2011; **11**: 20 [PMID: 21241523 DOI: 10.1186/1471-2407-11-20]

52 **Hassan MM**, Curley SA, Li D, Kaseb A, Davila M, Abdalla EK, Javle M, Moghazy DM, Lozano RD, Abbruzzese JL, Vauthey JN. Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. *Cancer* 2010; **116**: 1938-1946 [PMID: 20166205 DOI: 10.1002/cncr.24982]

53 **Singh S**, Singh PP, Singh AG, Murad MH, Sanchez W. Anti-diabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. *Am J Gastroenterol* 2013; **108**: 881-891; quiz 892 [PMID: 23381014 DOI: 10.1038/ajg.2013.5]

54 **Zhang H**, Gao C, Fang L, Zhao HC, Yao SK. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients: a meta-analysis. *Scand J Gastroenterol* 2013; **48**: 78-87 [PMID: 23137049 DOI: 10.3109/00365521.2012.719926]

55 **Lai SW**, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. *Am J Gastroenterol* 2012; **107**: 46-52 [PMID: 22085817 DOI: 10.1038/ajg.2011.384]

56 **Donadon V**, Balbi M, Valent F, Avogaro A. Glycated hemoglobin and antidiabetic strategies as risk factors for hepatocellular carcinoma. *World J Gastroenterol* 2010; **16**: 3025-3032 [PMID: 20572306 DOI: 10.3748/wjg.v16.i24.3025]

57 **Donadon V**, Balbi M, Mas MD, Casarin P, Zanette G. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. *Liver Int* 2010; **30**: 750-758 [PMID: 20331505 DOI: 10.1111/j.1478-3231.2010.02223.x]

58 **Chen CI**, Kuan CF, Fang YA, Liu SH, Liu JC, Wu LL, Chang CJ, Yang HC, Hwang J, Miser JS, Wu SY. Cancer risk in HBV patients with statin and metformin use: a population-based cohort study. *Medicine* (Baltimore) 2015; **94**: e462 [PMID: 25674734 DOI: 10.1097/MD.0000000000000462]

59 **Bhat M**, Chaiteerakij R, Harmsen WS, Schleck CD, Yang JD, Giama NH, Therneau TM, Gores GJ, Roberts LR. Metformin does not improve survival in patients with hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 15750-15755 [PMID: 25400459 DOI: 10.3748/wjg.v20.i42.15750]

60 **Chen TM**, Lin CC, Huang PT, Wen CF. Metformin associated with lower mortality in diabetic patients with early stage hepatocellular carcinoma after radiofrequency ablation. *J Gastroenterol Hepatol* 2011; **26**: 858-865 [PMID: 21251068 DOI: 10.1111/j.1440-1746.2011.06664.x]

61 **Zhang X**, Harmsen WS, Mettler TA, Kim WR, Roberts RO, Therneau TM, Roberts LR, Chaiteerakij R. Continuation of metformin use after a diagnosis of cirrhosis significantly improves survival of patients with diabetes. *Hepatology* 2014; **60**: 2008-2016 [PMID: 24798175 DOI: 10.1002/hep.27199]

62 **McKay SC**, Unger K, Pericleous S, Stamp G, Thomas G, Hutchins RR, Spalding DR. Array comparative genomic hybridization identifies novel potential therapeutic targets in cholangiocarcinoma. *HPB* (Oxford) 2011; **13**: 309-319 [PMID: 21492330 DOI: 10.1111/j.1477-2574.2010.00286.x]

63 **Chaiteerakij R**, Yang JD, Harmsen WS, Slettedahl SW, Mettler TA, Fredericksen ZS, Kim WR, Gores GJ, Roberts RO, Olson JE, Therneau TM, Roberts LR. Risk factors for intrahepatic cholangiocarcinoma: association between metformin use and reduced cancer risk. *Hepatology* 2013; **57**: 648-655 [PMID: 23055147 DOI: 10.1002/hep.26092]

64 **Ling S**, Feng T, Ke Q, Fan N, Li L, Li Z, Dong C, Wang C, Xu F, Li Y, Wang L. Metformin inhibits proliferation and enhances chemosensitivity of intrahepatic cholangiocarcinoma cell lines. *Oncol Rep* 2014; **31**: 2611-2618 [PMID: 24788596 DOI: 10.3892/or.2014.3151]

P- Reviewer: Chuang WL, Czaja MJ, Han T, Koch TR, Narciso-Schiavon JL
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1660

World J Hepatol 2015 June 28; 7(12): 1660-1670
ISSN 1948-5182 (online)

MINIREVIEWS

Induced immunity against hepatitis B virus

Zeinab Nabil Ahmed Said, Kouka Saadeldin Abdelwahab

Zeinab Nabil Ahmed Said, Kouka Saadeldin Abdelwahab, Department of Microbiology and Immunology, Faculty of Medicine (for girls), Al-Azhar University, Cairo 11754, Egypt

Author contributions: Said ZNA and Abdelwahab KS contributed equally to this review.

Conflict-of-interest: The authors report no potential conflicts of interest.

Correspondence to: Zeinab Nabil Ahmed Said, Professor, Department of Microbiology and Immunology, Faculty of Medicine (for girls), Al-Azhar University, Nasr City, Cairo 11754, Egypt. znabil58@yahoo.com
Telephone: +20-2-1006602418

Received: August 29, 2014
Peer-review started: August 30, 2014
First decision: December 17, 2014
Revised: January 15, 2015
Accepted: May 27, 2015
Article in press: May 28, 2015
Published online: June 28, 2015

Abstract

Prevention of hepatitis B virus (HBV) infection with its consequent development of HBV chronic liver disease and hepatocellular carcinoma is a global mandatory goal. Fortunately, safe and effective HBV vaccines are currently available. Universal hepatitis B surface antigen HBV vaccination coverage is almost done. Growing knowledge based upon monitoring and surveillance of HBV vaccination programs has accumulated and the policy of booster vaccination has been evaluated. This review article provides an overview of the natural history of HBV infection, immune responses and the future of HBV infection. It also summarizes the updated sources, types and uses of HBV vaccines, whether in the preclinical phase or in the post-field vaccination.

Key words: Hepatitis B surface antigen; Hepatitis B virus vaccines; Immunological memory; Hepatitis B virus; Booster and therapeutic vaccination

Core tip: Worldwide, it is estimated that more than 2 billion people have been infected with hepatitis B virus (HBV). Of these, approximately 240 million are chronically infected and at risk of serious illness or death from development of cirrhosis and subsequent progression to hepatocellular carcinoma (HCC), estimated to cause one million deaths each year worldwide. Prevention and control of HBV infection can therefore make a significant contribution to community health and to saving lives by preventing HCC. This review concerns the major advances in the field of HBV over the last few decades which have resulted in understanding the natural history of HBV infection and the development of effective vaccines against the virus. In the era of universal HBV vaccination coverage, the current growing body of knowledge regarding monitoring and surveillance of HBV vaccination programs and the policy of booster vaccination, several issues have to be evaluated regarding the vaccination policy and booster doses. In addition, it is worth evaluating vaccine-escape viral mutants, long-term protection and the therapeutic use of HBV vaccine as a promising new strategy for controlling chronic HBV infection.

Said ZNA, Abdelwahab KS. Induced immunity against hepatitis B virus. *World J Hepatol* 2015; 7(12): 1660-1670 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1660.

htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1660

INTRODUCTION

A serum antigen named Australia antigen was detected in sera from Australian aborigines[1]. Later it was found in sera from Down's syndrome, leukemia and hepatitis patients. It was identified as a marker of post-transfusion viral hepatitis and given the letter hepatitis B virus (HBV) to replace the previous term of serum hepatitis. Data accumulated to document that HBV has 7-9 genotypes with more or less defined geographical distribution[2]. The "a" component of hepatitis B surface antigen (HBsAg) is shared by all genotypes; however, infection by one genotype does not prevent subsequent infection by other types. Moreover, the antiviral effectiveness is related to HBV genotype and genotype D prevalent in the Middle Eastern countries, which is the most resistant to antivirals.

The virus morphology visualized by electron microscopy was described by Dane *et al*[3] and the complete HBV virion was named Dane particle, synonymous with HBV. The immunogen of HBV is a subviral unit of envelope proteins named HBsAg. During HBV replication, excess of the subviral components are produced. The hepatitis B core antigen (HBcAg) is restricted to the hepatocytes and to the full virion. Modified HBcAg, named hepatitis B e antigen (HBeAg), is released into the circulation. Moreover, excess HBsAg is released into the circulation in different morphological forms.

According to the 2010 Global Disease Burden estimates[4,5], both HBV and HCV caused 1.4 million deaths from acute infection, chronic infection, cirrhosis and hepatocellular carcinoma (HCC). It was reported that 240 million people are chronically infected with HBV and are at risk of serious illness or death from development of cirrhosis and subsequent progression to HCC. Later data showed that approximately 780000 persons die each year from hepatitis B infection[6].

The first anti HBV infectivity vaccine was HBsAg vaccine, which was produced by recombinant-DNA technology using yeasts. The r-DNA HBsAg vaccine produced robust immunity against the HBV infectivity and consequently prevented all the post HBV infection complications, including HCC. Prevention of HBV hepatitis and HCC is a significant contribution to global health and productivity. Progress in knowledge of HBV within the last few decades resulted in the understanding of the natural history of HBV infection, the development of sensitive assays for screening of blood donors for safe blood donation and monitoring of antiviral drugs, viral suppression and clearance[7], plus manufacturing improved r-DNA HBsAg vaccines. This progress paves the way for worldwide future elimination of HBV.

Natural history of HBV infection

Severity of HBV liver diseases shows great individual variations[8]. The outcome of infection and the pathogenesis of hepatic disease are determined by host factors and viral factors that play an essential role in virus clearance and persistence. It has been difficult to fully elucidate such factors on an experimental basis because of the restricted host range of HBV to man and chimpanzees[9]. Clinical research showed that there is a relationship between HBV genotype, natural history of infection and response to specific HBV antiviral treatment[10]. Both antibodies and cytotoxic T cells directed to different HBV antigens play an important role for decreasing viral load and clearing HBV-infected hepatocytes from the liver[11]. On the other hand, very weak or functionally impaired virus-specific immune responses have an essential role in the persistence of HBV infection[12]. Most primary infections of adults (70%-90%), whether symptomatic or not, ends up with efficient control of infection, with virus clearance from blood, liver and extrahepatic cells supporting HBV replication. The risk for chronicity decreases with increasing age at the time of exposure to HBV infection. HBV infection in nonimmune young children is mainly asymptomatic. Unlike adults who mostly do not develop chronic infection, neonates and infants who acquire HBV infection from their mothers at birth are most susceptible to the establishment of chronic HBV infection[13-15]. Currently, it is clearly emphasized that there are extrahepatic cells that support HBV replication and survival for several years or lifelong. These cells cause HBV infection of transplanted liver and rejection of the transplant.

HBV infected adults showed no clinically evident liver disease or mild acute hepatitis that terminated without long-term sequelae with development of lasting immunity to re-infection[16,17]. The other patients (10%-30%) who do not succeed in clearing the virus progress to chronic infection with continuous HBV replication. Host immune response plays an essential role in HBV-related hepatocyte damage because the virus itself is not cytolytic. The balance between host immune response and HBV replication in hepatocytes and in extrahepatic host cells is dynamic[18]. Fortunately, 70%-90% chronic HBV infected patients are asymptomatic without life-threatening effects on liver cells, while 10%-30% of patients develop liver cirrhosis[19] with consequent hepatic insufficiency and portal hypertension that make liver cirrhosis one of the most frightening consequences of chronic HBV infection[8]. Development of HCC is also a catastrophic result of chronic HBV infection with a lot of evidence supporting an association between HBV replication and the risk of development of HCC[18,20]. The HBx protein is a potent transactivator that activates host genes, including oncogenes[21].

Occult HBV infection

Occult HBV (OHBV) infection is a potential transmission source of post transfusion or organ transplantation HBV infection. To investigate the properties of hepatitis B surface antibody (HBsAb) in OHBV infection and its

affinity to different serotypes of HBsAg, Zhang *et al*[22] conducted long-term follow-up in 2 HBsAb positive patients with occult HBV infection where the HBsAb subtype was determined by performing neutralization experiments with different serotypes of HBsAg. They showed that the HBsAbs are mainly specific for common epitopes among different serotypes of HBsAg and are probably different than those produced by vaccine inoculation.

Immune response

Experimental infection of chimpanzees does not produce the same events as in human infection, which greatly limits the tools to study the natural life cycle of HBV in humans. Few well documented human HBV infections have shown that the virus itself is not cytolytic and the hepatolysis is immune-mediated. It was also shown that the HBsAg level varied with a different clinical or virological status. A low baseline level of HBsAg is associated with advanced liver fibrosis in HBeAg positive CHB patients[23]. Experimental studies demonstrated that acute HBV infected animal models developed silent innate immunity[21] and HBV does not immediately begin to replicate efficiently after inoculation[9,24], as it does in acutely infected humans. Following infection and up to 4-7 wk later, neither HBV antigens nor HBV-DNA are detectable in serum or liver[24-26]. The absence of early symptoms in HBV-infected patients is an indirect indication of the defective type I interferon (IFN) production during the early phase of HBV infection when exponential virus replication is going on[9].

The immunological events in the early phase of HBV replication mainly influence the differences in the adaptive immune response to HBV that characterizes resolved and chronic HBV infections[26]. Coordinated activation of the different branches of adaptive immunity is necessary for effective viral control[9]. Researchers showed that macrophages have an essential role in modulating HBV clearance, chronic hepatitis and the developed tissue damage, with M1-like macrophages promoting HBV clearance and M2-like macrophages impairing Th1 immune response and promoting tissue fibrosis/remodeling/wound healing[27]. Full recovery with elimination of HBV infected hepatocytes depends on active $CD8^+$ cytotoxic T lymphocytes. Following HBV infection, early immunological response failed to be activated as it is delayed until the exponential phase of replication[28]. In acute, self-limited hepatitis B infection, strong antiviral T-cell response is detected in peripheral blood. It includes both $CD4^+$ helper and $CD8^+$ cytotoxic T lymphocytes reactive with epitopes of multiple HBV antigens within the HBV core, polymerase and envelope proteins[8]. Virus-specific $CD8^+$ T cells have an essential role in HBV clearance[26]. Resolution of human HBV infection was believed to be solely dependent upon contact cytolysis of virus infected hepatocytes by $CD8^+$ cytotoxic T lymphocytes. However, researches on HBV transgenic mice and HBV-infected chimpanzees showed that T cell control of HBV replication is also influenced by cytokine-mediated noncytolytic mechanisms. $CD8^+$ lymphocytes produce different cytokines, including IFN-γ and tumor necrosis factor-α, that lead to inactivation of HBV in infected hepatocytes[29]. The cytolytic and noncytolytic action of virus-specific $CD8^+$ lymphocytes in its reaction with HBV-producing host cells adds up in acquired immune reactions against HBV infected hepatocytes. However, the noncytolytic mechanisms retain the more effective role[29].

Chronic HBV infected patients have impaired immune response to HBV[30,31]. In chronic HBV carriers, virus-specific T-cell responses are mainly attenuated; however, antibody responses are vigorous and sustained but free anti-HBs antibodies are undetectable because of excess circulating HBsAg[8]. Also, toll-like receptor (TLR) signaling in murine nonparenchymal liver cells (NPCs) is suppressed in the presence of HBsAg. This has been shown when peripheral blood mononuclear cells (PBMCs) from HBV infected patients and controls were stimulated by TLR ligands in the presence or absence of autologous serum. The expression of both TLR-mediated cytokine [*e.g.*, interleukin-6 (IL-6) and IL-10] and TLR3-induced IFN in PBMCs of HBV infected patients demonstrated a significant increase compared to the healthy volunteers, denoting a negative correlation between HBsAg and TLR3-mediated IFN-γ levels[32].

Six viral epitopes that are reactive with autologous HLA-A2 domains on cytotoxic T lymphocytes(CTL) were identified by Rehermann *et al*[33]. These epitopes are present in the highly conserved reverse transcriptase and RNase H domains of the viral polymerase protein. In acute HBV infected patients, the CTL response to polymerase is polyclonal, multi-specific and is mediated by $CD8^+$ T cells, but it is undetectable in chronic HBV infected patients or in healthy blood donors[33]. CTL responses against polymerase, core and envelope epitopes were identified up to one year following complete clinical recovery and seroconversion, indicating either the persistence of viral replication or the presence of long lasting memory CTL despite the absence of the viral antigens. It was shown that wild type viral DNA and RNA can persist indefinitely in trace amounts in serum and PBMC following complete clinical and serological recovery, despite a concomitant, vigorous and sustained polyclonal CTL response[33]. In order to explain the persistence of HBV, the authors further emphasized that the virus may retreat into immunologically privileged places from where it can seed the circulation and reach CTL inaccessible tissues, thereby maintaining the CTL response in apparently cured individuals and thus prolonging the liver disease in chronic HBV hepatitis patients[33]. In a large prospective clinic-based cohort of Asian chronic HBV patients, Desmond *et al*[34] identified significant associations between HLA types and HBV sequence variation at 41 sites within the HBV genome.

HBV VACCINES

Vaccination is the most effective measure to decrease the

worldwide HBV incidence and its complications, including liver cirrhosis and HCC. Worldwide, immunization has been an essential strategy for many countries to decrease the burden of HBV infection[5]. Economically, vaccination is an attractive option, both in terms of cost-effectiveness and benefit-cost ratios when compared with other health care interventions[35]. Commercial HBV vaccine supplies have been available for thirty years. HBV vaccine was the first vaccine against a chronic disease, the first vaccine to protect from a sexually transmitted infection and the first vaccine against a cancer[36]. The choice of a vaccine type and a schedule for doses and route of vaccination varies between countries. An ideal HBV vaccine schedule should protect against infection in infancy when the risk of becoming a chronic HBV carrier is high and in adolescence with common, high risk behaviors such as sex and drug abuse[37]. The CDC Advisory Committee on Immunization Practices (ACIP) recommends that all children should receive a birth dose of HBV vaccine and complete the vaccine series by 6-18 mo of age. It also recommends that older children and adolescents who did not previously receive the HBV vaccine should be vaccinated[38]. These policies were implemented by several countries with medium to high endemicity of HBV infection.

History of HBV vaccine development

The first HBV vaccine was prepared from the plasma of asymptomatic carriers of HBV in the form of purified inactivated HBsAg particles[39]. Later on, the r-HBsAg vaccines that contain the major (s) small protein spanning the hydrophilic amino acids 124-149 as the dominant immunogenic epitope were developed. HBV vaccinations induce neutralizing antibodies (anti-HBs) that are directed mainly towards the "a" determinant of HBsAg in all HBV genotypes from A to H[40]. The r-HBsAg vaccine elicits active synthesis of anti-HBs and prolonged immunological memory which provides continuous protection[41]. Persistent memory for 5 years or more is recognized from large, fast increases in anti-HBs level after booster vaccination, even in those who showed undetectable anti-HBs as measured by the available commercial kits. Using an *in vitro* enzyme linked immunosorbent assay (spot-ELISA), it was shown that the number of memory B lymphocytes able to induce anti-HBs does not decrease with decline of the anti-HBs level[41]. It was found that the value of immune memory and of the following secondary immune response can be estimated by the antibody response after primary vaccination. Both dose and structure of vaccine antigen influence the primary antibody response as well as the development of immune memory[42].

To increase the efficacy and prolong the duration of protection against HBV, second generation r-HBsAg vaccines, including the middle (pre-S_2) and the large (pre-S_1) proteins in HBV vaccine, were recognized. The immunogenicity of r-HBs 20 nm particles secreted by transfected Chinese hamster ovary (CHO) cells was compared with yeast-derived r-HBsAg vaccines[43]. The CHO-derived vaccine contains the small hepatitis B surface antigen (HBs protein) as the major component, together with pre-S_2 and pre-S_1 antigens, induced an augmented anti-HBs response in mice when compared with mice receiving the already used yeast-derived vaccines[43].

The current widely used r-HBsAg vaccines are a viral subunit produced by yeast that has been transfected with a plasmid that contains the *S* gene (codes for HBsAg) either as a single preparation or in combined form. Commercial r-HBsAg single antigen vaccines are Recombivax-HB (Merck) and Engerix-B (Glaxo). There are other approved combined vaccines against both HAV and HBV (Twinrix-Glaxo)[44]. Other formulations for infants are tetra or penta vaccines against diphtheria, tetanus, pertussis (whooping cough) and HBV for tetra vaccine and with the addition of injectable inactivated Salk polio (IPN) for the penta vaccine[45]. Combined Hepatitis B-Haemophilus influenzae type b (Hib) conjugate vaccine cannot be given before age of six weeks or after the age of seventy-one months[15]. Hexavalent combined vaccine, including diphtheria, tetanus, pertussis, HBV, Haemophilus b (Hib) and the three IPV serotypes antigens, is considered the most suitable combination vaccine for routine immunization[35,46]. Combined vaccines aid in improving compliance and simplifying complex pediatric immunization schedules, reduce storage requirements, reduce handling, costs of immunization programs and minimize the number of injections required, thereby reducing distress for infants and parents[46,47]. Their development is restricted because of their technical manufacturing complexity. High technical complexity increases production costs and therefore many manufacturers target them as premium products for developed countries[46].

Formulating of different types of HBV vaccine

Prophylactic vaccines: The need for HBV immunoprophylaxis was recognized as early as the late 1970s. The plasma derived vaccine was licensed in 1981. The vaccine contained a purified HBsAg single component that was obtained from sera of HBsAg carriers. Plasma derived HBV vaccines have been demonstrated to be highly immunogenic, efficacious and safe[48,49]. The long-term effect of plasma-derived HBV vaccine which was given to children born in 1986 was observed in a recent Chinese study. The vaccine showed a good long-term protective effect and there was no need for boosting the immunization 23 years later[50]. However, the use of this vaccine was dropped due to concern regarding the safety of a human blood-derived products, the inconsistency of a source of raw viral particles and the availability of new recombinant vaccines produced from yeast transfected with vector plasmid with DNA sequence coding for the production of soluble r-HBsAg proteins that are easily purified from the yeast proteins[45].

Yeast-derived recombinant hepatitis B vaccine contains a gene for the HBV surface antigen (S) which has been cloned into yeast cells that are cultured on

a wide scale to amplify the recombinant DNA coding for producing a large amount of the specified antigen (HBsAg) which is further purified, concentrated and combined with adjuvant to be ready for vaccination. Alum is included as an adjuvant in all licensed HBV vaccines[45]. HBsAg prevalence decreased dramatically after the implementation of yeast-derived r-HBsAg vaccine for 12 years for children in HBV-endemic areas in China, with no need for booster immunization[51]. Billions of doses of r-HBsAg vaccines have been administered worldwide, with a high record of immunogenicity and safety[44].

Years ago, several preclinical studies were conducted to evaluate the potential use of synthetic preS analogues for hepatitis B vaccination[52]; however, global adoption of these experimental vaccines was not achieved.

HBV DNA vaccines have been shown to be useful for both prophylaxis and treatment of HBV infection. It is one of the most effective ways to elicit protective immunity against infections. Preclinical studies in animal models, including mouse, chimpanzee, duck and woodchuck, were developed to evaluate the ability of DNA vaccines targeting hepadnaviral proteins to induce sustained strong immune responses in naïve animals and to enhance virus clearance and break immune tolerance in chronic HBV carriers[12]. HBV-DNA based vaccine is a plasmid DNA encoding the hepatitis HBVsAg that was shown to be immunologically effective, safe and well tolerated in chronic HBV carriers and non-responders to routinely used HBV vaccines[53,54]. The protective immunogenicity of a particle-mediated HBV-DNA vaccine in subjects who have responded suboptimally to conventional vaccination was evident[54]. In comparison with antigen-based HBV vaccines, plasmid DNA vaccine against HBV was evaluated in chimpanzees and protective anti-HBs antibody response was attained together with a strong anamnestic response achieved one year later[55]. It was well recognized that HBV DNA vaccine induces strong antigens that stimulate both humoral and cellular immunities that can be promoted by a joining of DNA that expresses IL-2 or IL-12 epitopes[56]. HBV-DNA based vaccines stimulated $CD8^+$CTL cells. Among the recently studied adjuvants, layered double hydroxide (LDH) nanoparticles as well as core-shell structure SiO_2@LDH nanoparticles were effectively proven adjuvants. Experimentally, SiO_2@LDH nanoparticles taken in by macrophages caused a higher dose-dependent expression of IFN-γ, IL-6, CD86 and MHC Ⅱ. Furthermore, *in vivo* immunization of BALB/c mice indicated that HBV DNA vaccine loaded-SiO_2@LDH nanoparticles not only induced increased serum antibody response compared to naked DNA vaccine and plain nanoparticles, but also obviously promoted T-cell proliferation and skewed T helper to Th1 polarization[57]; however, these experimental adjuvenated HBV DNA vaccines have not been adopted for humans so far.

Early trials to make use of genetically engineered plants as an alternative way for developing an HBV inexpensive and edible vaccine showed preservation of epitopes of HBsAg of both B- and T-cells when the antigen is expressed in a transgenic plant[58]. Also, HBVsAg encoding DNA gene was previously introduced into *Agrobacterium tumerifacience* LBA4404 that was used to get transgenic lupin (*Lupinus luteus L.*) and transgenic lettuce (*Lactuca sativa L.*) cv. Burpee Bibb expressing envelope surface protein. It was found that mice that were fed on HBV transgenic lupin tissue developed a significant amount of HBV specific antibodies. Meanwhile, human volunteers fed with transgenic lettuce plants expressing HBVsAg also developed a specific serum-IgG response to plant produced HBsAg[59]. Later on, a new modified HBV pre-S_1 protein gene was constructed and expressed by transgenic tomato plants[60]. The specific antigen expression from transgenic plants was proved by molecular techniques, including polymerase chain reaction (PCR) and reverse transcriptase PCR. Enzyme-linked immunoassays using a monoclonal antibody directed against human serum-derived HBsAg revealed that the highest amount of HBsAg was about 0.02% of the soluble protein in transgenic tomato fruit. The amount of HBsAg in mature fruit was found to be much more than that in small or medium fruit or leaf tissues. The ability of potato-derived HBV major surface antigen (P-HBsAg) that was orally administered to mice in different dosages (0.02 to 30 μg) to induce antibody responses was evaluated[61]. It was shown that all immunized mice developed specific serum IgG and fecal IgA antibodies against P-HBsAg even at low levels (< 5 μg), comparable to development of serum IgG anti-HBs following administration of a 0.5-μg yeast-derived HBsAg. Recently, a plant-derived prototype oral tri-component vaccine against HBV was evaluated for the potential of M/L-HBsAg expression in leaf tissue and the conditions of its processing for a prototype oral vaccine were assessed. Tobacco and lettuce carrying M- or L-HBsAg genes and resistant to the herbicide glufosinate were engineered and integration of the HBV-DNA transgenes was evaluated by PCR and Southern hybridizations[62].

Currently, several experimental trials for developing virus vector HBV vaccines are ongoing. Following a single immunization in mice, a recombinant vesicular stomatitis virus-based vaccine vector expressing the HBV pre-S_2 protein was able to efficiently promote a strong HBs-specific antibody response. It also developed robust CD8 T-cell activation, where response was broader in specificity and greater in magnitude than that acquired by a vaccinia virus-based vaccine vector or by recombinant protein immunization[63]. In addition, it was found that recombinant lentivectors could induce strong HBV HBsAg specific T cell responses and humoral immune responses. The HBS-Fc-lv lentivector could effectively break immune tolerance and elucidate strong HBsAg specific adaptive immune responses in HBsAg transgenic (Tg) mice with low serum level of HBsAg. The induction of HBsAg specific immune responses in TG mice accompanied seroconversion from HBsAg to anti-HBsAb[64]. Recently, Song *et al*[65] investigated the generation of recombinant influenza viruses that had

HBV B cell epitopes in its neuraminidase stalk region as a dual vaccine candidate against both HBV and influenza viruses. They successfully generated a chimeric influenza virus which contained 22 amino acid peptides derived from the surface and pre-surface HBV protein as foreign antigens[65].

Universal childhood immunization in the first year of life with three doses of HBV vaccine is a highly effective way for control and prevention of HBV infection[66]. Prevention of prenatal HBV infection is achieved by active and passive HBV immunization after birth as an intervention for preventing mother-to-child transmission of the HBV infection. A recent Taiwanese study was conducted in 2013 to compare the cost-effectiveness of strategies to control HBV that combine universal vaccination with hepatitis B immunoglobulin (HBIG) for neonates of carrier mothers. It was found that HBIG additional treatment to universal vaccination is likely to be cost-effective, particularly in settings with available healthcare infrastructure. Targeting HBIG in neonates of higher risk HBeAg-positive mothers may be preferred where willingness-to-pay is moderate. However, in very resource-limited settings, universal vaccination only is adequate[67]. CDC recommends vaccination of adults at high risk for infection, including dialysis patients, recipients of certain blood products, healthcare workers, household contacts and sex partners of persons with chronic HBV infection, those with a recent history of multiple sex partners, those with a sexually transmitted disease, IDUs and MSM[16].

Following vaccination, testing for antibodies is not needed for healthy people; however, it should be evaluated following vaccination in hemodialysis patients, those at occupational risk of infection, babies born to HBsAg-positive mothers, those with a family history of HBV carriers and human immunodeficiency virus positive people[66]. Long-term protection against HBV infection depends on the persistence of strong immunological memory. There is no need for boosters in immune competent individuals who have finished their vaccination course properly according to the recommended timelines. This was demonstrated in several studies conducted up to 20 years following the original immunization course. However, a booster dose is recommended for immunocompromised individuals based on serological monitoring[68].

The ACIP[69], the United States government, recommends that all children receive a birth dose of HBV vaccine and complete the vaccine series by 6-18 mo of age. Older children and adolescents who did not previously receive the HBV vaccine have to be vaccinated. A titer of anti-HBs antibodies to HBVsAg $\geqslant$ 10 IU/L is the marker of seroconversion to anti-HBs positivity following vaccination. It was found that the mean antibody level decreased significantly with increasing age[70,71]. Long-term follow-up studies have demonstrated that 10% to 50% of infants with seroprotective levels post third dose of vaccination had low or undetectable levels of antibody 5-15 years later[72]. Long-term protection due to persistent memory is obvious from quick rises in antibody after booster vaccination, even in those who have lost their anti-HBs. Recently, we conducted an Egyptian study aimed at estimating the seroprotection rate and evaluating the immune response to a booster dose in children and adolescents with an age range of 9 mo to 16 years who completed HBV vaccination during infancy. It was concluded from this Egyptian study that, in spite of the significant decline of level of antibodies over time, about half of the studied children have a seroprotective level of antibodies after primary compulsory vaccination. Moreover, the developed anamnestic response among children with a non-seroprotective level confirms immunological memory that can outlast the presence of protective level of antibodies[73]. Werner *et al*[74] found that HBsAg vaccine-induced immunity protects against new infection but does not induce sterilizing immunity in vaccinated healthcare workers with occupational exposure to HBV, as evidenced by detection of HBcore- and polymerase-specific CD8 (+) T cells[74].

According to current CDC recommendations[75-77], all healthcare providers and students should receive a 3 dose series of HBV vaccine followed by assessment of HBsAb to determine vaccination immunogenicity. Revaccination should be provided if indicated. Following revaccination (receiving a total of 6 doses), healthcare providers whose anti-HBs concentration is still not protective (< 10 mIU/mL) should be evaluated for HBsAg and anti-HBc to determine their infection status[54].

When considering offering a booster dose of the vaccine, Fitzsimons *et al*[78] recommended that vaccinees who were not tested for anti-HBs antibody one month following vaccination or those who have undetectable anti-HBs antibodies when tested should be potential recipients of it. Long-term protection is usually evaluated by 4 methods: the anamnestic response following administration of a booster dose, *in vitro* B and T cell activity evaluation, infection rate in vaccinated populations and seroepidemiological studies[68]. Estimation of the incidence of break-through infection (positive anti-HBc), as well as chronic carrier state (positive HBsAg) among previously vaccinated individuals, is used to determine the long-term protection provided by the HBV vaccine[79]. Many factors are related to HBV vaccination non response, including age above 40 years, male gender, impaired vaccine storage conditions, administration of the vaccine in buttocks, infections, obesity, drug abuse, smoking, chronic kidney/liver diseases, human immunodeficiency virus infection, celiac disease, thalassemia, type Ⅰ diabetes mellitus, Down's syndrome and other forms of mental retardation that are characterized by a poorer response than healthy subjects to HBV vaccination[66,80]. It has been shown that development of anti-HBs in hemodialysis patients is associated with gene polymorphisms of interleukins involved in the Th1 system[81]. Also, it was found that the administration of HBV recombinant vaccine by the intradermal route is very effective and could be a more useful strategy and an alternative to

conventional intramuscular vaccine in all non-responder patients[80]. As obesity is considered to be a major cause of decreasing the rate of antibody production by the HBV vaccine, recently it was reported that antibody titers can be raised in vaccinating obese youth by using long needles[82].

Therapeutic HBV vaccine: HBV vaccines have been recently identified as a promising therapeutic strategy for treatment and control HBV infection in HBV carriers and persistently infected patients[39,83]. Clinical trials of its therapeutic use in chronic HBV infection rely on using the conventional sAg based HBV vaccine. Specific treatment by the standard anti-HBV vaccine is effective in decreasing the replication of HBV and inhibiting the immune tolerance to HBsAg protein in about 50% of chronic active HBV patients[84]. However, it is well recognized that monotherapy with HBsAg-based immune therapy cannot lead to sustained control of HBV replication and/or liver damage[85]. New therapy strategies are currently shown to provide potent and durable antiviral immune responses in patients who can maintain long-term control of HBV replication[83]. Recent research concentrates on the clinical use of combined HBsAg- and HBcAg-based vaccines in CHB patients[85].

Using a duck HBV model, therapeutic DNA vaccination was proven to be able to enhance hepadnavirus intrahepatic covalently closed circular DNA clearance[86]. It was shown in humans that HBV DNA vaccination can specifically but transiently activate T-cell responses in some chronic HBV carriers who showed no response to the available HBV antiviral therapies[53]. Obeng-Adjei *et al*[11] evaluated the use of multivalent synthetic plasmids against HBV consensus core (HBc) and surface (HBs) antigens genotypes A and C for their immune potential in animal models and they found that it induced binding antibodies to HBsAg and robust cell-mediated immunity. The same responses to both HBc and HBs antigens were demonstrated by inoculation of HBc-HBs cocktails in mice and non-human primates. Besides the cytotoxic T-lymphocyte activities exhibited by the immunized mice, the vaccine-induced responses were broadly distributed across multiple antigenic epitopes[11]. Immune therapy with HBV-related antigens (HBsAg-based vaccine) has been used in CHB patients as a combination therapy with cytokines, growth factors and antiviral drugs, but proper designs of antigens, types of adjuvant, dose of vaccinations and routes of administration need further analyses for the development of an effective protocol of immune therapy for HBV infection[85]. GS-4774 is a safe and well-tolerated recombinant, heat-killed, yeast-based immunotherapy engineered to express HBV (HBV)-specific antigens and it is a promising therapeutic vaccine for chronic HBV infection[87]. Intensive research is currently concentrated on a better understanding of immune responses in hepatocytes, on mechanisms by which HBV evades innate immunity and on proper selection of patients susceptible to benefit from immune therapy, which could increase the efficacy of therapeutic vaccination[88].

What is the future HBV epidemiology after universal mass vaccination?

In 1992, the World Health Organization recommended that all countries integrate the hepatitis B vaccine into their childhood national immunization programs. By 2012, 181 countries implemented this vaccine in their national immunization program, with the global hepatitis B vaccine coverage estimated at 79%. 119 countries have reported > 90% vaccination coverage with consequent decrease in the prevalence of chronic HBV infection in children born since the r-HBsAg vaccine was included in infant immunization schedules[5]. It is well recognized that the use of highly immunogenic HBV vaccines produces long-lasting immunity. Some of the major challenges facing current HBV vaccines have been their inability to induce both humoral and cellular immunity to multiple antigenic targets and the induction of potent immune responses against the major genotypes of HBV[11].

The efficacy of universal immunization has been demonstrated in many countries, with a prominent decrease in the prevalence of HBV carriage in children. Moreover, HBV vaccination can protect children against fulminant hepatitis and HCC[89]. The success of the vaccination programs has also now been challenged by the discovery of mutant viruses showing amino acid substitutions in HBsAg, which may lead to evasion of vaccine-induced immunity[90]. Induction of immune escape mutants is one of the unwanted effects of the widely used HBV vaccine. It was first described in 1990 by Carman *et al*[91] when they observed the acquiring of HBV infection in 44 contacts of HBV carriers despite passive and active immunization according to the implemented standard schedules. There was partial loss of the common "a" determinant to which the vaccine-induced immunity is mainly directed. Globally, several S mutations that are potentially able to evade neutralizing anti-HBs immune response and thus infect vaccinated individuals have been recognized[40]. Inclusion of $Pres_2$ and $Pre\text{-}S_1$ epitopes may be recommended in order to reduce the emergence of such vaccine escape mutants. Meanwhile, it was shown that selection of pre-S/S mutants may demonstrate a relevant pathobiological and clinical impact. HBV mutants with an antigenically modified surface antigen may be potentially infectious for immune-protected patients and may account for those with occult HBV infection[92]. On the other hand, Romanò *et al*[40] confirmed that the overall effect of vaccine escape mutants is likely to be low and thus does not cause a public health threat or a need to modify the implemented HBV vaccination programs.

The effectiveness of universal hepatitis B vaccination is promising, although the coverage of vaccination varies between countries. The inclusion of r-HBsAg vaccine in the expanded programs of childhood vaccination had a major impact on protection against the disease and its complications and was successful in almost

eliminating childhood chronic HBV infection. However, this recombinant HBV yeast derived vaccine has a number of limitations that justify the development of new HBV vaccines: the need for multiple doses, lack of long-lasting immunity, incomplete protection in all vaccinees where a group of non-responders do exist and it is therapeutically ineffective[45,63].

REFERENCES

1 **Blumberg BS**, Alter HJ, Visnich S. A "new" antigen in leukemia sera. *JAMA* 1965; **191**: 541-546 [PMID: 14239025 DOI: 10.1001/jama.1965.03080070025007]

2 **Simmonds P**, Midgley S. Recombination in the genesis and evolution of hepatitis B virus genotypes. *J Virol* 2005; **79**: 15467-15476 [PMID: 16306618 DOI: 10.1128/JVI.79.24.15467-15476.2005]

3 **Dane DS**, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet* 1970; **1**: 695-698 [PMID: 4190997]

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

5 **World Health Organization**. Global policy report on the prevention and control of viral hepatitis. In WHO Member States. [Accessed 2013 July]. Available from: URL: http://www.who.int/csr/disease/hepatitis/global_report/en/

6 **World Health Organization.** Hepatitis B Fact sheet N°204. [Accessed 2015 March]. Available from: URL: http://www.who.int/mediacentre/factsheets/fs204/en/

7 **Lai M**, Liaw YF. Chronic hepatitis B: past, present, and future. *Clin Liver Dis* 2010; **14**: 531-546 [PMID: 20638030 DOI: 10.1016/j.cld.2010.05.003]

8 **Ganem D**, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118-1129 [PMID: 15014185 DOI: 10.1056/NEJMra031087]

9 **Bertoletti A**, Gehring AJ. The immune response during hepatitis B virus infection. *J Gen Virol* 2006; **87**: 1439-1449 [PMID: 16690908 DOI: 10.1099/vir.0.81920-0]

10 **Weber B**. Genetic variability of the S gene of hepatitis B virus: clinical and diagnostic impact. *J Clin Virol* 2005; **32**: 102-112 [PMID: 15653412 DOI: 10.1016/j.jcv.2004.10.008]

11 **Obeng-Adjei N**, Hutnick NA, Yan J, Chu JS, Myles DJ, Morrow MP, Sardesai NY, Weiner DB. DNA vaccine cocktail expressing genotype A and C HBV surface and consensus core antigens generates robust cytotoxic and antibody responses in mice and Rhesus macaques. *Cancer Gene Ther* 2013; **20**: 652-662 [PMID: 24310062 DOI: 10.1038/cgt.2013.65]

12 **Cova L**. Advances and challenges in the development of therapeutic DNA vaccines against hepatitis B virus infection. *Curr Gene Ther* 2014; **14**: 149-160 [PMID: 24828255 DOI: 10.2174/1566523214666140509102644]

13 **Heathcote EJ**. Demography and presentation of chronic hepatitis B virus infection. *Am J Med* 2008; **121**: S3-S11 [PMID: 19185072 DOI: 10.1016/j.amjmed.2008.09.024]

14 **Ott JJ**, Stevens GA, Wiersma ST. The risk of perinatal hepatitis B virus transmission: hepatitis B e antigen (HBeAg) prevalence estimates for all world regions. *BMC Infect Dis* 2012; **12**: 131 [PMID: 22682147 DOI: 10.1186/1471-2334-12-131]

15 **Centers for Disease Control and Prevention (CDC)**. The ABCs of Hepatitis Fact Sheet. [Updated 2014]. Available from: URL: http://www.cdc.gov/hepatitis/Resources/Professionals/PDFs/ABCTable.pdf

16 **Wright TL**, Lau JY. Clinical aspects of hepatitis B virus infection. *Lancet* 1993; **342**: 1340-1344 [PMID: 7694023 DOI: 10.1016/0140-6736(93)92250-W]

17 **Hoofnagle JH**. Serologic markers of hepatitis B virus infection. *Annu Rev Med* 1981; **32**: 1-11 [PMID: 7013654 DOI: 10.1146/annurev.me.32.020181.000245]

18 **Yim HJ**, Lok AS. Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology* 2006; **43**: S173-S181 [PMID: 16447285 DOI: 10.1002/hep.20956]

19 **Bertoletti A**, Gehring A. Immune response and tolerance during chronic hepatitis B virus infection. *Hepatol Res* 2007; **37** Suppl 3: S331-S338 [PMID: 17931183 DOI: 10.1111/j.1872-034X.2007.00221.x]

20 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174 [PMID: 12124405 DOI: 10.1056/NEJMoa013215]

21 **Fisicaro P**, Valdatta C, Boni C, Massari M, Mori C, Zerbini A, Orlandini A, Sacchelli L, Missale G, Ferrari C. Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. *Gut* 2009; **58**: 974-982 [PMID: 19201769 DOI: 10.1136/gut.2008.163600]

22 **Zhang ZH**, Zhao XP, Xia JB, Li X, Lu MJ, Yang DL. [Characterization of HBsAbs in occult hepatitis B virus infection]. *Zhonghua Gan Zang Bing Za Zhi* 2011; **19**: 506-510 [PMID: 22152241 DOI: 10.3760/cma.j.issn.1007-3418.2011.07.010]

23 **Cheng PN**, Tsai HW, Chiu YC, Ho CH, Wu IC, Chang TT. Clinical significance of serum HBsAg levels and association with liver histology in HBeAg positive chronic hepatitis B. *J Clin Virol* 2013; **57**: 323-330 [PMID: 23731848 DOI: 10.1016/j.jcv.2013.04.012]

24 **Fong TL**, Di Bisceglie AM, Biswas R, Waggoner JG, Wilson L, Claggett J, Hoofnagle JH. High levels of viral replication during acute hepatitis B infection predict progression to chronicity. *J Med Virol* 1994; **43**: 155-158 [PMID: 8083663 DOI: 10.1002/jmv.1890430210]

25 **Guidotti LG**, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells

during acute HBV infection. *Science* 1999; **284**: 825-829 [PMID: 10221919 DOI: 10.1126/science.284.5415.825]
26 **Thimme R**, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, Chisari FV. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J Virol* 2003; **77**: 68-76 [PMID: 12477811 DOI: 10.1128/JVI.77.1.68-76.2003]
27 **Murray PJ**, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* 2011; **11**: 723-737 [PMID: 21997792 DOI: 10.1038/nri3073]
28 **Wieland S**, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci USA* 2004; **101**: 6669-6674 [PMID: 15100412 DOI: 10.1073/pnas.0401771101]
29 **Phillips S**, Chokshi S, Riva A, Evans A, Williams R, Naoumov NV. CD8(+) T cell control of hepatitis B virus replication: direct comparison between cytolytic and noncytolytic functions. *J Immunol* 2010; **184**: 287-295 [PMID: 19949099 DOI: 10.4049/jimmunol.0902761]
30 **Stoop JN**, van der Molen RG, Baan CC, van der Laan LJ, Kuipers EJ, Kusters JG, Janssen HL. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. *Hepatology* 2005; **41**: 771-778 [PMID: 15791617 DOI: 10.1002/hep.20649]
31 **Penna A**, Chisari FV, Bertoletti A, Missale G, Fowler P, Giuberti T, Fiaccadori F, Ferrari C. Cytotoxic T lymphocytes recognize an HLA-A2-restricted epitope within the hepatitis B virus nucleocapsid antigen. *J Exp Med* 1991; **174**: 1565-1570 [PMID: 1720813 DOI: 10.1084/jem.174.6.1565]
32 **Jiang M**, Broering R, Trippler M, Poggenpohl L, Fiedler M, Gerken G, Lu M, Schlaak JF. Toll-like receptor-mediated immune responses are attenuated in the presence of high levels of hepatitis B virus surface antigen. *J Viral Hepat* 2014; **21**: 860-872 [PMID: 24498958 DOI: 10.1111/jvh.1221]
33 **Rehermann B**, Fowler P, Sidney J, Person J, Redeker A, Brown M, Moss B, Sette A, Chisari FV. The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. *J Exp Med* 1995; **181**: 1047-1058 [PMID: 7532675 DOI: 10.1084/jem.181.3.1047]
34 **Desmond CP**, Gaudieri S, James IR, Pfafferott K, Chopra A, Lau GK, Audsley J, Day C, Chivers S, Gordon A, Revill PA, Bowden S, Ayres A, Desmond PV, Thompson AJ, Roberts SK, Locarnini SA, Mallal SA, Lewin SR. Viral adaptation to host immune responses occurs in chronic hepatitis B virus (HBV) infection, and adaptation is greatest in HBV e antigen-negative disease. *J Virol* 2012; **86**: 1181-1192 [PMID: 22072755]
35 **Lyseng-Williamson KA**, Dhillon S. DTPa-HBV-IPV/Hib vaccine (Infanrix hexa™): a guide to its use in infants. *Paediatr Drugs* 2012; **14**: 337-343 [PMID: 22873778 DOI: 10.2165/11210000-000000000-00000]
36 **Lavanchy D**. Viral hepatitis: global goals for vaccination. *J Clin Virol* 2012; **55**: 296-302 [PMID: 22999800 DOI: 10.1016/j.jcv.2012.08.022]
37 **Mackie CO**, Buxton JA, Tadwalkar S, Patrick DM. Hepatitis B immunization strategies: timing is everything. *CMAJ* 2009; **180**: 196-202 [PMID: 19153395]
38 **Centers for Disease Control and Prevention (CDC)**. Updated CDC recommendations for the management of hepatitis B virus-infected health-care providers and students. *MMWR Recomm Rep* 2012; **61**: 1-12 [PMID: 22763928]
39 **Michel ML**, Tiollais P. Hepatitis B vaccines: protective efficacy and therapeutic potential. *Pathol Biol* (Paris) 2010; **58**: 288-295 [PMID: 20382485 DOI: 10.1016/j.patbio.2010.01.006]
40 **Romanò L,** Paladini S, Galli C, Raimondo G, Pollicino T, Zanetti AR. Hepatitis B vaccination: are escape mutant viruses a matter of concern? *Hum Vaccin Immunother* 2015; **11**: 53-57 [DOI: 10.4161/hv.34306]
41 **West DJ**, Calandra GB. Vaccine induced immunologic memory for hepatitis B surface antigen: implications for policy on booster vaccination. *Vaccine* 1996; **14**: 1019-1027 [PMID: 8879096 DOI: 10.1016/0264-410X(96)00062-X]
42 **Banatvala J**, Van Damme P, Oehen S. Lifelong protection against hepatitis B: the role of vaccine immunogenicity in immune memory. *Vaccine* 2000; **19**: 877-885 [PMID: 11115711 DOI: 10.1016/S0264-410X(00)00224-3]
43 **Shouval D**, Ilan Y, Adler R, Deepen R, Panet A, Even-Chen Z, Gorecki M, Gerlich WH. Improved immunogenicity in mice of a mammalian cell-derived recombinant hepatitis B vaccine containing pre-S1 and pre-S2 antigens as compared with conventional yeast-derived vaccines. *Vaccine* 1994; **12**: 1453-1459 [PMID: 7533967 DOI: 10.1016/0264-410X(94)90155-4]
44 **Wolters B**, Müller T, Ross RS, Clauberg R, Werfel U, Roggendorf H, Siggelkow C, Hausen T, Roggendorf M. Comparative evaluation of the immunogenicity of combined hepatitis A and B vaccine by a prospective and retrospective trial. *Hum Vaccin* 2009; **5**: 248-253 [PMID: 19276678 DOI: 10.4161/hv.5.4.7051]
45 **Shouval D**. Hepatitis B vaccines. *J Hepatol* 2003; **39** Suppl 1: S70-S76 [PMID: 14708681 DOI: 10.1016/S0168-8278(03)00152-1]
46 **Mahmood K**, Pelkowski S, Atherly D, Sitrin RD, Donnelly JJ. Hexavalent IPV-based combination vaccines for public-sector markets of low-resource countries. *Hum Vaccin Immunother* 2013; **9**: 1894-1902 [PMID: 23787559 DOI: 10.4161/hv.25407]
47 **Nolan T**, Lambert S, Roberton D, Marshall H, Richmond P, Streeton C. DTPa-HBV-IPV vaccine for primary vaccination of infants. *J Paediatr Child Health* 2007; **43**: 587-592 [PMID: 17688642 DOI: 10.1111/j.1440-1754.2007.01139.x]
48 **Shaw FE**, Graham DJ, Guess HA, Milstien JB, Johnson JM, Schatz GC, Hadler SC, Kuritsky JN, Hiner EE, Bregman DJ. Postmarketing surveillance for neurologic adverse events reported after hepatitis B vaccination. Experience of the first three years. *Am J Epidemiol* 1988; **127**: 337-352 [PMID: 2962488]
49 **McMahon BJ**, Helminiak C, Wainwright RB, Bulkow L, Trimble BA, Wainwright K. Frequency of adverse reactions to hepatitis B vaccine in 43,618 persons. *Am J Med* 1992; **92**: 254-256 [PMID: 1532114 DOI: 10.1016/0002-9343(92)90073-K]
50 **Zhang HZ**, Wu WS, Su F, Sun CM, Jiang MB, Zhang GH, Wang XY, Xu ZY. [Valuation on the immunization efficacy on the 23 years who had received plasma-derived HBV vaccine as newborns]. *Zhonghua Liuxingbingxue Zazhi* 2012; **33**: 207-209 [PMID: 22575145]
51 **Shen L**, Wang F, Wang F, Cui F, Zhang S, Zheng H, Zhang Y, Liang X, Bi S. Efficacy of yeast-derived recombinant hepatitis B vaccine after being used for 12 years in highly endemic areas in China. *Vaccine* 2012; **30**: 6623-6627 [PMID: 22959987 DOI: 10.1016/j.vaccine.2012.08.067]
52 **Neurath AR**, Kent SB, Parker K, Prince AM, Strick N, Brotman B, Sproul P. Antibodies to a synthetic peptide from the preS 120-145 region of the hepatitis B virus envelope are virus neutralizing. *Vaccine* 1986; **4**: 35-37 [PMID: 2421497 DOI: 10.1016/S0264-410X(86)80001-9]
53 **Mancini-Bourgine M**, Fontaine H, Scott-Algara D, Pol S, Bréchot C, Michel ML. Induction or expansion of T-cell responses by a hepatitis B DNA vaccine administered to chronic HBV carriers. *Hepatology* 2004; **40**: 874-882 [PMID: 15382173 DOI: 10.1002/hep.20408]
54 **Rottinghaus ST**, Poland GA, Jacobson RM, Barr LJ, Roy MJ. Hepatitis B DNA vaccine induces protective antibody responses in human non-responders to conventional vaccination. *Vaccine* 2003; **21**: 4604-4608 [PMID: 14575774 DOI: 10.1016/S0264-410X(03)00447-X]
55 **Davis HL**, McCluskie MJ, Gerin JL, Purcell RH. DNA vaccine for hepatitis B: evidence for immunogenicity in chimpanzees and comparison with other vaccines. *Proc Natl Acad Sci USA* 1996; **93**: 7213-7218 [PMID: 8692971 DOI: 10.1073/pnas.93.14.7213]
56 **Du DW**, Jia ZS, Li GY, Zhou YY. HBV DNA vaccine with adjuvant cytokines induced specific immune responses against HBV infection. *World J Gastroenterol* 2003; **9**: 108-111 [PMID: 12508362]
57 **Wang J**, Zhu R, Gao B, Wu B, Li K, Sun X, Liu H, Wang S. The enhanced immune response of hepatitis B virus DNA vaccine using

SiO2@LDH nanoparticles as an adjuvant. *Biomaterials* 2014; **35**: 466-478 [PMID: 24099705 DOI: 10.1016/j.biomaterials.2013.09.060]

58 **Thanavala Y**, Yang YF, Lyons P, Mason HS, Arntzen C. Immunogenicity of transgenic plant-derived hepatitis B surface antigen. *Proc Natl Acad Sci USA* 1995; **92**: 3358-3361 [PMID: 7724566 DOI: 10.1073/pnas.92.8.3358]

59 **Kapusta J**, Modelska A, Figlerowicz M, Pniewski T, Letellier M, Lisowa O, Yusibov V, Koprowski H, Plucienniczak A, Legocki AB. A plant-derived edible vaccine against hepatitis B virus. *FASEB J* 1999; **13**: 1796-1799 [PMID: 10506582]

60 **Lou XM**, Yao QH, Zhang Z, Peng RH, Xiong AS, Wang HK. Expression of the human hepatitis B virus large surface antigen gene in transgenic tomato plants. *Clin Vaccine Immunol* 2007; **14**: 464-469 [PMID: 17314228]

61 **Youm JW**, Won YS, Jeon JH, Moon KB, Kim HC, Shin KS, Joung H, Kim HS. Antibody responses in mice stimulated by various doses of the potato-derived major surface antigen of hepatitis B virus. *Clin Vaccine Immunol* 2010; **17**: 2029-2032 [PMID: 20943879]

62 **Pniewski T**, Kapusta J, Bociąg P, Kostrzak A, Fedorowicz-Strońska O, Czyż M, Gdula M, Krajewski P, Wolko B, Płucienniczak A. Plant expression, lyophilisation and storage of HBV medium and large surface antigens for a prototype oral vaccine formulation. *Plant Cell Rep* 2012; **31**: 585-595 [PMID: 22246107 DOI: 10.1007/s00299-011-1223-7]

63 **Cobleigh MA**, Buonocore L, Uprichard SL, Rose JK, Robek MD. A vesicular stomatitis virus-based hepatitis B virus vaccine vector provides protection against challenge in a single dose. *J Virol* 2010; **84**: 7513-7522 [PMID: 20504927 DOI: 10.1128/JVI.00200-10]

64 **Hong Y**, Peng Y, Mi M, Xiao H, Munn DH, Wang GQ, He Y. Lentivector expressing HBsAg and immunoglobulin Fc fusion antigen induces potent immune responses and results in seroconversion in HBsAg transgenic mice. *Vaccine* 2011; **29**: 3909-3916 [PMID: 21421003 DOI: 10.1016/j.vaccine.2011.03.025]

65 **Song JM**, Lee KH, Seong BL. Recombinant influenza viruses as delivery vectors for hepatis B virus epitopes. *Clin Exp Vaccine Res* 2012; **1**: 77-82 [PMID: 23596580 DOI: 10.7774/cevr.2012.1.1.77]

66 **Kwon SY**, Lee CH. Epidemiology and prevention of hepatitis B virus infection. *Korean J Hepatol* 2011; **17**: 87-95 [PMID: 21757978 DOI: 10.3350/kjhep.2011.17.2.87]

67 **Chen SC**, Toy M, Yeh JM, Wang JD, Resch S. Cost-effectiveness of augmenting universal hepatitis B vaccination with immunoglobin treatment. *Pediatrics* 2013; **131**: e1135-e1143 [PMID: 23530168 DOI: 10.1542/peds.2012-1262]

68 **Leuridan E**, Van Damme P. Hepatitis B and the need for a booster dose. *Clin Infect Dis* 2011; **53**: 68-75 [PMID: 21653306 DOI: 10.1093/cid/cir270]

69 **Centers for Disease Control and Prevention (CDC)**. A comprehensive immunization strategy to eliminate transmission of hepatitis B virusinfection in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP) Part 1: Immunization of Infants, Children, and Adolescents. Available from: URL: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5416a1.htm

70 **Shaaban FA**, Hassanin AI, Samy SM, Salama SI, Said ZN. Long-term immunity to hepatitis B among a sample of fully vaccinated children in Cairo, Egypt. *East Mediterr Health J* 2007; **13**: 750-757 [PMID: 17955755]

71 **Abushady EA**, Gameel MM, Klena JD, Ahmed SF, Abdel-Wahab KS, Fahmy SM. HBV vaccine efficacy and detection and genotyping of vaccineé asymptomatic breakthrough HBV infection in Egypt. *World J Hepatol* 2011; **3**: 147-156 [PMID: 21860674]

72 **Gilca V**, De Serres G, Boulianne N, De Wals P, Murphy D, Trudeau G, Massé R, Duval B. Antibody kinetics among 8-10 years old respondents to hepatitis B vaccination in a low endemic country and the effect of a booster dose given 5 or 10 years later. *Vaccine* 2009; **27**: 6048-6053 [PMID: 19683086 DOI: 10.1016/j.vaccine.2009.07.100]

73 **Salama II**, Sami SM, Salama SI, Foud WA, Abdel Hamid AT, Said ZN. Persistence of protection to hepatitis B vaccine and response to booster dose among children and adolescents in Dakahleya-Egypt. *Egypt J Immunol* 2014; **21**: 13-26 [PMID: 25204041]

74 **Werner JM**, Abdalla A, Gara N, Ghany MG, Rehermann B. The hepatitis B vaccine protects re-exposed health care workers, but does not provide sterilizing immunity. *Gastroenterology* 2013; **145**: 1026-1034 [PMID: 23916846 DOI: 10.1053/j.gastro.2013.07.044]

75 **Centers for Disease Control and Prevention (CDC)**. Immunization of health-care personnel: recommendations of the Advisory Committee on Immunization Practices (ACIP). [Accessed 2011 Nov 25]. Available from: URL: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6007a1.htm

76 **Centers for Disease Control and Prevention (CDC)**. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices. Recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: immunization of adults. [Accessed, 2006 Dec 8]. Available from: URL: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5516a1.htm

77 **Centers for Disease Control and Prevention (CDC)**. Recommended adult immunization schedule-United States-2015. Available from: URL: http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf

78 **Fitzsimons D**, François G, Hall A, McMahon B, Meheus A, Zanetti A, Duval B, Jilg W, Böcher WO, Lu SN, Akarca U, Lavanchy D, Goldstein S, Banatvala J, Damme PV. Long-term efficacy of hepatitis B vaccine, booster policy, and impact of hepatitis B virus mutants. *Vaccine* 2005; **23**: 4158-4166 [PMID: 15964484]

79 **Poorolajal J**, Mahmoodi M, Majdzadeh R, Nasseri-Moghaddam S, Haghdoost A, Fotouhi A. Long-term protection provided by hepatitis B vaccine and need for booster dose: a meta-analysis. *Vaccine* 2010; **28**: 623-631 [PMID: 19887132 DOI: 10.1016/j.vaccine.2009.10.068]

80 **Filippelli M**, Lionetti E, Gennaro A, Lanzafame A, Arrigo T, Salpietro C, La Rosa M, Leonardi S. Hepatitis B vaccine by intradermal route in non responder patients: an update. *World J Gastroenterol* 2014; **20**: 10383-10394 [PMID: 25132754 DOI: 10.3748/wjg.v20.i30.10383]

81 **Grzegorzewska AE**, Wobszal PM, Mostowska A, Jagodziński PP. Antibodies to hepatitis B virus surface antigen and interleukin 12 and interleukin 18 gene polymorphisms in hemodialysis patients. *BMC Nephrol* 2012; **13**: 75 [PMID: 22863216 DOI: 10.1186/1471-2369-13-75]

82 **Middleman AB**, Anding R, Tung C. Effect of needle length when immunizing obese adolescents with hepatitis B vaccine. *Pediatrics* 2010; **125**: e508-e512 [PMID: 20142295 DOI: 10.1542/peds.2009-1592]

83 **Liu J**, Kosinska A, Lu M, Roggendorf M. New therapeutic vaccination strategies for the treatment of chronic hepatitis B. *Virol Sin* 2014; **29**: 10-16 [PMID: 24452539 DOI: 10.1007/s12250-014-3410-5]

84 **Couillin I**, Pol S, Mancini M, Driss F, Bréchot C, Tiollais P, Michel ML. Specific vaccine therapy in chronic hepatitis B: induction of T cell proliferative responses specific for envelope antigens. *J Infect Dis* 1999; **180**: 15-26 [PMID: 10353856 DOI: 10.1086/314828]

85 **Akbar SM**, Al-Mahtab M, Uddin MH, Khan MS. HBsAg, HBcAg, and combined HBsAg/HBcAg-based therapeutic vaccines in treating chronic hepatitis B virus infection. *Hepatobiliary Pancreat Dis Int* 2013; **12**: 363-369 [PMID: 23924493]

86 **Thermet A**, Buronfosse T, Werle-Lapostolle B, Chevallier M, Pradat P, Trepo C, Zoulim F, Cova L. DNA vaccination in combination or not with lamivudine treatment breaks humoral immune tolerance and enhances cccDNA clearance in the duck model of chronic hepatitis B virus infection. *J Gen Virol* 2008; **89**: 1192-1201 [PMID: 18420797 DOI: 10.1099/vir.0.83583-0]

87 **Gaggar A**, Coeshott C, Apelian D, Rodell T, Armstrong BR, Shen G, Subramanian GM, McHutchison JG. Safety, tolerability and immunogenicity of GS-4774, a hepatitis B virus-specific therapeutic vaccine, in healthy subjects: a randomized study. *Vaccine* 2014; **32**:

4925-4931 [PMID: 25045824 DOI: 10.1016/j.vaccine.2014.07.027]
88 **Michel ML**, Bourgine M, Fontaine H, Pol S. Therapeutic vaccines in treating chronic hepatitis B: the end of the beginning or the beginning of the end? *Med Microbiol Immunol* 2015; **204**: 121-129 [PMID: 25511871]
89 **Kao JH**, Chen DS. Global control of hepatitis B virus infection. *Lancet Infect Dis* 2002; **2**: 395-403 [PMID: 12127351]
90 **Lazarevic I**. Clinical implications of hepatitis B virus mutations: recent advances. *World J Gastroenterol* 2014; **20**: 7653-7664 [PMID: 24976703 DOI: 10.3748/wjg.v20.i24.7653]
91 **Carman WF**, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; **336**: 325-329 [PMID: 1697396]
92 **Pollicino T**, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. *J Hepatol* 2014; **61**: 408-417 [PMID: 24801416 DOI: 10.1016/j.jhep.2014.04.041]

P- Reviewer: Kaplan DE, Luo GH, Novo E **S- Editor**: Tian YL
L- Editor: Roemmele A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1671

World J Hepatol 2015 June 28; 7(12): 1671-1678
ISSN 1948-5182 (online)

Occult hepatitis B virus infection in Egypt

Ashraf Elbahrawy, Alshimaa Alaboudy, Walid El Moghazy, Ahmed Elwassief, Ahmed Alashker, Abdallah Mahmoud Abdallah

Ashraf Elbahrawy, Ahmed Elwassief, Ahmed Alashker, Abdallah Mahmoud Abdallah, Department of Internal Medicine, Al-Azhar School of Medicine, Al-Azhar University, Cairo 11884, Egypt
Alshimaa Alaboudy, Department of Gastroenterology and Hepatology, University of Alberta, Alberta T6G 2R3, Canada
Alshimaa Alaboudy, Department of Tropical Medicine and Gastroenterology, Sohag University, Sohag 11432, Egypt
Walid El Moghazy, Department of HPB Surgery and Transplantation, University of Alberta, Alberta T6G 2R3, Canada
Walid El Moghazy, Department of Surgery, Sohag University, Sohag 11432, Egypt

Author contributions: Elbahrawy A, Alaboudy A, El Moghazy W and Elwassief A designed and wrote the review; Alashker A and Abdallah AM contributed to analysis and interpretation of data.

Conflict-of-interest: None to declare.

Correspondence to: Ashraf Elbahrawy, Assistant Professor, Department of Internal Medicine, Al-Azhar School of Medicine, Al-Azhar University, Nasr City, Cairo 11884, Egypt. bahrawy3@hotmail.com
Telephone: +20-2-25109140
Fax: +20-2-25109140

Received: November 29, 2014
Peer-review started: December 2, 2014
First decision: January 8, 2015
Revised: January 27, 2015
Accepted: May 8, 2015
Article in press: May 11, 2015
Published online: June 28, 2015

Abstract

The emerging evidence of the potentially clinical importance of occult hepatitis B virus (HBV) infection (OBI) increases the interest in this topic. OBI may impact in several clinical contexts, which include the possible transmission of the infection, the contribution to liver disease progression, the development of hepatocellular carcinoma, and the risk of reactivation. There are several articles that have published on OBI in Egyptian populations. A review of MEDLINE database was undertaken for relevant articles to clarify the epidemiology of OBI in Egypt. HBV genotype D is the only detectable genotype among Egyptian OBI patients. Higher rates of OBI reported among Egyptian chronic HCV, hemodialysis, children with malignant disorders, and cryptogenic liver disease patients. There is an evidence of OBI reactivation after treatment with chemotherapy. The available data suggested that screening for OBI must be a routine practice in these groups of patients. Further studies needed for better understand of the epidemiology of OBI among Egyptian young generations after the era of hepatitis B vaccination.

Key words: Hepatitis B virus; Occult hepatitis B virus infection; Hepatitis C virus; Egypt; Blood donors; Hemodialysis; Hepatitis B virus reactivation

Core tip: Hepatitis B virus (HBV) genotype D is the only detectable genotype among Egyptian occult HBV infection (OBI) patients. Higher rates of OBI reported among Egyptian chronic HCV, hemodialysis, children with malignant disorders, and cryptogenic liver disease patients. There is an evidence of OBI reactivation after treatment with chemotherapy. The available data suggested that screening for OBI must be a routine practice in these groups of patients. Further studies are needed to understand the epidemiology of OBI among

Egyptian young generations after the era of hepatitis B vaccination.

Elbahrawy A, Alaboudy A, El Moghazy W, Elwassief A, Alashker A, Abdallah AM. Occult hepatitis B virus infection in Egypt. *World J Hepatol* 2015; 7(12): 1671-1678 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1671.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1671

INTRODUCTION

The detection of hepatitis B virus (HBV) nucleic acid in the blood or liver of hepatitis B surface antigen (HBsAg) negative patients is called occult HBV infection (OBI). The molecular basis of OBI usually attributed to the long-term persistence viral covalently-closed-circular DNA in the nuclei of the hepatocytes[1,2]. The majority of the OBI cases infected with replication-competent HBV showing strong suppression of replication and gene expression[1]. The causes of this suppression had not yet clarified, although host immune response and epigenetic factors may play crucial roles. As a consequence of HBV suppression, the viral load is very low (usually below 200 IU/mL) or even undetectable in OBI cases[1].

Patients with OBI can be either seropositive or seronegative. Seropositive OBI is characterized by positivity of anti-hepatitis B core antibody (anti-HBc) with or without the presence of anti-hepatitis B surface antibody (anti-HBs) while the seronegative OBI s characterized by the negativity of both antibodies. Sero-positive OBI constitutes the vast majority of OBI which can be quite explained by the larger proportion of resolved HBV infection[3]. More than 20% of the occult carriers are seronegative for all HBV markers[1,3]. Whether OBI-seronegative persons lack circulating antibodies due to the progressive antibodies disappearance in the years after acute infection resolution or it occurs from the beginning of HBV infection is unknown[1,4]. Similarly, the difference in terms of clinical impact between OBI-seropositive and OBI-seronegative individuals is entirely obscure. What is known that OBI shows long-lasting specific T-cell immune response against HBV epitopes with a profile that is different between these two subsets of individuals? In fact, while *ex vivo* responses are similarly weak, *in vitro* T-cell expansion following specific peptide stimulation is more efficient among seropositive OBI cases than seronegative OBI cases[1,5].

The only reliable diagnostic marker of OBI is HBV-DNA detection. No standardized assays for the detection of OBI in liver tissue are available[1]. It is strongly recommended to utilize a highly sensitive[1] and specific approach, for both blood and liver analysis based on "nested" or "real time" polymerase chain reaction (PCR) techniques. In addition, the use of primers specific for different HBV genomic regions and complementary to highly conserved (genotype shared) nucleotide sequences highly suggested[1]. Anti-HBc should be used, as a less than ideal surrogate marker if highly sensitive HBV-DNA testing is not feasible[1,6].

Worldwide, the prevalence of OBI is quite variable[7]. This variability depends on the sensitivity of the HBV DNA detection assay, the sample size, and whether it tested in the liver or the serum[8,9]. There is a growing evidence of a positive correlation between prevalence of OBI and the endemicity of HBV infection[1,3]. HCV infected patients appear to be a category of individuals with a higher prevalence of occult HBV[1,3]. In particular, HBV-DNA is detectable in about one-third of HBsAg-negative HCV carriers in the Mediterranean countries[1,3]. In addition, it was suggested that OBI is highly prevalent in HCV-infected patients with the advanced liver disease even in areas with less HBV spread[1]. Prevalence of OBI appears to be fairly elevated even in patients with the cryptogenic liver disease, particularly in those with cirrhosis[1]. Among blood donors, OBI appears to be a rare occurrence in the western world. It is a frequent incident in the developing countries[1]. OBI rate in hepatitis B (HB)-vaccinated children varies in different risk groups, according to the local incidence of HBV, and irrespective[10] to anti-HBs sero-status.

The emerging evidence of the potentially considerable clinical importance of occult HBV infection is the main reason for increasing interest in this topic[11]. OBI may impact in several clinical contexts. The possible transmission of the infection[11], the contribution to liver disease progression[11], the development of hepatocellular carcinoma (HCC)[11], and the risk of reactivation are the most relevant contexts[12-14].

In 2013, the Food and Drug Administration had drawn attention to the possible fatal risk of hepatitis B reactivation in patients receiving anti-CD20 drugs[12]. HBV reactivation is known to occur with a wide variety of immune-suppressive therapies and may occur in the context of cancer treatment[12], immunosuppressive therapy for autoimmune disease[12] and transplantation. It is a potentially lethal condition and yet is preventable[12].

HBV reactivation does occur in persons who have anti-HBc[12] with and without anti-HBs and no detectable HBsAg in serum[12]. Among 100 patients undergoing chemotherapy for non-Hodgkins lymphoma, reactivation was noted in 2 of 45 (4%) anti-HBc positive/HBsAg negative patients[12]. In a study[12] from the Brigham and Women's Hospital in Boston. Sixty-one patients identified with resolved HBV infection before a hematopoietic stem cell transplant (HSCT), (HBsAg negative, anti-HBc positive)[12]. Of these, 12 (20%) developed reverse seroconversion[12]. A recent systematic literature review identified reports of 257 patients with active or recovered HBV infection[12] treated with anti-tumor necrosis factor[14]. Reactivation detected in 5% of those who were HBsAg negative but anti-HBc positive[14]. These data hint that all patients undergoing chemotherapy, immunosuppressive therapy, HSCT or solid organ transplantation should screen for prior HBV infection[11]. The use of antiviral treatment appears to diminish the

Table 1 General, and epidemiological characteristics of included studies *n* (%)

Ref.	Study period	Studied population	Location of the study	Age group	Total number	OBI rate
Elrashidy *et al*[10]	2013-2014	Healthy/diabetic children	Lower Egypt	Children	170	0 (0)
Raouf *et al*[20]	2011-2013	HCV positive cancer	Lower Egypt	Children	50	16 (32)
Kishk *et al*[21]	ND	HCV	Lower Egypt	Adults	162	3 (1.85)
Elkady *et al*[22]	2010-2011	Hematologic malignancy	Upper Egypt	Adults	53	1 (1.88)
Said *et al*[34]	ND	HBD	Lower Egypt	Adults	3167	52 (1.64)
El-Ghitany *et al*[31]	ND	HBD and HCV-BD	Lower Egypt	Adults	504	21 (4.16)
Youssef *et al*[23]	ND	CLD	Lower Egypt	Children	24	7 (29.2)
Abu El Makarem *et al*[18]	ND	HD	Upper Egypt	Adults	145	6 (4.1)
Elgohry *et al*[19]	ND	HD	Lower Egypt	Adult	93	25 (26.9)
Shaker *et al*[27]	ND	Thalasemics	Lower Egypt	Children	80	26 (32.5)
Selim *et al*[30]	2008-2009	HCV	Lower Egypt	Adult	60	23 (38.3)
Hassan *et al*[54]	ND	HCC	Lower Egypt	Adult	40	25 (62.5)
Emara *et al*[32]	ND	HCV	Lower Egypt	Adult	155	6 (3.9)
Antar *et al*[25]	2007-2008	HBD	ND	Adult	1021	5 (0.48)
El-Sherif *et al*[29]	2005-2006	HCV	Lower Egypt	Adult	100	16 (16)
Youssef *et al*[26]	ND	CLD	Lower Egypt	Adult	204	119 (58.3)
El-Zayadi *et al*[33]	2005	HBD	Upper/lower Egypt	Adult	712	9 (1.26)
Said *et al*[28]	ND	Hematologic malignancy and disorders	Lower Egypt	Children	100	21 (21)
[1]El-Sherif *et al*[39]	1998-1999	HBD	Lower Egypt	Adults	150	2 (1.3)

[1]Data taken by direct contact with authors. ND: Not determined; OBI: Occult HBV; HBD: Healthy blood donors; HCV-BD: Blood donors positive for HCV; CLD: Cryptogenic liver disease; HD: Hemodialysis; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus.

risk of severe or fatal reactivation of HB infection [12].

Egypt had a high prevalence of HCV (17.5%)[15,16] and intermediate endemicity for HBV infection[16]. The rising prevalence of OBI in Egypt is not surprising. Several reports published on OBI in Egyptian populations. We find that it will prudent to enumerate those studies, and review them to clarify the overall prevalence of OBI and determine the size of that problem in Egypt.

GEOGRAPHIC DISTRIBUTION OF OBI IN EGYPT

Lehman *et al*[17] were unable to compare the HBV prevalence between Upper and Lower Egypt due to the inadequate number of HBV prevalence studies in Upper Egypt. Similarly, only two studies[18,19] addressed OBI prevalence in Upper Egypt (Table 1). Lower OBI rate (4.1%)[18] reported among Upper Egypt hemodialysis (HD) patients compared with HD patients in Lower Egypt (26.9%)[19]. HCV prevalence was significantly higher in patients from Lower Egypt according to Lehman *et al*[17], which in turn confirms the positive correlation between HCV and OBI frequencies.

OBI GENOTYPES AMONG EGYPTIANS

The genetic background of OBI studied by Raouf *et al*[20]. The researchers recruited only children with cancer and the results pointed out that the incidence of OBI in those patients was 32% (Table 1). All infections were with genotype D, and 5 out of 16 OBI patients showed some mutation in the HBV sequence. Two patients had a mutation in the surface gene, and a third one had mutation with a significant shift from the wild-type genetic sequence and those three mutations were presumed to be the cause of OBI. The remaining two mutations were considered silent and not the cause of OBI. In addition Kishk *et al*[21] concluded that HBV genotype D was the only detectable genotype in Egyptian OBI patients with chronic HCV[21]. Among 53 HBsAg negative patients with hematologic malignancies, 5 of them (9.4%) experienced HBV reactivation. OBI was confirmed in one of them (1.88%) (Table 1), and all five patients had Genotype D1[22]. HBV-DNA detected in 7/24 HBsAg-negative children with symptomatic hepatic dysfunction. The G1896A mutation found in 3/7 (43%) HBsAg-negative children and all classified as genotype D[23]. Classically it had been concluded that HBV genotype D is more common in the Mediterranean area, the Middle East and India[24]. The similarity of OBI genotype and the reported HBV genotype in Egypt could support the assumption that OBI infection is part of the natural history of HBV rather than a distinct entity of infection.

OBI DISTRIBUTION AMONG DIFFERENT AGE GROUPS

HBV prevalence is decreasing in Egyptian young generations[10], which may attributed to universal HBV vaccination. The average prevalence of HBV in Egyptian adults is 8% while the average prevalence in children is 1.6%[10]. OBI prevalence among Egyptian adults ranges from 0.48%[25] to 58.3%[26], with lower prevalence among blood donors and higher prevalence among chronic liver disease patients (Table 1). Unexpectedly the prevalence of OBI in children was high according to available data. OBI detected in 32%[20], 32.5%[27], 21%[28] of HCV-positive cancer children, thalassemic and children with hematologic malignancy and disorders

respectively (Table 1). These high OBI rates could be attributed to the fact that; these studies assessed OBI in children with multiple risks for HBV transmission, includes poly-transfusion and immunosuppression. In addition, many children in these studies were HCV co-infected, which may explain the higher OBI prevalence among tested children. Going with our notion Elrashidy *et al*[10], were unable to detect OBI among HB-vaccinated healthy children (*n* = 107).

OBI IN HCV EGYPTIAN PATIENTS

As HBV and HCV share many risk factors and the same transmission routes, OBI detection in HCV patients is not surprising. Indeed, El-Sherif *et al*[29] showed that; among HBsAg-negative patients, the number of patients with parenteral antischistosomal therapy (PAT) is significantly higher in anti-HBc-positive HCV-positive patients, compared with anti-HBc-negative HCV-positive patients[29]. They concluded that PAT transmitted both HCV and HBV in many Egyptians. OBI prevalence in Egyptian HCV-positive patients is 1.85% to 38.3% according to the available data[21,30] (Table 1). This wide range of OBI may be related to different study designs as well as different HBV-DNA detection methods. Moreover, it may related to the liver disease severity and the immunity of the studied patients.

Occult HBV prevalence correlated with the severity of liver disease in HCV patient[1], and it inflicted an adverse impact on HCV outcome[29]. Selim *et al*[30] found that OBI prevalence was higher among patients with alanine aminotransferase (ALT) flare (63.3%) in contrast with normal ALT patients (13.3%). One hundred chronic HCV patients negative for HBsAg subdivided into two groups according to anti-HBc seroreactivity and tested for OBI by El-Sherif *et al*[29]. Group A included 71 patients positive for anti-HBc and group B included 29 patients negative for anti-HBc. HCV- patients positive for anti-HBc have more severe liver disease compared with anti-HBc negative patients[29]. Although HBV-DNA in the serum detected in 22.5% of anti-HBc-positive chronic HCV patients, it was not detected in any of anti-HBc-negative patients[29]. There was no significant difference in the clinical and laboratory parameters tested, between anti-HBc-positive patients with and without HBV-DNA in the serum[29]. Kishk *et al*[21] examined the difference between HCV-infected and HCV/OBI dually infected patients in terms of proinflammatory markers, and histopathological picture. Although they used small sample size (3 patients) their results highlighted that OBI associated with higher Necro inflammatory markers and worse histopathological picture. A clear example came from El-Ghitany *et al*[31] who compared OBI prevalence between two groups of apparently healthy blood donors, they found no significant difference between OBI prevalence among both HCV-positive (3.2%) and HCV-negative (5.1%) cohorts.

One cannot perform firm conclusion about the OBI impact on response to HCV antiviral therapy from the available literature data. Two studies, with a small number of OBI patients (6 patients), addressed the impact of OBI on the response of HCV patients to antiviral therapy. Emara *et al*[32] concluded that; detection of OBI in chronic HCV-positive patients (*n* = 3) has no impact on response to combined pegylated interferon/ribavirin therapy. Similarly, Kishk *et al*[21]. 2014 reported OBI patients (*n* = 3) were responsive to combine pegylated interferon/ribavirin therapy after 12 wk.

OBI IN EGYPTIAN BLOOD DONORS

Occult HBV infection extensively explored in blood donors[1] where it appears to occur quite rarely in the western world and more frequently in developing countries[1]. According to available data, OBI was detected in 1.26%[33] to 4.16%[31] of Egyptian blood donors (Table 1). The detection rate of OBI in Egyptian HBsAg-negative blood donors, without known anti-HBc serostatus, is relatively low (4.16%)[31]. This rate markedly increased when OBI tested in anti-HBc positive donors (14.3%)[34]. This high rate of OBI among anti-HBc-positive donors is not consistent with the findings of many published studies[35-38], which ranged from 0%-6%. This high prevalence may attributed to the high sensitivity of used assay. Out of five studies, three[25,33,39] used nested PCR. The fourth one[34] used a real time PCR with very low detection limit (3.8 IU/mL) (Table 2), increasing the sensitivity of OBI detection.

Little data are available about the infectivity of OBI after a blood transfusion, although the overall OBI transmission rate is 28% according to a recent European study[40]. A lower rate detected in a Taiwanese study[41] (18.2%). The lower transmission rate detected among Japanese (3%) may related to exclusion of patients with detectable levels of HBV-DNA in mini pool nucleic acid test and those with high titer anti-HBc, and many recipients were immune[42]. The infectivity of OBI assessed in a small number of Egyptian blood recipients[34], where 11/34 recipients received anti-HBc positive blood, two of them were HBV-DNA positive, and none developed post-transfusion hepatitis. Like other lookback studies; this study reported several difficulties and limitations in addition to the small sample.

These data indicated that screening Egyptian blood donors for HBsAg only is not safe and the need for additional blood safety measures to reduce post-transfusion HBV transmission. Anti-HBc screening of Egyptian blood donors (in addition to HBsAg) is not practical. It could result in discarding a significant amount of blood, where HBV-DNA not detected in 82.8%[25], 88%[33], 90%[39], 93.7%[34] of HBsAg-negative/anti-HBc-positive blood donors according to available data. On the other hand missing anti-HBc/HBV-DNA-definite units may have serious implication if used in blood transfusion[39]. Based on the high OBI rate among blood donors, we believe that HBV-DNA testing should introduce in Egypt's blood banks. Whether to test HBV-DNA in a mini pool, in all blood donors or in anti-HBc-positive donors only is

Table 2 Methods of hepatitis B virus DNA detection in included studies

Ref.	HBV DNA detected in	PCR method	DNA limit of detection
Elrashidy *et al*[10]	Serum	Nested	ND
Raouf *et al*[20]	Serum	Real-time and Nested	ND
Kishk *et al*[21]	Serum	Real-time and Nested	ND
Elkady *et al*[22]	Serum	Real-time	20 IU/mL
Said *et al*[34]	Serum	Real-time	3.8 IU/mL
El-Ghitany *et al*[31]	Serum	Semi-nested	45 copies/mL
Youssef *et al*[23]	Serum	Real-time	10 copies/mL
Abu El Makarem *et al*[18]	Serum	Nested and Real-time for HBV-DNA-positive by nested PCR	6 IU/mL for real-time PCR
Elgohry *et al*[19]	Serum	Real-time	ND
Shaker *et al*[27]	Serum	Real-time	ND
Selim *et al*[30]	Serum	Semi-nested	45 copies/mL
Hassan *et al*[54]	Liver tissue	Nested	ND
Emara *et al*[32]	Serum	Real-time	12 IU/mL
Antar *et al*[25]	Serum	Real-time	ND
El-Sherif *et al*[29]	Serum	Real-time	35 copies/mL
Youssef *et al*[26]	Serum	Nested	ND
El-Zayadi *et al*[33]	Serum	Nested	ND
Said *et al*[28]	Serum	Nested	ND
[1]El-Sherif *et al*[39]	Serum	Nested	ND

[1]Data taken by direct contact with authors. ND: Not determined; PCR: Polymerase chain reaction; HBV: Hepatitis B virus.

debatable and attractive area for research. Similarly, the relatively high OBI rate among Egyptian blood donors justifies further lookback and traceback extensive study of OBI infectivity among blood recipients.

OBI IN EGYPTIAN THALASSEMIC

Populations at high risk of parenterally transmitted infection have widely investigated for OBI. Thalassemic, are at increased risk of infectious disease transmission. The prevalence of OBI among Egyptian thalassemic children was found to be 32.5% (26/80)[27] (Table 1). The prevalence of OBI was 0% and 31.4% among Iranian[43] and Indian[44] thalassemic respectively. This variation may mostly related to small samples size.

OBI IN EGYPTIAN HEMODIALYSIS PATIENTS

The OBI prevalence in Egyptian hemodialysis (HD)-patients range from 4.1% to 26.9%[18,19] (Table 1). Studies on HD-patients have provided widely divergent results. The highest OBI prevalence (58%) in HD-patients reported from Spain[45]. The results reported from Turkey varied from 0% to 27.5%[46,47]. Lower prevalence (3.8%) was reported by American investigators[48]. Studies from Greece showed that 0.9%-20.4% of HD-patients suffered from OBI[49,50]. The prevalence of OBI among HD-patients seems to be multi-factorial and could not be explained by one factor. It is not region specific, where different studies reported contradictory results from the same county. The difference in methods sensitivity and specificity in the various studies could be the main cause responsible for the discrepant findings.

Age, sex, history of hepatitis, blood transfusion, schistosomal antibodies, liver enzymes, and serum albumin level not associated with OBI in Egyptian hemodialysis patients. In addition, no significant differences in the age, duration of hemodialysis, biochemical parameters, and serological markers of HBV, or HBV-DNA between patients with and without HCV infection. In contrast, the presence of HBV-DNA significantly associated with anti-HBc (P = 0.003)[18]. These data support the role of anti-HBc as a useful surrogate marker for OBI in HD-patients whenever HBV-DNA testing is not available.

OBI IN EGYPTIAN CHILDREN WITH HEMATOLOGIC MALIGNANCIES

Raouf *et al*[20] and Said *et al*[28] tested OBI in children with hematologic malignancies, and the results pointed out that the incidence of OBI in those patients was 21%-32%, respectively (Table 1). The majority of OBI patients were seronegative to anti-HBc which point that anti-HBc is not sufficient to exclude OBI at least in patients with hematologic malignancies. It believed that hematological malignancies associated with an immune deficiency that could preclude the synthesis of anti-HBs and anti-HBc. Another interesting finding is the higher OBI prevalence in HCV-infected patients.

OBI IN CRYPTOGENIC LIVER DISEASE PATIENTS

Occult HBV infection is becoming an important disease entity as a cause of liver disease in HBsAg negative patients. OBI diagnosed in 58.3%[26] of Egyptian patients with symptomatic hepatitis (Table 1). Lower OBI

frequency (30%) reported by Chemin *et al*[51] among patients with chronic non-A non-E hepatitis in France. This difference may related to the difference of HBV prevalence in general populations of both countries.

OBI IN EGYPTIAN HCC PATIENTS

Occult HBV infection may play a direct oncogenic role through both its integration into the host genome and the maintained transcriptional activity. The prevalence of OBI in HBsAg-negative and anti-HBC-negative HCC patients varies among the different population. It ranges from 16% in the United States, which has a low prevalence of chronic hepatitis B, to 70% in endemic areas like china[52,53]. A high rate of OBI was found among Egyptian HCC patients 62.55% (25/40)[54] (Table 1). This high frequency of OBI in Egyptian patients with HCC cannot explain the level of HBV endemicity in Egypt. The small samples size in addition to combined OBI and HCV infection may partially explain the higher OBI frequency among studied HCC patients.

REACTIVATION OF OBI AFTER CHEMOTHERAPY

Among 53 patients with hematological malignancies[22], negative for HBsAg before the start of and throughout the chemotherapy course. Thirty-five (66%) were anti-HBc-negative, and the remaining 18 (34%) patients were anti-HBc-positive. Five of the 53 (9.4%) patients with hematologic malignancies experienced HBV reactivation[22], one of them (1.88%) developed reactivation from an occult HBV infection (Table 1).

ROLE OF HEPATITIS B-VACCINE IN PROTECTION AGAINST OBI

Because of immune incompetence in diabetic patients compared with healthy control, OBI detected in 11% of diabetic patients *vs* 3% of healthy controls[55]. Elrashidy *et al*[10] tested the incidence of OBI in HB-vaccinated healthy (n = 107) and diabetic (n = 63) children. None of the tested children had evidence of OBI or remote HBV infection (anti-HBc) which indicate that HB vaccination had been protective against OBI[10] (Table 1).

CONCLUSION

Like HCV and HBV; nationwide epidemiological study providing exact data regarding OBI situation in Egypt is lacking. Our review represented a trial to synthesize the published OBI results in Egypt. The current report highlights the increase in OBI prevalence in Egyptian HCV-positive patients parallel to the progression of liver disease. Screening for OBI in HCV-positive patients may detect those vulnerable to more progression of their liver disease. Further testing of the impact of OBI on HCV antiviral therapy and transplanted patients is needed. In addition screening for OBI must be a routine practice in blood donors and poly transfused as well as cryptogenic liver disease patients. A definitive assessment of OBI among Egyptian blood donors needed for testing all HBsAg-negative blood donors, instead of only testing anti-HBc-positive patients. Further evaluation of the OBI epidemiology among Egyptian young generations after the era of HBV vaccination is needed. As well, studies of OBI in Upper Egypt should be encouraged.

ACKNOWLEDGMENTS

Here the authors would like to thank Professor Kouka Saad Eldeen Abdel Wahab, Professor of Virology, Al-Azhar University, Cairo, Egypt, for critical discussion of our review.

REFERENCES

1 **Raimondo G**, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. *J Hepatol* 2007; **46**: 160-170 [PMID: 17112622 DOI: 10.1016/j.jhep.2006.10.007]
2 **Levrero M**, Pollicino T, Petersen J, Belloni L, Raimondo G, Dandri M. Control of cccDNA function in hepatitis B virus infection. *J Hepatol* 2009; **51**: 581-592 [PMID: 19616338 DOI: 10.1016/j.jhep.2009.05.022]
3 **Torbenson M**, Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002; **2**: 479-486 [PMID: 12150847]
4 **Michalak TI**, Mulrooney PM, Coffin CS. Low doses of hepadnavirus induce infection of the lymphatic system that does not engage the liver. *J Virol* 2004; **78**: 1730-1738 [PMID: 14747538]
5 **Zerbini A**, Pilli M, Boni C, Fisicaro P, Penna A, Di Vincenzo P, Giuberti T, Orlandini A, Raffa G, Pollicino T, Raimondo G, Ferrari C, Missale G. The characteristics of the cell-mediated immune response identify different profiles of occult hepatitis B virus infection. *Gastroenterology* 2008; **134**: 1470-1481 [PMID: 18355815 DOI: 10.1053/j.gastro.2008.02.017]
6 **Raimondo G**, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, Craxì A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D, Smedile A, Squadrito G, Trépo C, Villa E, Will H, Zanetti AR, Zoulim F. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008; **49**: 652-657 [PMID: 18715666 DOI: 10.1016/j.jhep.2008.07.014]
7 **Ramezani A**, Banifazl M, Eslamifar A, Sofian M, Aghakhani A. Occult hepatitis B infection in different high risk patients. *Hepat Mon* 2012; **12**: 467-468 [PMID: 23008730 DOI: 10.5812/hepatmon.7094]
8 **Emara MH**. Occult hepatitis B: the Egyptian situation. *Trop Gastroenterol* 2012; **33**: 242-250 [PMID: 23923349]
9 **Shetty K**, Hussain M, Nei L, Reddy KR, Lok AS. Prevalence and significance of occult hepatitis B in a liver transplant population with chronic hepatitis C. *Liver Transpl* 2008; **14**: 534-540 [PMID: 18324677 DOI: 10.1002/lt.21284]
10 **Elrashidy H**, El-Didamony G, Elbahrawy A, Hashim A, Alashker A, Morsy MH, Elwassief A, Elmestikawy A, Abdallah AM, Mohammad AG, Mostafa M, George NM, Abdelhafeez H. Absence of occult hepatitis B virus infection in sera of diabetic children and adolescents following hepatitis B vaccination. *Hum Vaccin Immunother* 2014; **10**: 2336-2341 [PMID: 25424940 DOI: 10.4161/hv.29521]
11 **Raimondo G**, Pollicino T, Romanò L, Zanetti AR. A 2010 update on occult hepatitis B infection. *Pathol Biol* (Paris) 2010; **58**: 254-257 [PMID: 20303674 DOI: 10.1016/j.patbio.2010.02.003]
12 **Di Bisceglie AM**, Lok AS, Martin P, Terrault N, Perrillo RP, Hoofnagle JH. Recent US Food and Drug Administration

warnings on hepatitis B reactivation with immune-suppressing and anticancer drugs: just the tip of the iceberg? *Hepatology* 2015; **61**: 703-711 [PMID: 25412906 DOI: 10.1002/hep.27609]
13 **Hammond SP**, Borchelt AM, Ukomadu C, Ho VT, Baden LR, Marty FM. Hepatitis B virus reactivation following allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2009; **15**: 1049-1059 [PMID: 19660717 DOI: 10.1016/j.bbmt.2009.05.001]
14 **Pérez-Alvarez R**, Díaz-Lagares C, García-Hernández F, Lopez-Roses L, Brito-Zerón P, Pérez-de-Lis M, Retamozo S, Bové A, Bosch X, Sanchez-Tapias JM, Forns X, Ramos-Casals M. Hepatitis B virus (HBV) reactivation in patients receiving tumor necrosis factor (TNF)-targeted therapy: analysis of 257 cases. *Medicine* (Baltimore) 2011; **90**: 359-371 [PMID: 22033451 DOI: 10.1097/MD.0b013e3182380a76]
15 **Karoney MJ**, Siika AM. Hepatitis C virus (HCV) infection in Africa: a review. *Pan Afr Med J* 2013; **14**: 44 [PMID: 23560127 DOI: 10.11604/pamj.2013.14.44.2199]
16 **Attia MA**. Prevalence of hepatitis B and C in Egypt and Africa. *Antivir Ther* 1998; **3**: 1-9 [PMID: 10726051]
17 **Lehman EM**, Wilson ML. Epidemiology of hepatitis viruses among hepatocellular carcinoma cases and healthy people in Egypt: a systematic review and meta-analysis. *Int J Cancer* 2009; **124**: 690-697 [PMID: 18973270 DOI: 10.1002/ijc.23937]
18 **Abu El Makarem MA**, Abdel Hamid M, Abdel Aleem A, Ali A, Shatat M, Sayed D, Deaf A, Hamdy L, Tony EA. Prevalence of occult hepatitis B virus infection in hemodialysis patients from egypt with or without hepatitis C virus infection. *Hepat Mon* 2012; **12**: 253-258 [PMID: 22690232 DOI: 10.5812/hepatmon.665]
19 **Elgohry I**, Elbanna A, Hashad D. Occult hepatitis B virus infection in a cohort of Egyptian chronic hemodialysis patients. *Clin Lab* 2012; **58**: 1057-1061 [PMID: 23163124]
20 **Raouf HE**, Yassin AS, Megahed SA, Ashour MS, Mansour TM. Seroprevalence of occult hepatitis B among Egyptian paediatric hepatitis C cancer patients. *J Viral Hepat* 2015; **22**: 103-111 [PMID: 24754376 DOI: 10.1111/jvh.12260]
21 **Kishk R**, Atta HA, Ragheb M, Kamel M, Metwally L, Nemr N. Genotype characterization of occult hepatitis B virus strains among Egyptian chronic hepatitis C patients. *East Mediterr Health J* 2014; **20**: 130-138 [PMID: 24945562]
22 **Elkady A**, Aboulfotuh S, Ali EM, Sayed D, Abdel-Aziz NM, Ali AM, Murakami S, Iijima S, Tanaka Y. Incidence and characteristics of HBV reactivation in hematological malignant patients in south Egypt. *World J Gastroenterol* 2013; **19**: 6214-6220 [PMID: 24115819 DOI: 10.3748/wjg.v19.i37.6214]
23 **Youssef A**, Yano Y, El-Sayed Zaki M, Utsumi T, Hayashi Y. Characteristics of hepatitis viruses among Egyptian children with acute hepatitis. *Int J Oncol* 2013; **42**: 1459-1465 [PMID: 23404231 DOI: 10.3892/ijo.2013.1822]
24 **Kumar GT**, Kazim SN, Kumar M, Hissar S, Chauhan R, Basir SF, Sarin SK. Hepatitis B virus genotypes and hepatitis B surface antigen mutations in family contacts of hepatitis B virus infected patients with occult hepatitis B virus infection. *J Gastroenterol Hepatol* 2009; **24**: 588-598 [PMID: 19207682 DOI: 10.1111/j.1440-1746.2008.05727.x]
25 **Antar W**, El-Shokry MH, Abd El Hamid WA, Helmy MF. Significance of detecting anti-HBc among Egyptian male blood donors negative for HBsAg. *Transfus Med* 2010; **20**: 409-413 [PMID: 20573069 DOI: 10.1111/j.1365-3148.2010.01021.x]
26 **Youssef A**, Yano Y, Utsumi T, abd El-alah EM, abd El-Hameed Ael-E, Serwah Ael-H, Hayashi Y. Molecular epidemiological study of hepatitis viruses in Ismailia, Egypt. *Intervirology* 2009; **52**: 123-131 [PMID: 19468235 DOI: 10.1159/000219385]
27 **Shaker O**, Ahmed A, Abdel Satar I, El Ahl H, Shousha W, Doss W. Occult hepatitis B in Egyptian thalassemic children. *J Infect Dev Ctries* 2012; **6**: 340-346 [PMID: 22505444]
28 **Said ZN**, El-Sayed MH, El-Bishbishi IA, El-Fouhil DF, Abdel-Rheem SE, El-Abedin MZ, Salama II. High prevalence of occult hepatitis B in hepatitis C-infected Egyptian children with haematological disorders and malignancies. *Liver Int* 2009; **29**: 518-524 [PMID: 19192168 DOI: 10.1111/j.1478-3231.2009.01975.x]
29 **El-Sherif A**, Abou-Shady M, Abou-Zeid H, Elwassief A, Elbahrawy A, Ueda Y, Chiba T, Hosney AM. Antibody to hepatitis B core antigen as a screening test for occult hepatitis B virus infection in Egyptian chronic hepatitis C patients. *J Gastroenterol* 2009; **44**: 359-364 [PMID: 19271112 DOI: 10.1007/s00535-009-0020-3]
30 **Selim HS**, Abou-Donia HA, Taha HA, El Azab GI, Bakry AF. Role of occult hepatitis B virus in chronic hepatitis C patients with flare of liver enzymes. *Eur J Intern Med* 2011; **22**: 187-190 [PMID: 21402251 DOI: 10.1016/j.ejim.2010.12.001]
31 **El-Ghitany EM**, Farghaly AG, Hashish MH. Occult hepatitis B virus infection among hepatitis C virus seropositive and seronegative blood donors in Alexandria, Egypt. *J Egypt Public Health Assoc* 2013; **88**: 8-13 [PMID: 23528526 DOI: 10.1097/01.EPX.0000422774.29308.b3]
32 **Emara MH**, El-Gammal NE, Mohamed LA, Bahgat MM. Occult hepatitis B infection in egyptian chronic hepatitis C patients: prevalence, impact on pegylated interferon/ribavirin therapy. *Virol J* 2010; **7**: 324 [PMID: 21083926 DOI: 10.1186/1743-422X-7-324]
33 **El-Zayadi AR**, Ibrahim EH, Badran HM, Saeid A, Moneib NA, Shemis MA, Abdel-Sattar RM, Ahmady AM, El-Nakeeb A. Anti-HBc screening in Egyptian blood donors reduces the risk of hepatitis B virus transmission. *Transfus Med* 2008; **18**: 55-61 [PMID: 18279193 DOI: 10.1111/j.1365-3148.2007.00806.x]
34 **Said ZN**, Sayed MH, Salama II, Aboel-Magd EK, Mahmoud MH, Setouhy ME, Mouftah F, Azzab MB, Goubran H, Bassili A, Esmat GE. Occult hepatitis B virus infection among Egyptian blood donors. *World J Hepatol* 2013; **5**: 64-73 [PMID: 23646231 DOI: 10.4254/wjh.v5.i2.64]
35 **Amini S**, Mahmoodi MF, Andalibi S, Solati AA. Seroepidemiology of hepatitis B, delta and human immunodeficiency virus infections in Hamadan province, Iran: a population based study. *J Trop Med Hyg* 1993; **96**: 277-287 [PMID: 8411302]
36 **Ks M**, R A, P A. The prevalence of the hepatitis B core antibody and the occult hepatitis B infection among voluntary blood donors in chennai, India. *J Clin Diagn Res* 2012; **6**: 1710-1712 [PMID: 23373034 DOI: 10.7860/JCDR/2012/4826.2636]
37 **Makroo RN**, Chowdhry M, Bhatia A, Arora B, Rosamma NL. Hepatitis B core antibody testing in Indian blood donors: A double-edged sword! *Asian J Transfus Sci* 2012; **6**: 10-13 [PMID: 22623835 DOI: 10.4103/0973-6247.95043]
38 **Kleinman SH**, Kuhns MC, Todd DS, Glynn SA, McNamara A, DiMarco A, Busch MP. Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-HBc: implications for transfusion transmission and donor screening. *Transfusion* 2003; **43**: 696-704 [PMID: 12757519]
39 **El-Sherif AM**, Abou-Shady MA, Al-Hiatmy MA, Al-Bahrawy AM, Motawea EA. Screening for hepatitis B virus infection in Egyptian blood donors negative for hepatitis B surface antigen. *Hepatol Int* 2007; **1**: 469-470 [PMID: 19669344 DOI: 10.1007/s12072-007-9017-2]
40 **Allain JP**, Mihaljevic I, Gonzalez-Fraile MI, Gubbe K, Holm-Harritshøj L, Garcia JM, Brojer E, Erikstrup C, Saniewski M, Wernish L, Bianco L, Ullum H, Candotti D, Lelie N, Gerlich WH, Chudy M. Infectivity of blood products from donors with occult hepatitis B virus infection. *Transfusion* 2013; **53**: 1405-1415 [PMID: 23362802 DOI: 10.1111/trf.12096]
41 **Wang JT**, Lee CZ, Chen PJ, Wang TH, Chen DS. Transfusion-transmitted HBV infection in an endemic area: the necessity of more sensitive screening for HBV carriers. *Transfusion* 2002; **42**: 1592-1597 [PMID: 12473140]
42 **Satake M**, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K. Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. *Transfusion* 2007; **47**: 1197-1205 [PMID: 17581154 DOI: 10.1111/j.1537-2995.2007.01276.x]
43 **Arababadi MK**, Hassanshahi G, Yousefi H, Zarandi ER, Moradi M, Mahmoodi M. No detected hepatitis B virus-DNA in thalassemic

patients infected by hepatitis C virus in Kerman province of Iran. *Pak J Biol Sci* 2008; **11**: 1738-1741 [PMID: 18819629]
44 **Singh H**, Pradhan M, Singh RL, Phadke S, Naik SR, Aggarwal R, Naik S. High frequency of hepatitis B virus infection in patients with beta-thalassemia receiving multiple transfusions. *Vox Sang* 2003; **84**: 292-299 [PMID: 12757503]
45 **Cabrerizo M**, Bartolomé J, De Sequera P, Caramelo C, Carreño V. Hepatitis B virus DNA in serum and blood cells of hepatitis B surface antigen-negative hemodialysis patients and staff. *J Am Soc Nephrol* 1997; **8**: 1443-1447 [PMID: 9294837]
46 **Goral V**, Ozkul H, Tekes S, Sit D, Kadiroglu AK. Prevalence of occult HBV infection in haemodialysis patients with chronic HCV. *World J Gastroenterol* 2006; **12**: 3420-3424 [PMID: 16733862]
47 **Yakaryilmaz F**, Gurbuz OA, Guliter S, Mert A, Songur Y, Karakan T, Keles H. Prevalence of occult hepatitis B and hepatitis C virus infections in Turkish hemodialysis patients. *Ren Fail* 2006; **28**: 729-735 [PMID: 17162434 DOI: 10.1080/08860220600925602]
48 **Siagris D**, Christofidou M, Triga K, Pagoni N, Theocharis GJ, Goumenos D, Lekkou A, Thomopoulos K, Tsamandas AC, Vlachojannis J, Labropoulou-Karatza C. Occult hepatitis B virus infection in hemodialysis patients with chronic HCV infection. *J Nephrol* 2006; **19**: 327-333 [PMID: 16874693]
49 **Fabrizi F**, Bunnapradist S, Lunghi G, Aucella F, Martin P. Epidemiology and clinical significance of hepatotropic infections in dialysis patients. Recent evidence. *Minerva Urol Nefrol* 2004; **56**: 249-257 [PMID: 15467503]
50 **Mina P**, Georgiadou SP, Rizos C, Dalekos GN, Rigopoulou EI. Prevalence of occult hepatitis B virus infection in haemodialysis patients from central Greece. *World J Gastroenterol* 2010; **16**: 225-231 [PMID: 20066742]
51 **Chemin I**, Zoulim F, Merle P, Arkhis A, Chevallier M, Kay A, Cova L, Chevallier P, Mandrand B, Trépo C. High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology. *J Hepatol* 2001; **34**: 447-454 [PMID: 11322208]
52 **Bréchot C**, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; **34**: 194-203 [PMID: 11431751 DOI: 10.1053/jhep.2001.25172]
53 **Kannangai R**, Molmenti E, Arrazola L, Klein A, Choti M, Thomas DL, Torbenson M. Occult hepatitis B viral DNA in liver carcinomas from a region with a low prevalence of chronic hepatitis B infection. *J Viral Hepat* 2004; **11**: 297-301 [PMID: 15230851 DOI: 10.1111/j.1365-2893.2004.00502.x]
54 **Hassan ZK**, Hafez MM, Mansor TM, Zekri AR. Occult HBV infection among Egyptian hepatocellular carcinoma patients. *Virol J* 2011; **8**: 90 [PMID: 21371325 DOI: 10.1186/1743-422X-8-90]
55 **Demir M**, Serin E, Göktürk S, Ozturk NA, Kulaksizoglu S, Ylmaz U. The prevalence of occult hepatitis B virus infection in type 2 diabetes mellitus patients. *Eur J Gastroenterol Hepatol* 2008; **20**: 668-673 [PMID: 18679070 DOI: 10.1097/MEG.0b013e3282f55e1e]

P- Reviewer: Balaban YH, Ramos S, Sazci A, Zhang X
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1679

World J Hepatol 2015 June 28; 7(12): 1679-1684
ISSN 1948-5182 (online)

MINIREVIEWS

Influence of gut bacteria on development and progression of non-alcoholic fatty liver disease

Ali Abdul-Hai, Ali Abdallah, Stephen DH Malnick

Ali Abdul-Hai, Ali Abdallah, Stephen DH Malnick, Division of Internal Medicine, Kaplan Medical Center, Affiliated to the Hebrew University, Rehovot 76100, Israel

Author contributions: All the authors were involved in the writing of various sections of the manuscript; Malnick SDH conceived the idea of writing the review.

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

Correspondence to: Dr. Stephen DH Malnick, Division of Internal Medicine, Kaplan Medical Center, Affiliated to the Hebrew University, 1 Pasternak, Rehovot 76100, Israel. stephen@malnick.net
Telephone: +972-89-441371
Fax: +972-89-441852

Received: December 17, 2014
Peer-review started: December 18, 2014
First decision: March 6, 2015
Revised: April 8, 2015
Accepted: April 16, 2015
Article in press: April 20, 2015
Published online: June 28, 2015

Abstract

The intestine of the human contains a dynamic population of microbes that have a symbiotic relationship with the host. In addition, there is an effect of the intestinal microbiota on metabolism and digestion. Non-alcoholic fatty liver disease (NAFLD) is a common cause worldwide of hepatic pathology and is thought to be the hepatic manifestation of the metabolic syndrome. In this review we examine the effect of the human microbiome on the components and pathogenesis of the metabolic syndrome. We are now on the threshold of therapeutic interventions on the human microbiome in order to effect human disease including NAFLD.

Key words: Microbiome; Metabolic syndrome; Stool transplantation; Non-alcoholic fatty liver disease

Core tip: The human intestine contains more bacterial cells than mammalian cells. These have a symbiotic relationship with the host. Non-alcoholic fatty liver disease is the hepatic manifestation of the metabolic syndrome and a major cause of hepatic morbidity as a consequence of the obesity epidemic. We examine the effect of the human microbiome on the components of the metabolic syndrome and fatty liver and mention the possibility of therapeutic interventions in humans.

Abdul-Hai A, Abdallah A, Malnick SDH. Influence of gut bacteria on development and progression of non-alcoholic fatty liver disease. *World J Hepatol* 2015; 7(12): 1679-1684 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1679.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1679

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of the metabolic syndrome. The metabolic syndrome is defined by clear clinical and laboratory criteria (Table 1). NAFLD encompasses a range of liver damage ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and its complications. NAFLD is present in approximately

Table 1 The definitions of the metabolic syndrome

	NCEP ATP Ⅲ	IDF
Absolutely required	None	Central obesity (waist circumference) ⩾ 94 cm in males or ⩾ 80 cm in females European origin ⩾ 90 cm in males or ⩾ 80 cm in females
Criteria	Any three of the five criteria below	Central obesity plus two of the four criteria below
Obesity	(1) waist circumference > 40 inches in males, or > 35 inches in females	
Hyperglycemia	(2) fasting glucose ⩾ 100 mg/dL or treated for DM	(1) fasting glucose ⩾ 100 mg/dL
Dyslipidemia	(3) TG ⩾ 150 mg/dL or treated for dyslipidemia Or (4) HDL cholesterol < 40 mg/dL in males, or < 50 mg/dL in females or under treatment	(2) TG ⩾ 150 mg/dL or treated for dyslipidemia Or (3) HDL cholesterol < 40 mg/dL in males, or < 50 mg/dL in females or under treatment
Hypertension	(5) > 130 mmHg systolic or > 85 mmHg diastolic or treated for HTN	(4) > 130 mmHg systolic or > 85 mmHg diastolic or treated for HTN

NCEP ATP Ⅲ: National Cholesterol and Education Program - Adult Treatment Panel Ⅲ; IDF: International diabetes federation; DM: Diabetes mellitus; TG: TG: Triglycerides; HTN: Hypertension.

1/3 of the United States population, who have isolated steatosis of the liver[1]. Of the patients with NAFLD, approximately 30% have NASH[2]. NASH refers to those patients who have developed liver inflammation and fibrosis. It is those patients with NASH who may develop stage 3 or 4 fibrosis (cirrhosis)[3].

Many factors including diet, sedentary lifestyle and genetics have been shown to influence the progression from steatosis through NASH to cirrhosis. However, not all people who are obese develop NAFLD and neither are all patients with NAFLD obese.

GUT MICROBIOTA

The intestinal microbiome is attracting an increasing amount of attention[4]. It is becoming apparent that there is a symbiotic relationship between the intestine and its microbiota and that disturbance in this relationship can be associated with the pathogenesis of many disorders. The most striking example of such an association is *Clostridium difficile* infection, for which fecal transplantation from healthy donors is now an accepted treatment[5].

Distinct gut microbiota profiles are linked with specific metabolomes. Ninety-five percent of the gut microbiota of humans consists of the *Firmicutes*, *Bacteroidetes* and *Actinobacteria* phyla. The species level of the human microbiota, however, has higher diversity, with approximately 200 highly prevalent and up to 1000 less common bacterial species[6]. In humans as in mice, each individual has an unique bacterial species profile[7]. The gut bacteria may alter in response to a high fat diet (HFD), which could be responsible for some of the responses to an HFD.

Bacteria from human stools can be transferred to germ free (GF) mice and result in a similar microbiome in the host mice[8]. This can result in the appearance of human gut enzymatic activities in GF rodents after human fecal transplantation[9,10].

Recently, the transfer of human gut microbiome from obesity discordant twins to GF mice was shown to result in the transfer of the adiposity phenotype of the donor twin[11]. Thus, the transfer of human fecal microbiota to GF mice may result in the development of human diseases and provide an experimental study system.

INTESTINAL MICROBIOTA ARE RELATED TO OBESITY AND INSULIN RESISTANCE

The gut microbiota is now recognized as contributing to obesity and NAFLD[12]. GF mice have been found to gain less weight than conventional mice after being fed a high sugar and fat diet in spite of a higher amount of food consumption[13,14]. Furthermore, GF mice on an HFD develop an increase in insulin sensitivity[15] and GF mice colonized with conventional mouse intestinal microbiota develop an increase in body fat content[13]. There are, however, wide variations in the development of HFD-associated features[16,17], but the responsible factors are still undefined.

The insulin resistance index can be transferred by gut microbiota transplantation[18]. Gut microbiota affects both macrophage fat accumulation and systemic glucose metabolism by different mechanisms[19]. In a diet-induced obesity mouse model, administration of antibiotics improved fasting glycemia and insulin resistance independently of both food intake or adiposity[20]. Furthermore the improved insulin sensitivity correlated with less hepatic lipogenesis and steatosis in the antibiotic-treated mice[21]. Taken together, these findings suggest that the gut microbiota influences both host glucose metabolism and liver function.

A study in humans showed that transfer of intestinal microbiota from lean donors to males with the metabolic syndrome resulted in increased insulin sensitivity[21]. Dietary factors and changes in diet influence the composition of the microbiome. The intestinal microbiota of obese individuals has a different microbial diversity compared to lean persons. They have less *Bacteroides* and more *Firmicutes*[22]. Furthermore, an HFD increases the proportion of Gram-negative to

Gram-positive microbes, resulting in the production of lipopolysaccharide (LPS) which is responsible for inflammation[23]. Gram-positive microbes are increased following the administration of prebiotics[24]. A prebiotic is a nondigestible food substrate which increases the growth of intestinal bacteria that can result in health benefits for the host.

The intestinal microbiome in obesity has an increased capacity to extract energy from the host diet. Bacterial enzymes extract calories from otherwise indigestible dietary polysaccharides[25]. Enteric bacteria suppress the synthesis and secretion of small intestinal fasting-induced adipocyte factor, resulting in an increased activity of lipoprotein lipase and increased liver triglyceride[13,14].

GF lean mice that were resistant to becoming obese on a fat-enriched diet had an increase of phosphorylated adenosine monophosphate-activated protein kinase (AMPK) in both the skeletal muscle and liver. AMPK phosphorylates acetyl coenzyme A (CoA) carboxylase, resulting in decreased malonyl CoA levels. Malonyl CoA controls the rate-limiting step of long-chain fatty acyl CoA entry to the mitochondria by blocking carnitine palmitoyltransferase which promotes the oxidation of fatty acid and results in a lower storage of fat[14,26].

Thus, the intestinal microbiome has an effect on both obesity and insulin resistance, as well as hepatic fat content.

GUT MICROBIOTA AND NAFLD

In view of the intimate connection between the metabolic syndrome with its concomitant insulin resistance and NAFLD, it is expected that there is an effect of the intestinal microbiome on NAFLD.

The fecal microbiota in NAFLD and NASH patients has been examined using quantitative polymerase chain reaction (PCR) and deep sequencing of a conserved region in the bacterial 16S ribosomal RNA gene[27-30]. A recent review provides a summary of the changes in the intestinal microbiota associated with NAFLD and NASH[12]. Many of these studies have variable and often contradictory findings. This may be due to differences in patient mix, methodology and documentation of liver disease.

In addition to the mixture of bacteria in the colon, patients with obesity or NAFLD have more small intestinal bacterial overgrowth[31,32]. Small intestinal bacterial overgrowth was found in 50% of patients with NASH, significantly more than that in a control population[33]. The intestinal permeability and bacterial overgrowth were shown to be related to the degree of hepatic steatosis but not inflammation or fibrosis[31].

However, it is not clear if the assessment of small bowel bacterial overgrowth by breath tests is accurate since an estimate of total fecal bacterial count by real-time PCR did not detect any difference between healthy controls and patients with NAFLD and NASH[28].

Possible mediators of the link between the enteric microbiome and the host include alcohol, choline and endotoxins. Obese animals have been shown to have higher levels of alcohol in breath tests than thin animals[34]. Alcohol reaches the liver *via* the portal blood and can cause triglyceride accumulation in hepatocytes[35]. In addition, alcohol may provide the "second hit" to the liver for making the transformation from steatosis to steatohepatitis[36].

Choline may also be involved in the development of NAFLD and NASH. It is well known that choline deficiency may result in chronic liver disease[37]. In animal models choline-deficient diets were utilized, but it is now known that choline deficiency can exist while there is a diet that is not deficient. HFDs produce intestinal microbiota that converts dietary choline into methylamines. This results in a reduction of serum level of phosphatidylcholine which can cause NASH[26]. Phosphatidylcholine is important for the production of very low-density lipoprotein (VLDL)[38] and thus choline deficiency secondary to the intestinal microbiome will result in lower hepatic secretion of VLDL and result in triglyceride accumulation in hepatocytes.

The products of the intestinal microbiota are also implicated in the development of NAFLD and NASH. Endotoxemia has been found in patients with NASH[39]. Toll-like receptor 4, a receptor for LPS, in hematopoietic-derived cells is necessary for the development of hepatic steatosis but not for obesity in mice[40]. Mice that are deficient in sensing pathogen-associated molecular patterns (PAMPs) or downstream signaling are resistant to NASH[41,42].

The microbial products reach the liver *via* the portal vein and cause inflammation. Mice that are genetically obese are more sensitive to endotoxin-induced hepatotoxicity and develop steatohepatitis after being exposed to low doses of LPS[43]. NAFLD patients have an increased intestinal permeability and changes in the intestinal tight junctions, as compared to healthy individuals[31]. The increased permeability, in combination with bacterial overgrowth, increases the hepatic exposure to endotoxins.

Alteration of the fecal microbiome by administration of probiotics has been shown to decrease the amount of intrahepatic triglyceride content in addition to a decrease in *Firmicutes* and an increase in *Bacteroidetes*[30]. A meta-analysis of the published trials of probiotics in patients with NAFLD, showed a reduction in serum transaminases, total cholesterol, tumor necrosis factor-α and an improvement in insulin resistance[44].

Dysbiosis can induce intestinal inflammation. Indeed GF mice are protected from inflammation of the small intestine[45]. Mice deficient in Nlrp3 and Nlrp6 are unable to form cytoplasmic multiprotein complexes composed of nucleotide-binding domain and leucine-rich repeat-containing proteins (NLR) family, inflammasomes. Inflammasomes are sensors of exogenous PAMPs that regulate cleavage of precursors of inflammatory cytokines including pro-interleukin 1 beta (pro-IL1β) and pro-IL18. In mice, loss of Nlrp3 and Nlrp6 inflammasomes is associated with intestinal dysbiosis and colonic inflammation *via* CCL5. Dysbiosis is linked to an increase in *Prevotella*[46]. The consequent translocation of bacteria

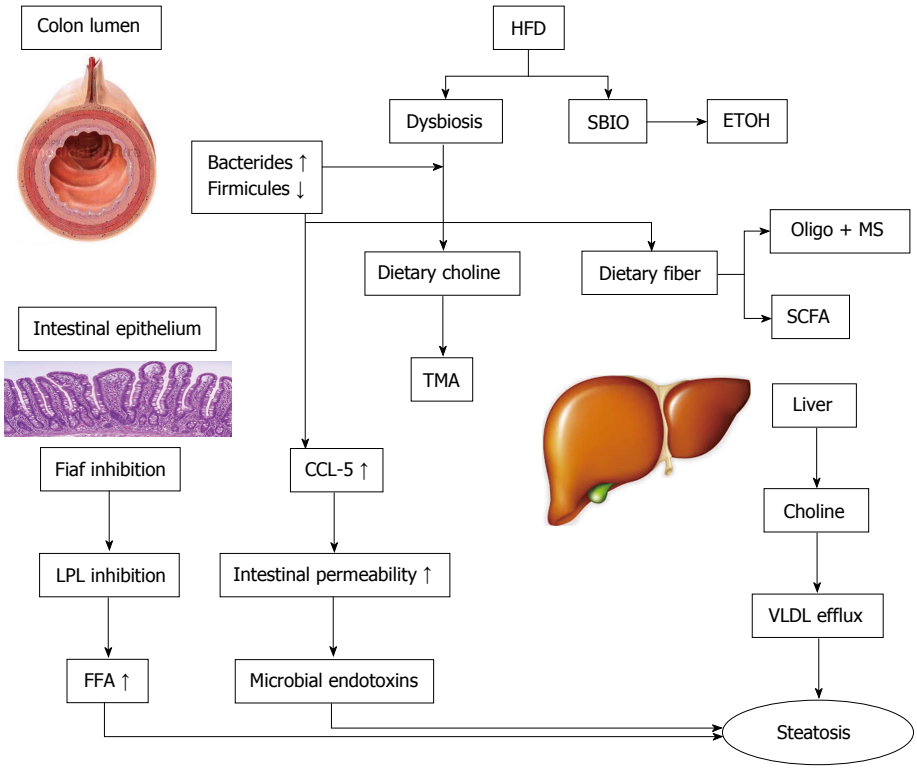

Figure 1 The effect of the intestinal microbiota on non-alcoholic fatty liver disease. High fat diets (HFD) produce dysbiosis and small bowel intestinal overgrowth (SBIO). There is an increase in energy extraction and fermentation of dietary fibers to oligo- and mono-saccahrides and short chain fatty acids (SCFA). There is also an increase in ethanol (ETOH) production. The microbiota metabolize choline to trimethylamine (TMA). There is a choline deficiency which decreases very low-density lipoprotein (VLDL) efflux and hepatic steatosis. In addition the intestinal microbiota suppresses the production of fasting induced adipocyte factor (Fiaf) in intestinal epithelia, which increases the activity of lipoprotein lipase and the levels of free fatty acids (FFA). Dysbiosis results in a disruption of tight junctions in the enterocytes *via* chemokine (C-C motif) ligand 5 (CCL-5). The resulting increase in intestinal permeability results in the translocation of microbial products to the liver and inflammation; MS: Monosaccharides; LPL: Lipoprotein lipase.

leads to an increase in bacterial products including LPS and bacterial DNA in the portal vein. The ensuing hepatic inflammatory response promotes progression of NAFLD to NASH (Figure 1). This change in phenotype can be transmitted by co-housing wild-type and NASH-prone mice[46].

Thus, intestinal dysbiosis can induce colonic inflammation and bacterial translocation which accelerates the progression of simple steatosis to NASH. As a result of these findings attention is beginning to be directed at fecal microbiota transplantation (FMT). FMT was first used in China more than 1500 years ago[47]. In 1958, 4 cases of treatment of pseudomembranous colitis by fecal enemas were reported[48]. This is now an established treatment[49]. At present there is only one report of FMT for metabolic syndrome. Vrieze *et al*[21] reported 18 patients with the metabolic syndrome who underwent a stool transplant that was either autologous or from lean healthy volunteers. Six weeks following the FMT there was a significant increase in insulin sensitivity together with an increase in the levels of butyrate-producing intestinal microbiota.

In summary, there appears to be an effect of the fecal microbiome on the development of the metabolic syndrome and its hepatic manifestation NAFLD and NASH. Further investigation of this relationship will increase our understanding of this connection. There is evidence that manipulation of the fecal microbiome may result in a change in the metabolic syndrome and an improvement in the features of NAFLD. This needs to be explored further in order to investigate if there will be an improvement in clinically significant end points.

REFERENCES

1 **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]

2 **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]

3 **Caldwell S**, Argo C. The natural history of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 162-168 [PMID: 20460906]

4 **Nicholson JK**, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. *Science* 2012; **336**: 1262-1267 [PMID: 22674330 DOI: 10.1126/science.1223813]

5 **Floch MH**. The power of poop: probiotics and fecal microbial transplant. *J Clin Gastroenterol* 2012; **46**: 625-626 [PMID: 22874805 DOI: 10.1097/MCG.0b013e3182667a93]

6 **Ley RE**, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat Rev Microbiol* 2008; **6**: 776-788 [PMID: 18794915 DOI: 10.1038/

nrmicro1978]
7 **Zoetendal EG**, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol* 1998; **64**: 3854-3859 [PMID: 9758810]
8 **Turnbaugh PJ**, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 2009; **1**: 6ra14 [PMID: 20368178 DOI: 10.1126/scitranslmed.3000322]
9 **Bowey E**, Adlercreutz H, Rowland I. Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human flora associated rats. *Food Chem Toxicol* 2003; **41**: 631-636 [PMID: 12659715]
10 **Gérard P**, Béguet F, Lepercq P, Rigottier-Gois L, Rochet V, Andrieux C, Juste C. Gnotobiotic rats harboring human intestinal microbiota as a model for studying cholesterol-to-coprostanol conversion. *FEMS Microbiol Ecol* 2004; **47**: 337-343 [PMID: 19712322 DOI: 10.1016/S0168-6496(03)00285-X]
11 **Ridaura VK**, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013; **341**: 1241214 [PMID: 24009397 DOI: 10.1126/science.1241214]
12 **Schnabl B**, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 2014; **146**: 1513-1524 [PMID: 24440671 DOI: 10.1053/j.gastro.2014.01.020]
13 **Bäckhed F**, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; **101**: 15718-15723 [PMID: 15505215 DOI: 10.1073/pnas.0407076101]
14 **Bäckhed F**, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* 2007; **104**: 979-984 [PMID: 17210919 DOI: 10.1073/pnas.0605374104]
15 **Rabot S**, Membrez M, Bruneau A, Gérard P, Harach T, Moser M, Raymond F, Mansourian R, Chou CJ. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *FASEB J* 2010; **24**: 4948-4959 [PMID: 20724524 DOI: 10.1096/fj.10-164921]
16 **Jang I**, Hwang D, Lee J, Chae K, Kim Y, Kang T, Kim C, Shin D, Hwang J, Huh Y, Cho J. Physiological difference between dietary obesity-susceptible and obesity-resistant Sprague Dawley rats in response to moderate high fat diet. *Exp Anim* 2003; **52**: 99-107 [PMID: 12806884]
17 **Li H**, Xie Z, Lin J, Song H, Wang Q, Wang K, Su M, Qiu Y, Zhao T, Song K, Wang X, Zhou M, Liu P, Zhao G, Zhang Q, Jia W. Transcriptomic and metabonomic profiling of obesity-prone and obesity-resistant rats under high fat diet. *J Proteome Res* 2008; **7**: 4775-4783 [PMID: 18828625]
18 **Le Roy T**, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, Martin P, Philippe C, Walker F, Bado A, Perlemuter G, Cassard-Doulcier AM, Gérard P. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut* 2013; **62**: 1787-1794 [PMID: 23197411 DOI: 10.1136/gutjnl-2012-303816]
19 **Caesar R**, Reigstad CS, Bäckhed HK, Reinhardt C, Ketonen M, Lundén GÖ, Cani PD, Bäckhed F. Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice. *Gut* 2012; **61**: 1701-1707 [PMID: 22535377 DOI: 10.1136/gutjnl-2011-301689]
20 **Membrez M**, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, Corthesy I, Macé K, Chou CJ. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J* 2008; **22**: 2416-2426 [PMID: 18326786 DOI: 10.1096/fj.07-102723]
21 **Vrieze A**, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; **143**: 913-6.e7 [PMID: 22728514 DOI: 10.1053/j.gastro.2012.06.031]
22 **Turnbaugh PJ**, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008; **3**: 213-223 [PMID: 18407065 DOI: 10.1016/j.chom.2008.02.015]
23 **Cani PD**, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; **56**: 1761-1772 [PMID: 17456850 DOI: 10.2337/db06-1491]
24 **Cani PD**, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* 2007; **50**: 2374-2383 [PMID: 17823788 DOI: 10.1007/s00125-007-0791-0]
25 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312 DOI: 10.1038/nature05414]
26 **Dumas ME**, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci USA* 2006; **103**: 12511-12516 [PMID: 16895997 DOI: 10.1073/pnas.0601056103]
27 **Raman M**, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, Greenwood R, Sikaroodi M, Lam V, Crotty P, Bailey J, Myers RP, Rioux KP. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2013; **11**: 868-875.e1-3 [PMID: 23454028]
28 **Mouzaki M**, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013; **58**: 120-127 [PMID: 23401313]
29 **Zhu L**, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* 2013; **57**: 601-609 [PMID: 23055155 DOI: 10.1002/hep.26093]
30 **Wong VW**, Tse CH, Lam TT, Wong GL, Chim AM, Chu WC, Yeung DK, Law PT, Kwan HS, Yu J, Sung JJ, Chan HL. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis--a longitudinal study. *PLoS One* 2013; **8**: e62885 [PMID: 23638162 DOI: 10.1371/journal.pone.0062885]
31 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]
32 **Sabaté JM**, Jouët P, Harnois F, Mechler C, Msika S, Grossin M, Coffin B. High prevalence of small intestinal bacterial overgrowth in patients with morbid obesity: a contributor to severe hepatic steatosis. *Obes Surg* 2008; **18**: 371-377 [PMID: 18286348 DOI: 10.1007/s11695-007-9398-2]
33 **Wigg AJ**, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2001; **48**: 206-211 [PMID: 11156641]
34 **Cope K**, Risby T, Diehl AM. Increased gastrointestinal ethanol

production in obese mice: implications for fatty liver disease pathogenesis. *Gastroenterology* 2000; **119**: 1340-1347 [PMID: 11054393]

35 **Hartmann P**, Chen WC, Schnabl B. The intestinal microbiome and the leaky gut as therapeutic targets in alcoholic liver disease. *Front Physiol* 2012; **3**: 402 [PMID: 23087650 DOI: 10.3389/fphys.2012.00402]

36 **Day CP**. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002; **16**: 663-678 [PMID: 12406438]

37 **Blumberg H**, McCollum EV. The prevention by choline of liver cirrhosis in rats on high fat, low protein diets. *Science* 1941; **93**: 598-599 [PMID: 17795968 DOI: 10.1126/science.93.2425.598]

38 **Jiang XC**, Li Z, Liu R, Yang XP, Pan M, Lagrost L, Fisher EA, Williams KJ. Phospholipid transfer protein deficiency impairs apolipoprotein-B secretion from hepatocytes by stimulating a proteolytic pathway through a relative deficiency of vitamin E and an increase in intracellular oxidants. *J Biol Chem* 2005; **280**: 18336-18340 [PMID: 15734742 DOI: 10.1074/jbc.M500007200]

39 **Farhadi A**, Gundlapalli S, Shaikh M, Frantzides C, Harrell L, Kwasny MM, Keshavarzian A. Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in non-alcoholic steatohepatitis. *Liver Int* 2008; **28**: 1026-1033 [PMID: 18397235 DOI: 10.1111/j.1478-3231.2008.01723.x]

40 **Saberi M**, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, Verma IM, Olefsky JM. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab* 2009; **10**: 419-429 [PMID: 19883619 DOI: 10.1016/j.cmet.2009.09.006]

41 **Miura K**, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, Olefsky JM, Brenner DA, Seki E. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology* 2010; **139**: 323-34.e7 [PMID: 20347818 DOI: 10.1053/j.gastro.2010.03.052]

42 **Rivera CA**, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* 2007; **47**: 571-579 [PMID: 17644211 DOI: 10.1016/j.jhep.2007.04.019]

43 **Yang SQ**, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 1997; **94**: 2557-2562 [PMID: 9122234]

44 **Ma YY**, Li L, Yu CH, Shen Z, Chen LH, Li YM. Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. *World J Gastroenterol* 2013; **19**: 6911-6918 [PMID: 24187469]

45 **Ding S**, Chi MM, Scull BP, Rigby R, Schwerbrock NM, Magness S, Jobin C, Lund PK. High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. *PLoS One* 2010; **5**: e12191 [PMID: 20808947 DOI: 10.3748/wjg.v19.i40.6911]

46 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]

47 **Zhang F**, Luo W, Shi Y, Fan Z, Ji G. Should we standardize the 1,700-year-old fecal microbiota transplantation? *Am J Gastroenterol* 2012; **107**: 1755; author reply p.1755-p.1756 [PMID: 23160295 DOI: 10.1038/ajg.2012.251]

48 **Eiseman B**, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 1958; **44**: 854-859 [PMID: 13592638]

49 **van Nood E**, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013; **368**: 407-415 [PMID: 23323867 DOI: 10.1056/NEJMoa1205037]

P- Reviewer: Abdel-Salam OME, Balaban YH, Hsieh SY, Julie NL, Rajeshwari K, Wong GLH
S- Editor: Ji FF **L- Editor**: Wang TQ **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1685

World J Hepatol 2015 June 28; 7(12): 1685-1693
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Efficacy of tolvaptan in patients with refractory ascites in a clinical setting

Takamasa Ohki, Koki Sato, Tomoharu Yamada, Mari Yamagami, Daisaku Ito, Koki Kawanishi, Kentaro Kojima, Michiharu Seki, Nobuo Toda, Kazumi Tagawa

Takamasa Ohki, Koki Sato, Tomoharu Yamada, Mari Yamagami, Daisaku Ito, Koki Kawanishi, Kentaro Kojima, Michiharu Seki, Nobuo Toda, Kazumi Tagawa, Department of Gastroenterology, Mitsui Memorial Hospital, Tokyo 101-8643, Japan

Author contributions: All authors contributed to this paper.

Ethics approval: This is a retrospective study. We have no statement about institutional review board.
Informed consent: For this type of retrospective study formal consent is not required.
Conflict-of-interest: We have no conflict of interests.
Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at following e-mail address (anb72547@nifty.com). Participants gave informed consent for data sharing.

Correspondence to: Takamasa Ohki, MD, PhD, Department of Gastroenterology, Mitsui Memorial Hospital, 1 Kandaizumicho, Chiyoda-ku, Tokyo 101-8643, Japan. anb72547@nifty.com
Telephone: +81-3-38629111

Received: March 5, 2015
Peer-review started: March 6, 2015
First decision: April 17, 2015
Revised: May 11, 2015
Accepted: June 9, 2015
Article in press: June 11, 2015
Published online: June 28, 2015

Abstract

AIM: To elucidate the efficacies of tolvaptan (TLV) as a treatment for refractory ascites compared with conventional treatment.

METHODS: We retrospectively enrolled 120 refractory ascites patients between January 1, 2009 and September 31, 2014. Sixty patients were treated with oral TLV at a starting dose of 3.75 mg/d in addition to sodium restriction (> 7 g/d), albumin infusion (10-20 g/wk), and standard diuretic therapy (20-60 mg/d furosemide and 25-50 mg/d spironolactone) and 60 patients with large volume paracentesis in addition to sodium restriction (less than 7 g/d), albumin infusion (10-20 g/wk), and standard diuretic therapy (20-120 mg/d furosemide and 25-150 mg/d spironolactone). Patient demographics and laboratory data, including liver function, were not matched due to the small number of patients. Continuous variables were analyzed by unpaired *t*-test or paired *t*-test. Fisher's exact test was applied in cases comparing two nominal variables. We analyzed factors affecting clinical outcomes using receiver operating characteristic curves and multivariate regression analysis. We also used multivariate Cox's proportional hazard regression analysis to elucidate the risk factors that contributed to the increased incidence of ascites.

RESULTS: TLV was effective in 38 (63.3%) patients. The best cut-off values for urine output and reduced urine osmolality as measures of refractory ascites improvement were > 1800 mL within the first 24 h and > 30%, respectively. Multivariate regression analysis indicated that > 25% reduced urine osmolality [odds ratio (OR) = 20.7; $P < 0.01$] and positive hepatitis C viral antibodies (OR = 5.93; $P = 0.05$) were positively correlated with an improvement of refractory ascites, while the total bilirubin level per 1.0 mg/dL (OR = 0.57;

P = 0.02) was negatively correlated with improvement. In comparing the TLV group and controls, only the serum sodium level was significantly lower in the TLV group (133 mEq/L *vs* 136 mEq/L; *P* = 0.02). However, there were no significant differences in the other parameters between the two groups. The cumulative incidence rate was significantly higher in the control group with a median incidence time of 30 d in the TLV group and 20 d in the control group (*P* = 0.01). Cox hazard proportional multivariate analysis indicated that the use of TLV (OR = 0.58; *P* < 0.01), uncontrolled liver neoplasms (OR = 1.92; *P* < 0.01), total bilirubin level per 1.0 mg/dL (OR = 1.10; *P* < 0.01), and higher sodium level per 1.0 mEq/L (OR = 0.94; *P* < 0.01) were independent factors that contributed to incidence.

CONCLUSION: Administration of TLV results in better control of refractory ascites and reduced the incidence of additional invasive procedures or hospitalization compared with conventional ascites treatments.

Key words: Refractory ascites; Tolvaptan; Paracentesis; Decompensated cirrhosis

Core tip: Tolvaptan (TLV) was effective in 38 (63.3%) refractory ascites patients. The best cut-off values for urine output and reduced urine osmolality as measures of refractory ascites improvement were > 1800 mL within the first 24 h and > 30%, respectively. The cumulative incidence rate was significantly higher in the control group with a median incidence time of 30 d in the TLV group and 20 d in the control group. Administration of TLV results in better control of refractory ascites and reduced the incidence of additional invasive procedures or hospitalization compared with conventional ascites treatments.

Ohki T, Sato K, Yamada T, Yamagami M, Ito D, Kawanishi K, Kojima K, Seki M, Toda N, Tagawa K. Efficacy of tolvaptan in patients with refractory ascites in a clinical setting. *World J Hepatol* 2015; 7(12): 1685-1693 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1685.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1685

INTRODUCTION

Hepatic edema and ascites are common complications in decompensated liver cirrhosis patients[1,2]. Refractory ascites is defined as non-responsiveness to sodium dietary restriction and high dose diuretic therapy that occurs in 15%-20% of all ascites patients[3]. Refractory ascites is also associated with a poor quality of life (QOL) and prognosis due to restricted treatment options[4,5].

Tolvaptan (TLV) is a new oral, selective vasopressin V2 receptor antagonist originally developed for the treatment of hypervolemic or euvolemic hyponatremia in patients with heart failure, cirrhosis or syndrome of inappropriate antidiuretic hormone[6,7]. Inhibition of the vasopressin V2 receptor by TLV prevents the insertion of aquaporin 2 water channels into the apical cell membrane of the collecting duct, which increases free water excretion without significantly affecting urinary sodium or potassium secretion[8]. This allows for reduced water retention with elevated serum sodium levels, which is an ideal outcome in decompensated liver cirrhosis patients with refractory ascites.

In Japan, the addition of TLV to conventional diuretic therapy has been useful for the treatment of refractory ascites. A phase 3 study showed a remarkable reduction in ascites with a median loss of 2 kg body weight compared with the placebo controls. However, the administration of TLV was limited to 7 d because of the study design[9], and since decompensated cirrhosis is a progressive disease, even transient improvement of refractory ascites could eventually result in uncontrolled ascites.

Our principal objective was to conduct an observational retrospective study to elucidate the clinical outcomes of TLV. These outcomes included assessing the safety and efficacy of long-term administration, determining the effectiveness cut-off level, and identifying factors that contribute to improved refractory ascites in a clinical setting. In addition, since decompensated cirrhosis is a progressive disease, we examined the time to progression by comparing TLV to conventional treatment.

MATERIALS AND METHODS

Study design

A single center, open label, observational retrospective study was conducted in Mitsui Memorial Hospital (Tokyo, Japan) between January 1st 2009 and September 30 2014. The last follow-up date was October 31 2014.

Inclusion criteria

This study enrolled liver cirrhosis patients 20-80 years of age with refractory ascites who had been receiving loop diuretic and/or anti-aldosterone agents. Refractory ascites was defined as follows: existence of ascites detected by ultrasound under the treatment of a loop diuretic at a daily dose equivalent to ⩾ 40 mg/d furosemide and ⩾ 25 mg/d spironolactone, a loop diuretic at a daily dose equivalent to ⩾ 20 mg/d furosemide and ⩾ 50 mg/d spironolactone, or a loop diuretic alone at a daily dose equivalent to ⩾ 60 mg/d furosemide. Patients were required to be hospitalized or to be available for hospitalization during the treatment period. Exclusion criteria were existence of hepatic encephalopathy, inability to take oral medication, or end stage renal disease on hemodialysis.

Patients

We enrolled 60 refractory ascites patients treated with

TLV until September 30, 2014. We included another 60 refractory ascites patients treated with conventional large volume paracentesis as a control group from our liver disease database between January 1, 2009 and September 30, 2012. Patient demographics and laboratory data, including liver function, were not matched due to the small number of patients. The final analysis included 120 patients.

Therapeutic protocol

Sixty patients received oral TLV at a starting dose of 3.75 mg/d in addition to sodium restriction (> 7 g/d), albumin infusion (10-20 g/wk), and standard diuretic therapy (20-60 mg/d furosemide and 25-50 mg/d spironolactone). Patients could drink water without restriction. The dose of TLV was increased to 7.5 mg/d if insufficient effects were seen. Because the effect of TLV is closely related to serum creatinine levels[9], in some patients with poor renal function, the TLV dose was increased to 15.0 mg/d as directed by the primary physician. TLV was discontinued if patients had encephalopathy, hematemesis, hemodialysis or side effects of > grade 3 assessed using the common terminology criteria for adverse events (CTCAE) version 4.0 or if the patients were unable to take the medication orally. If there were no improvements with TLV, the patients received large-volume paracentesis as a rescue treatment for refractory ascites.

The control patients received conventional large volume paracentesis as a treatment for refractory ascites in addition to sodium restriction (less than 7 g/d), albumin infusion (10-20 g/wk), and standard diuretic therapy (20-120 mg/d furosemide and 25-150 mg/d spironolactone). In all cases, total paracentesis was achieved by removal of all ascites by supplementing 10-20 g albumin per each liter exceeding 5 L. If ascites re-accumulated during the follow-up period, large-volume paracentesis was repeated.

Efficacy assessment

In the TLV-treated group, the primary endpoint was improvement of symptoms, such as bloating sensation or respiratory discomfort, or a > 2-kg reduction in body weight. We also assessed factors that contributed to the effectiveness of TLV by comparing the TLV-treated group with the controls. The primary endpoint was the cumulative incidence rate. In the controls, all ascites was removed transiently by large volume paracentesis. The cumulative incidence rate was defined as the necessity of an additional invasive procedure to treat refractory ascites, including large volume paracentesis, or admission for the treatment of refractory ascites. The secondary endpoint was overall survival rate.

Safety assessment

Patients were monitored throughout the study period, and any incidences of adverse events or deaths were recorded. Adverse events were evaluated using CTCAE version 4.0.

Statistical analysis

Data was expressed as medians (25-75th percentile range) or means ± SD deviations, unless otherwise indicated. Continuous variables were analyzed by unpaired *t*-test or paired *t*-test. Fisher's exact test was applied in cases comparing two nominal variables. We applied receiver operating characteristic (ROC) curve analysis to determine the ideal cut-off levels that indicate the potency of TLV. Univariate and multivariate logistic regression analyses were used to assess the predictors for improvement of refractory ascites by TLV. The cumulative incidence rate and survival rate were estimated using the Kaplan-Meier method compared with the log-rank test. We used univariate and multivariate Cox's proportional hazard regression analysis to elucidate the risk factors that contributed to the increased incidence of ascites. Differences with a $P < 0.05$ were considered statistically significant. Data processing and analysis were performed using StatView version 5 (SAS institute, Cary, NC, United States).

RESULTS

Characteristics of patients treated with TLV

Sixty patients were treated with TLV. The demographics and other baseline characteristics of these patients are shown in Table 1. The mean dosing period was 54 d, and the mean observational period was 168 d. There were 27 (45.0%) Child-Pugh class C patients and 26 (43.3%) patients who had uncontrollable liver neoplasms, defined as TNM stage 3, 4a or 4b. There were four patients with a small amount of ascites who had severe hepatic hydrothorax. The mean estimated glomerular filtration rate (eGFR) was 43.1 mL/min per 1.73 m^2, which indicated the existence of moderate chronic kidney disease.

Changes after administration of TLV

Body weight was significantly reduced during the treatment period. The median reduction in bodyweight was 3 kg ($P < 0.01$), and 38 (63.3%) patients had improved bloating sensation or respiratory discomfort or achieved a > 2-kg weight reduction (Figure 1). The serum sodium concentration increased, peaking 3 d after administration of TLV. The median elevated serum sodium concentration was 4.5 mEq/L ($P < 0.01$, Figure 2). The eGFR was decreased significantly after administration of TLV from 43.1 mL/min per 1.73 m^2 to 38.1 mL/min per 1.73 m^2 ($P < 0.01$, Figure 3). The minimum urine osmolality was markedly reduced 7 d after administration of TLV with a 34% reduction in the urine osmolality rate (Table 2).

Follow-up of TLV treated patients

The median follow-up period was 168 d. During this follow-up period, 33 of 38 patients (86.8%) treated with TLV who improved then experienced exacerbated symptoms, such as bloating sensation or respiratory discomfort, or an increase in body weight, which

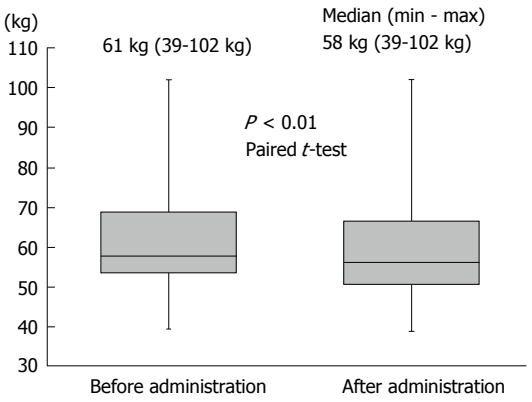

Figure 1 The median reduction in body weight was 3 kg ($P < 0.01$) during the tolvaptan treatment period.

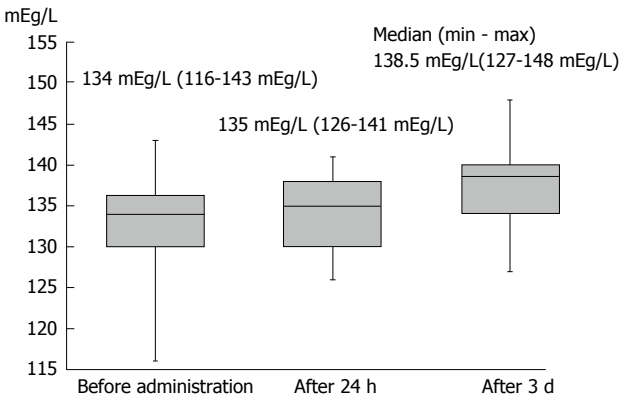

Figure 2 The serum sodium concentration peaked 3 d after administration of tolvaptan. The median elevated serum sodium concentration was 4.5 mEq/L.

Table 1 Baseline characteristics of the patients treated with tolvaptan and controls

Characteristic	TLV group (n = 60)	Controls (n = 60)	P
Age (yr)	67.1 ± 11.2	69.5 ± 9.0	0.21
Male	46 (76.7%)	46 (76.7%)	1.00
Bodyweight (kg)	61 (54-69)	64 (55-73)	0.58
HCV antibody positive	36 (60.0%)	35 (58.3%)	1.00
Child-Pugh class C	27 (45.0%)	24 (40.0%)	0.71
Refractory ascites	56 (93.3%)	60 (100%)	0.12
Hepatic hydrothorax	32 (53.3%)	29 (48.3%)	0.72
Liver neoplasms stage 3, 4a, or 4b	26 (43.3%)	25 (41.7%)	1.00
Serum albumin (g/dL)	2.8 (2.5-3.1)	2.8 (2.5-3.1)	0.98
Total bilirubin (mg/dL)	2.7 (0.7-3.1)	2.8 (1.1-3.6)	0.81
ALT (IU/L)	42 (20-44)	36 (20-53)	0.27
Serum creatinine (mg/dL)	1.40 (0.90-1.61)	1.45 (0.78-1.59)	0.30
eGFR (mL/min per 1.73 m^2)	43.1 (31.0-62.7)	49.8 (33.5-67.8)	0.95
Serum sodium (mEq/L)	133 (130-136)	136 (132-139)	0.02
Platelet count (× 10^3/μL)	114 (58-147)	95 (69-139)	0.80
Prothrombin activity (%)	58.7 (45.0-70.0)	57.3 (42.3-70.2)	0.71

ALT: Alanine aminotransferase; eGFR: Estimated glomerular filtration rate; HCV: Hepatitis C virus; TLV: Tolvaptan.

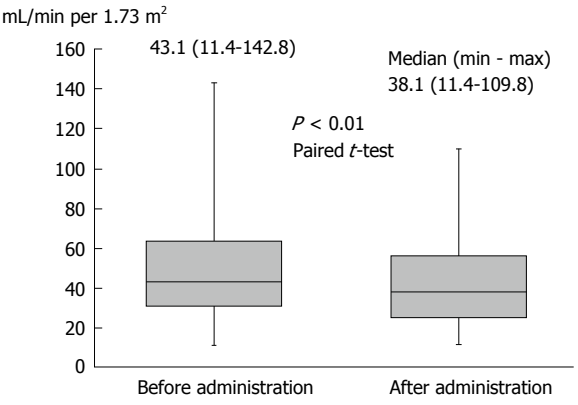

Figure 3 The estimated glomerular filtration rate significantly decreased after administration of tolvaptan from 43.1 to 38.1 mL/min per 1.73 m^2.

indicates the progression of ascites. The median time to progression was 48 d. Although administration of TLV transiently improved refractory ascites, decompensated cirrhosis is a progressive disease that will eventually lead to the development of uncontrolled ascites.

Table 2 Changes in the urine volume and osmolality after administration of tolvaptan

	TLV group (n = 60)
24 h urine volume (mL)	1844 (1200-2400)
24 h water intake (mL)	1231 (894-1463)
Pre-urine osmolality (OSM)	417 (366-487)
Time to achieve the minimum urine osmolality (d)	7 (2-8)
The minimum urine osmolality (OSM)	274 (230-311)
Pre-post-urine osmolality rate (%)	66 (55-79)

OSM: Osmole; TLV: Tolvaptan.

TLV adverse events during the hospitalization period

Patients received TLV while hospitalized for 6-8 d. Adverse events were observed in 19 (31.7%) patients during the hospitalization period. The most common adverse event was thirst. Polydipsia was observed in 14 (23.3%) patients (CTCAE grade 1 to 2). There were no other severe side-effects higher than grade 3 as defined by CTCAE version 4 during the hospitalization period; CTCAE grade 2 tachycardia was seen in 1 patient,

Table 3 Comparison of baseline characteristics (effective *vs* ineffective with tolvaptan)

Characteristic	Effective (n = 38)	Ineffective (n = 22)	P
Dosing period (d)	73 (12-109)	22 (7-36)	0.02
TLV (mg/d)	7.5 (7.5-7.5)	7.5 (7.5-7.5)	0.36
Age (yr)	66.7 ± 11.1	67.0 ± 11.4	0.95
Male	33 (86.8%)	13 (59.1%)	0.02
Bodyweight (kg)	62 (54-68)	60 (48-71)	0.58
[1]Bodyweight (kg)	3.6 (2.1-4.7)	0.2 (0.1-0.8)	< 0.01
HCV antibody positive	27 (71.1%)	9 (40.9%)	0.03
Child-Pugh class C	11 (28.9%)	16 (72.7%)	< 0.01
Refractory ascites	35 (92.1%)	32 (95.5%)	1.00
Hepatic hydrothorax	21 (55.3%)	11 (50.0%)	0.79
Liver neoplasms stage 3, 4a, or 4b	11 (28.9%)	15 (68.2%)	< 0.01
Serum albumin (g/dL)	2.9 (2.6-3.2)	2.7 (2.3-3.0)	0.20
Total bilirubin (mg/dL)	1.7 (0.7-1.9)	4.5 (1.5-6.3)	< 0.01
ALT (IU/L)	37 (20-41)	50 (25-77)	0.18
Serum creatinine (mg/dL)	1.53 (0.89-2.09)	1.17 (0.95-1.40)	0.11
eGFR (mL/min per 1.73 m^2)	49.8 (26.7-62.7)	51.7 (34.6-62.5)	0.80
Serum sodium (mEq/L)	134 (132-138)	131 (128-136)	0.03
Platelet count (× 10^3/μL)	107 (58-144)	127 (65-190)	0.27
Prothrombin activity (%)	61.5 (46.3-73.2)	53.8 (41.2-64.2)	0.10

[1]Reduction. ALT: Alanine aminotransferase; eGFR: Estimated glomerular filtration rate; HCV: Hepatitis C virus; TLV: Tolvaptan.

Table 4 Comparison of the changes after administration of tolvaptan (effective *vs* ineffective with tolvaptan)

	Effective (n = 38)	Ineffective (n = 22)	P
[1]Bodyweight	3.6 (2.1-4.7)	0.2 (0.1-0.8)	< 0.01
24 h urine volume (mL)	2154 (1448-2516)	1352 (871-1675)	< 0.01
24 h water intake (mL)	1238 (800-1429)	1235 (1000-1463)	0.96
Pre-urine osmolality (OSM)	445 (411-485)	417 (335-495)	0.42
Time to achieve the minimum urine osmolality (d)	7 (3-9)	4 (1-6)	0.02
The minimum urine osmolality (OSM)	251 (213-288)	313 (256-359)	< 0.01
Pre-post-urine osmolality rate (%)	58 (51-69)	78 (66-90)	< 0.01

[1]Reduction. OSM: Osmole.

CTCAE grade 2 fatigue in 1 patient, CTCAE grade 2 cough in 1 patient, and CTCAE grade 2 acute kidney injury in 1 patient. One patient discontinued TLV due to the necessity for frequent blood tests. During the entire follow-up period, 8 (13.3%) patients developed hepatic encephalopathy. However, it is difficult to determine whether this was due to adverse events or the natural course of decompensated cirrhosis.

Comparison of TLV effectiveness

There were 38 patients who had improved symptoms, such as a bloating sensation or respiratory discomfort, or a loss of 2 kg body weight. There were significant differences in TLV effectiveness related to the proportion of male patients, comorbidity with hepatitis C virus (HCV), severe liver dysfunction, and uncontrolled liver neoplasms. Blood tests showed significantly higher levels of serum bilirubin and lower levels of sodium in patients in whom TLV was ineffective (Table 3).

Comparison of the changes between effective and ineffective patients after administration of TLV

We evaluated the changes in urine volume and osmolality between the two groups after administration of TLV (Table 4). Urine volume recorded at 24 h after administration of TLV was significantly higher in the effective group (2154 mL *vs* 1352 mL; $P < 0.01$). The minimum urine osmolality was also significantly lower in the effective group (251 osmole *vs* 313 osmole; $P < 0.01$). The time to reach minimum urine osmolality was significantly longer in the effective group (median: 7 d *vs* 4 d; $P = 0.02$).

eGFR analysis to determine the cut-off level

Figure 4A indicated that a reduction in urine osmolality over 25% was the single best cut-off level to clarify the improvement of refractory ascites with 89.5% sensitivity and 59.1% specificity. A combined measure of urine > 1800 mL within the first 24 h and a reduction in urine osmolality > 30% were the best cut-off levels to clarify refractory ascites improvements with 84.2% sensitivity and 81.8% specificity (Figure 4B).

Multivariate regression analysis to elucidate the factor contributing to improved refractory ascites

Multivariate regression analysis was performed to

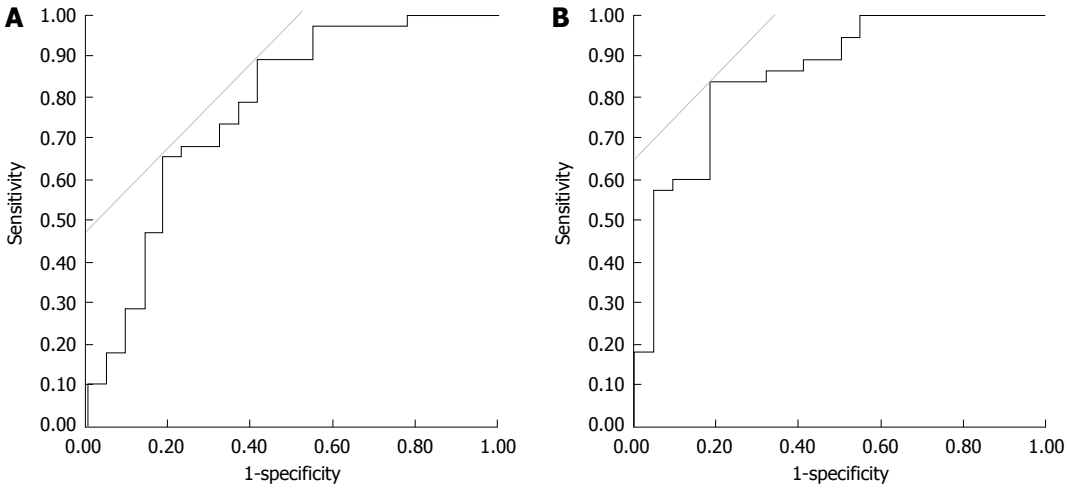

Figure 4 Receiver operating characteristic analysis. A: A reduction in urine osmolality > 25% was the single best cut-off level for improvement of refractory ascites with 89.5% sensitivity and 59.1% specificity; B: A combination of urine output > 1800 mL within the first 24 h and a 30% reduction in urine osmolality were the best cut-off levels for improvement of refractory ascites with 84.2% sensitivity and 81.8% specificity.

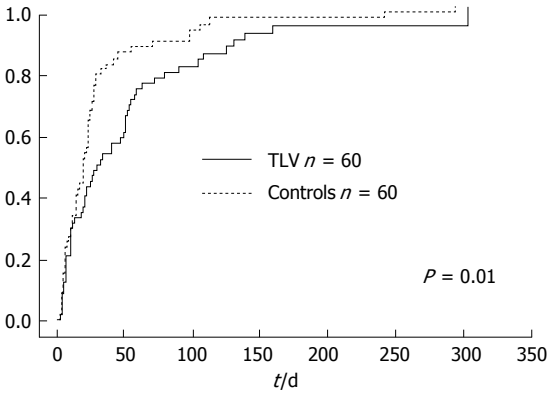

Figure 5 The cumulative incidence rate was significantly higher in the control group, with a median incidence time of 30 d in the tolvaptan group and 20 d in the control group.

evaluate the factors found to be significant in univariate analysis. As a result, a reduction in urine osmolality > 25% [odds ratio (OR) = 20.7; $P < 0.01$] and the presence of positive HCV antibodies (OR = 5.93; $P = 0.05$) were positively correlated with an improvement of refractory ascites, while the total bilirubin level per 1.0 mg/dL (OR = 0.57; $P = 0.02$) was negatively correlated with improvement (Table 5).

Comparing the patients backgrounds between the TLV group and historical controls

Due to the small number of patients, their backgrounds and laboratory data, including liver function, were not matched. In comparing the TLV group and controls, only the serum sodium level was significantly lower in the TLV group (133 mEq/L *vs* 136 mEq/L; $P = 0.02$). However, there were no significant differences in the other parameters between the two groups (Table 1).

Cumulative incidence rate

The cumulative incidence rate was defined as the need for an additional invasive procedure to treat refractory ascites, including large volume paracentesis, or hospital admission for the treatment of refractory ascites. The cumulative incidence rate was significantly higher in the control group, with a median incidence time of 30 d in the TLV group and 20 d in the control group ($P = 0.01$, Figure 5).

Table 5 Multivariate regression analysis assessing the effectiveness of tolvaptan

Variables	HR (95%CI)	*P*
Reduction of urine osmolality over 25%	20.7 (3.26-132)	< 0.01
Age (yr)	1.00 (0.93-1.08)	0.91
HCV antibody positive	5.93 (1.01-34.8)	0.05
Uncontrollable liver neoplasms	0.68 (0.03-1.20)	0.21
Total bilirubin (per 1.0 mg/dL)	0.57 (0.35-0.93)	0.02
Na (per 1.0 mEq/mL)	0.99 (0.84-1.17)	0.93

HCV: Hepatitis C virus.

Factors affecting the incidence of refractory ascites

We used univariate and multivariate Cox's proportional hazard regression analysis to elucidate the risk factors predicting incidence. Cox hazard proportional multivariate analysis indicated that the use of TLV (OR = 0.58; $P < 0.01$), uncontrolled liver neoplasms (OR = 1.92; $P < 0.01$), a total bilirubin level per 1.0 mg/dL (OR = 1.10; $P < 0.01$), and a higher sodium level per 1.0 mEq/L (OR = 0.94; $P < 0.01$) were independent factors contributing to the incidence of refractory ascites (Table 6).

Cumulative survival rate

There was no significant difference in cumulative survival rate between the TLV group (median survival time = 121 d) and control group (median survival time = 123 d; $P = 0.57$, Figure 6).

DISCUSSION

This study demonstrated that administration of TLV

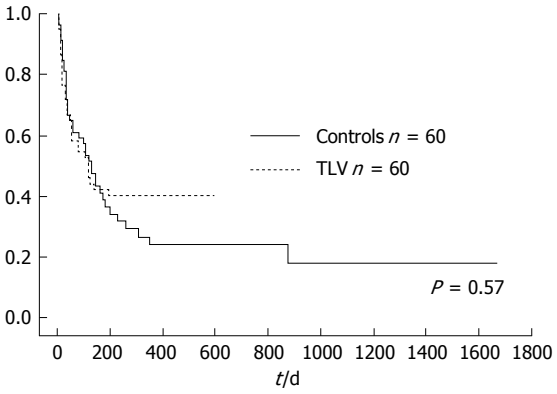

Figure 6 There was no significant difference in cumulative survival rate between the groups, with a median survival time of 121 d in the tolvaptan group and 123 d in the control group.

Table 6 Cox's proportional hazard multivariate regression analysis assessing the factors contributing to the incidence of refractory ascites

Variables	HR (95%CI)	*P*
Use of TLV	0.58 (0.39-0.87)	< 0.01
Age (yr)	1.01 (0.99-1.03)	0.19
Uncontrollable liver neoplasms	1.92 (1.23-2.94)	< 0.01
ALT (IU/L)	1.00 (0.98-1.01)	0.11
Total bilirubin (mg/dL)	1.10 (1.03-1.18)	< 0.01
Na (mEq/mL)	0.94 (0.91-0.98)	< 0.01

HCV: Hepatitis C virus; ALT: Alanine aminotransferase; TLV: Tolvaptan.

improved refractory ascites in 38 (63.3%) patients. Before the introduction of TLV, treatment of refractory ascites was initially based on invasive procedures, such as paracentesis, concentrated ascites reinfusion therapy, the Denver® shunt, or transjugular intrahepatic portosystemic shunt[10-12]. Although, increased doses of loop diuretic agents or spironolactone were also allowed, the effectiveness was limited and renal function deteriorated[13]. TLV is a less invasive novel oral aquaresis treatment that complements conventional refractory ascites therapies. The effectiveness of TLV is limited, however, because decompensated liver cirrhosis is a progressive disease, although compared with conventional large volume paracentesis treatment, TLV may prolong the time to disease progression. Thus, TLV may be an important therapy that transiently improves refractory ascites to avoid early invasive treatment or hospitalization.

Determining the best timing of TLV administration is difficult, since TLV was not effective in 22 cases of patients with either uncontrolled liver neoplasms or severe liver dysfunction Child-Pugh class C. In this study, we defined refractory ascites as follows: existence of ascites detected by ultrasound under the treatment of a loop diuretic at a daily dose equivalent to ⩾ 40 mg/d furosemide and ⩾ 25 mg/d spironolactone, a loop diuretic at a daily dose equivalent to ⩾ 20 mg/d furosemide and ⩾ 50 mg/d spironolactone, or a loop diuretic alone at a daily dose equivalent to ⩾ 60 mg/d furosemide. If refractory ascites was not controlled with these doses of standard diuretic medicines, TLV administration should be considered. If TLV is initiated later, its effects may be restricted due to liver dysfunction or progression of liver neoplasms.

The change in urine osmolality is an important factor to consider when evaluating the effectiveness of TLV. It has been reported that a reduction in the rate of urine osmolality can predict TLV effectiveness in chronic heart failure patients[14]. In these patients, urine osmolality was measured before and 4-6 h after administration of TLV. However, in refractory ascites patients, our current study showed that the minimum urine osmolality was reached at a median of 7 d after TLV administration (Table 2), which indicated that the reduction rate in urine osmolality was a promising measure of TLV effectiveness. However, the sensitivity and specificity were more accurate using the combination of 24-h urine volume and urine osmolality reduction rate after the administration of TLV. The mechanisms underlying this difference remain unknown, although it has been reported that elevated intra-abdominal pressure due to refractory ascites might affect renal function[15]. Treating refractory ascites with TLV may lead to a gradual improvement of glomerular blood flow. Thus, the decrease in urine osmolality was slower in refractory ascites patients compared with the change in chronic heart failure patients. At any rate, the results showed that it was difficult to predict the effectiveness of TLV in a short-term study.

TLV is thought to be a relatively safe drug with little impact on renal function[9,16]. However, in our study, TLV administration deteriorated renal function. Although there is a possibility that TLV could lead to dehydration and decrease eGFR, progressive liver disease induces renal impairment, a phenomenon known as hepatorenal syndrome[17]. The control patients also had a significant decrease in eGFR during the follow-up period. These patients were treated with large-volume paracentesis under infusion of albumin, which was also reported to have less impact on renal function[18]. In a comprehensive manner, the decrease in eGFR did not depend on TLV but on progressive liver disease itself. Thus, we concluded that TLV could be used safely in patients with refractory ascites.

An elevation in the serum sodium level is a major side effect of TLV[19]. Advanced liver cirrhosis tends to result in hyponatremia, and TLV is used as a treatment option for hyponatremia in patients with euvolemic hyponatremia[20]. In this study, we did not experience any side effects of hypernatremia in patients treated with TLV. Although the median level of serum sodium was elevated to a maximum of 138.5 mEq/L, this was preferable to hyponatremia in advanced liver cirrhosis patients. Comorbidity with hyponatremia has a high risk for mortality[21]. In this current study, there were no significant differences in the cumulative survival rate between the TLV and control group. However, the

patients' backgrounds between the two groups were not matched, and it was difficult to elucidate the true effect of treating hyponatremia with TLV. A further prospective study is needed to clarify the outcome of improving hyponatremia on the cumulative survival rate.

There were no severe adverse events that exceeded CTCAE grade 2 during the hospitalization period. The most common side effect was thirst observed in 14 (23.3%) patients, which was similar to previous reports[22,23]. Hypernatremia was also reported as an adverse effect of TLV treatment[20]. However, there were no patients with a severe elevation in serum sodium. During the follow-up period after hospital discharge, hepatic encephalopathy occurred in 8 patients. It is difficult to clarify whether the cause of encephalopathy was administration of TLV or part of the natural course of severe liver dysfunction. On the whole, TLV is a safe treatment for refractory ascites patients with advanced liver cirrhosis.

This study has several limitations. It was not a randomized retrospective study, and the control group was not matched to the TLV group. However, this study was conducted in a realistic clinical setting. We propose that the clinical outcomes of TLV will have significant meaning for the treatment of refractory ascites, and the current results revealed that the best new indicators to predict efficacy of TLV were a 24-h urine volume > 1800 mL and > 30% urine osmolality reduction rate, as well as prolongation of progression-free survival. Thus, this retrospective study will serve as a reference for using TLV in refractory ascites patients.

In conclusion, administration of TLV achieved not only better control of refractory ascites but also improved QOL by avoiding additional invasive procedures, including paracentesis, or the need for hospitalization compared with conventional ascites treatments.

COMMENTS

Background

Hepatic edema and ascites are common complications in decompensated liver cirrhosis patients. In Japan, the addition of tolvaptan (TLV) to conventional diuretic therapy has been useful for the treatment of refractory ascites. The principal objective was to conduct an observational retrospective study to elucidate the safety and efficacy of long-term administration, determining the effectiveness cut-off level, and identifying factors that contribute to improved refractory ascites in a clinical setting. In addition, since decompensated cirrhosis is a progressive disease, the authors examined the time to progression by comparing TLV to conventional treatment.

Research frontiers

TLV was reported as a new oral, selective vasopressin V2 receptor antagonist originally developed for the treatment of hypervolemic or euvolemic hyponatremia in patients with heart failure, cirrhosis or syndrome of inappropriate antidiuretic hormone. A phase 3 study showed a remarkable reduction in ascites with a median loss of 2 kg body weight compared with the placebo controls. However, the administration of TLV was limited to 7 d because of the study design.

Innovations and breakthroughs

The authors concluded that administration of TLV resulted in better control of refractory ascites and reduced the incidence of additional invasive procedures or hospitalization compared with conventional ascites treatments. The authors also determined the best values for urine output and reduced urine osmolality as measures of refractory ascites.

Applications

This study may provide a new treatment option for refractory ascites. TLV could use safely in refractory ascites patients with over 60% of effectiveness. Administration of TLV may be considered before invasive procedure such as paracentesis.

Peer-review

This is an interesting topic.

REFERENCES

1 **Ginès P**, Cárdenas A, Arroyo V, Rodés J. Management of cirrhosis and ascites. *N Engl J Med* 2004; **350**: 1646-1654 [PMID: 15084697]

2 **Moore KP**, Wong F, Gines P, Bernardi M, Ochs A, Salerno F, Angeli P, Porayko M, Moreau R, Garcia-Tsao G, Jimenez W, Planas R, Arroyo V. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. *Hepatology* 2003; **38**: 258-266 [PMID: 12830009]

3 **Runyon BA**. Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. *Hepatology* 2013; **57**: 1651-1653 [PMID: 23463403]

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

5 **Planas R**, Montoliu S, Ballesté B, Rivera M, Miquel M, Masnou H, Galeras JA, Giménez MD, Santos J, Cirera I, Morillas RM, Coll S, Solà R. Natural history of patients hospitalized for management of cirrhotic ascites. *Clin Gastroenterol Hepatol* 2006; **4**: 1385-1394 [PMID: 17081806]

6 **Vaidya C**, Ho W, Freda BJ. Management of hyponatremia: providing treatment and avoiding harm. *Cleve Clin J Med* 2010; **77**: 715-726 [PMID: 20889809]

7 **Orlandi C**, Zimmer CA, Gheorghiade M. Tolvaptan for the treatment of hyponatremia and congestive heart failure. *Future Cardiol* 2006; **2**: 627-634 [PMID: 19804253]

8 **Decaux G**, Soupart A, Vassart G. Non-peptide arginine-vasopressin antagonists: the vaptans. *Lancet* 2008; **371**: 1624-1632 [PMID: 18468546]

9 **Sakaida I**, Kawazoe S, Kajimura K, Saito T, Okuse C, Takaguchi K, Okada M, Okita K. Tolvaptan for improvement of hepatic edema: A phase 3, multicenter, randomized, double-blind, placebo-controlled trial. *Hepatol Res* 2014; **44**: 73-82 [PMID: 23551935]

10 **Sanyal AJ**, Genning C, Reddy KR, Wong F, Kowdley KV, Benner K, McCashland T. The North American Study for the Treatment of Refractory Ascites. *Gastroenterology* 2003; **124**: 634-641 [PMID: 12612902]

11 **Ginès P**, Arroyo V, Vargas V, Planas R, Casafont F, Panés J, Hoyos M, Viladomiu L, Rimola A, Morillas R. Paracentesis with intravenous infusion of albumin as compared with peritoneovenous shunting in cirrhosis with refractory ascites. *N Engl J Med* 1991; **325**: 829-835 [PMID: 1875966]

12 **Zaak D**, Paquet KJ, Kuhn R. Prospective study comparing human albumin vs. reinfusion of ultrafiltrate-ascitic fluid after total paracentesis in cirrhotic patients with tense ascites. *Z Gastroenterol* 2001; **39**: 5-10 [PMID: 11215366]

13 **Peacock WF**, Costanzo MR, De Marco T, Lopatin M, Wynne J, Mills RM, Emerman CL. Impact of intravenous loop diuretics on outcomes of patients hospitalized with acute decompensated heart failure: insights from the ADHERE registry. *Cardiology* 2009; **113**: 12-19 [PMID: 18931492]

14 **Imamura T**, Kinugawa K, Shiga T, Kato N, Muraoka H, Minatsuki S, Inaba T, Maki H, Hatano M, Yao A, Kyo S, Nagai R. Novel criteria of urine osmolality effectively predict response to tolvaptan in decompensated heart failure patients--association between non-responders and chronic kidney disease. *Circ J* 2013; **77**: 397-404 [PMID: 23131721]

15 **Mullens W**, Abrahams Z, Skouri HN, Francis GS, Taylor DO,

Starling RC, Paganini E, Tang WH. Elevated intra-abdominal pressure in acute decompensated heart failure: a potential contributor to worsening renal function? *J Am Coll Cardiol* 2008; **51**: 300-306 [PMID: 18206740]

16 **Zhang X**, Wang SZ, Zheng JF, Zhao WM, Li P, Fan CL, Li B, Dong PL, Li L, Ding HG. Clinical efficacy of tolvaptan for treatment of refractory ascites in liver cirrhosis patients. *World J Gastroenterol* 2014; **20**: 11400-11405 [PMID: 25170228]

17 **Arroyo V**, Ginès P, Gerbes AL, Dudley FJ, Gentilini P, Laffi G, Reynolds TB, Ring-Larsen H, Schölmerich J. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. *Hepatology* 1996; **23**: 164-176 [PMID: 8550036]

18 **Ginès P**, Titó L, Arroyo V, Planas R, Panés J, Viver J, Torres M, Humbert P, Rimola A, Llach J. Randomized comparative study of therapeutic paracentesis with and without intravenous albumin in cirrhosis. *Gastroenterology* 1988; **94**: 1493-1502 [PMID: 3360270]

19 **Ambrosy A**, Goldsmith SR, Gheorghiade M. Tolvaptan for the treatment of heart failure: a review of the literature. *Expert Opin Pharmacother* 2011; **12**: 961-976 [PMID: 21401442]

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

21 **Umemura T**, Shibata S, Sekiguchi T, Kitabatake H, Nozawa Y, Okuhara S, Kimura T, Morita S, Komatsu M, Matsumoto A, Tanaka E. Serum sodium concentration is associated with increased risk of mortality in patients with compensated liver cirrhosis. *Hepatol Res* 2014; Epub ahead of print [PMID: 25163635]

22 **Sakaida I**, Yamashita S, Kobayashi T, Komatsu M, Sakai T, Komorizono Y, Okada M, Okita K. Efficacy and safety of a 14-day administration of tolvaptan in the treatment of patients with ascites in hepatic oedema. *J Int Med Res* 2013; **41**: 835-847 [PMID: 23685892]

23 **Okita K**, Kawazoe S, Hasebe C, Kajimura K, Kaneko A, Okada M, Sakaida I. Dose-finding trial of tolvaptan in liver cirrhosis patients with hepatic edema: A randomized, double-blind, placebo-controlled trial. *Hepatol Res* 2014; **44**: 83-91 [PMID: 23530991]

P- Reviewer: De Ponti F, Enomoto H, He JY, Qin JM
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1694

World J Hepatol 2015 June 28; 7(12): 1694-1700
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Downstaging disease in patients with hepatocellular carcinoma outside up-to-seven criteria: Strategies using degradable starch microspheres transcatheter arterial chemo-embolization

Antonio Orlacchio, Fabrizio Chegai, Stefano Merolla, Simona Francioso, Costantino Del Giudice, Mario Angelico, Giuseppe Tisone, Giovanni Simonetti

Antonio Orlacchio, Fabrizio Chegai, Stefano Merolla, Costantino Del Giudice, Giovanni Simonetti, Department of Diagnostic and Molecular Imaging, Radiation Therapy and Interventional Radiology, University Hospital Tor Vergata, 00133 Rome, Italy
Simona Francioso, Mario Angelico, Liver Unit, University Hospital Tor Vergata, 00133 Rome, Italy
Giuseppe Tisone, Organ Transplantation Unit, University Hospital Tor Vergata, 00133 Rome, Italy

Author contributions: Orlacchio A, Chegai F, Francioso S, Angelico M, Tisone G and Simonetti G designed research; Orlacchio A, Chegai F, Merolla S and Del Giudice C performed research; Orlacchio A, Chegai F, Merolla S and Francioso S contributed new reagents or analytic tools; Orlacchio A, Chegai F and Francioso S analyzed data; Orlacchio A, Chegai F and Francioso S wrote the paper.

Ethics approval: This study was approved by the Ethics Committee of our institution, No. 175/13.
Informed consent: All patients declared the informed consent.
Conflict-of-interest: All authors declare no conflict of interest.
Data sharing: Thecnical appendix, statistical code and data set available from the corresponding auhor at aorlacchio@uniroma2.it. The present data are anonymized without risk of identification.

Correspondence to: Antonio Orlacchio, MD, Department of Diagnostic and Molecular Imaging, Radiation Therapy and Interventional Radiology, University Hospital Tor Vergata, Viale Oxford 81, 00133 Rome, Italy. aorlacchio@uniroma2.it
Telephone: +39-6-20902400
Fax: +39-6-20902404

Received: November 28, 2014
Peer-review started: November 29, 2014
First decision: January 8, 2015
Revised: January 20, 2015
Accepted: May 26, 2015
Article in press: May 27, 2015
Published online: June 28, 2015

Abstract

AIM: To evaluate the downstaging rates in hepatitis C virus-patients with hepatocellular carcinoma (HCC), treated with degradable starch microspheres transcatheter arterial chemoembolization (DSM-TACE), to reach new-Milan-criteria (nMC) for transplantation.

METHODS: This study was approved by the Ethics Committee of our institution. From September 2013 to March 2014 eight patients (5 men and 3 women) with liver cirrhosis and multinodular HCC, that did not meet nMC at baseline, were enrolled in this study. Patients who received any other type of treatment such as termal ablation or percutaneous ethanol injection were excluded. DSM-TACE was performed in all patients using EmboCept® S and doxorubicin. Baseline and follow-up computed tomography or magnetic resonance imaging was assessed measuring the longest enhancing axial dimension of each tumor according to the modified Response Evaluation Criteria In Solid Tumors measure-

ments, and medical records were reviewed.

RESULTS: DSM-TACE was successfully performed in all patients without major complication. We treated 35 lesions (mean 4.3 per patient). Six of eight patients (75%) had their HCC downstaged to meet nMC. Every patient whose disease was downstaged eventually underwent transplantation. The six patients who received transplant were still living at the time of this writing, without recurrence of HCC. Baseline age (P = 0.25), Model for End-stage Liver Disease score (P = 0. 77), and α-fetoprotein level (P = 1.00) were similar between patients with and without downstaged HCC.

CONCLUSION: DSM-TACE represents a safely and effective treatment option with similar safety and efficacy of conventional chemoembolization and could be successfully performed also for downstaging disease in patients without nMC, allowing them to reach liver transplantation.

Key words: Hepatocellular carcinoma; Transcatheter arterial chemoembolization; Liver transplantation; Degradable starch microspheres; New-Milan-criteria; Recurrence-free survival; Locoregional therapies

Core tip: Liver transplantation (LT) is the standard of care for select patients with hepatocellular carcinoma (HCC) and cirrhosis and recently more transplant centers use the new Milan criteria to assess the candidacy of HCC patients for LT. This manuscript reports a preliminary experience on the HCC treatment in liver transplant candidates without new-Milan-criteria, using a new technique of transcatheter arterial chemoembolization with degradable starch microspheres transcatheter arterial chemoembolization (DSM-TACE). Providing a temporary embolization DSM-TACE allows to treat more patients with an impaired liver function reducing toxicity due to standard arterial embolization. Moreover good down-staging rates after repeated DSM-TACE were successfully achieved.

Orlacchio A, Chegai F, Merolla S, Francioso S, Del Giudice C, Angelico M, Tisone G, Simonetti G. Downstaging disease in patients with hepatocellular carcinoma outside up-to-seven criteria: Strategies using degradable starch microspheres transcatheter arterial chemo-embolization. *World J Hepatol* 2015; 7(12): 1694-1700 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1694.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1694

INTRODUCTION

Currently, trans-arterial chemoembolization (TACE) represents the most accepted and widely used treatment for large or multinodular hepatocellular carcinoma (HCC), notably in patients with a relatively preserved liver function without vascular invasion and/or extra-hepatic spread[1].

HCC ranks as the third most common cause of cancer-related death and is the sixth most common form of cancer worldwide[2].

The purpose of TACE is to reach high and lasting concentrations of chemotherapeutic drugs in the tumor site in order to increase their uptake by neoplastic cells, reducing at the meantime systemic side effects.

Nevertheless, liver transplantation (LT) remains the ideal treatment for small HCC resulting from chronic liver disease. Given the difference between donor organ availability and need, rigorous inclusion criteria, named the Milan criteria, have been originally adopted to warrant tumor free survival after LT. They was defined as having a single tumor 5 cm or less in diameter in patients with single HCC, or up to 3 separate lesions < 3 cm and without microscopic vascular invasion or extra-hepatic spread[3].

The excessive intransigence of those criteria has been a matter of debate, with some groups questioning their restrictive settings[4,5]. Afterwards, the tendency of the Milan group was to make the previous criteria less stringent, so were developed new ones called the up-to-seven criteria [new-Milan-criteria (nMC)]: HCCs with seven as the sum of the size of the largest tumor (in cm) and the number of tumors[6]. Several studies have then evaluated their reliability and usefulness in assessing the possible candidates for LT among patients with HCC[7,8]. Nevertheless, albeit have been analyzed all over the world, up-to-seven criteria have not been as widely accepted as the Milan criteria.

In prospective studies on selected patients with HCC, loco-regional treatments has been shown both to downstage the disease and to confer acceptable disease free survival after liver trasplantation. Several locoregional treatment for downstaging may be used[9,10]. Green *et al*[10] using doxorubicin-eluting bead transarterial chemoembolization showed that a treatment with drug eluting beads (DEB) has a high likelihood (77%) of downstaging the disease so that it can meet Milan criteria.

Nevertheless, by interrupting blood flow to the tumor, TACE induces necrosis at the site of disease but it may create conditions that permit or even encourage angiogenesis[11].

A recent study performed by Pieper *et al*[12] in a swine model demonstrated that using degradable starch microspheres (DSMs) a short term embolization can be achieved (30 min), without significant hystological differences in damage between treated or untreated liver. Thus, using DSMs it is possible to avoid the paradoxical angiogenetic effect due to ischemia[13], emphasizing the effects of arterial chemotherapy.

As reported by Furuse *et al*[14] in their work, DSM-TACE should not be employed as a single session therapy but needs to be performed at least three times until there was evidence of disease progression or unacceptable

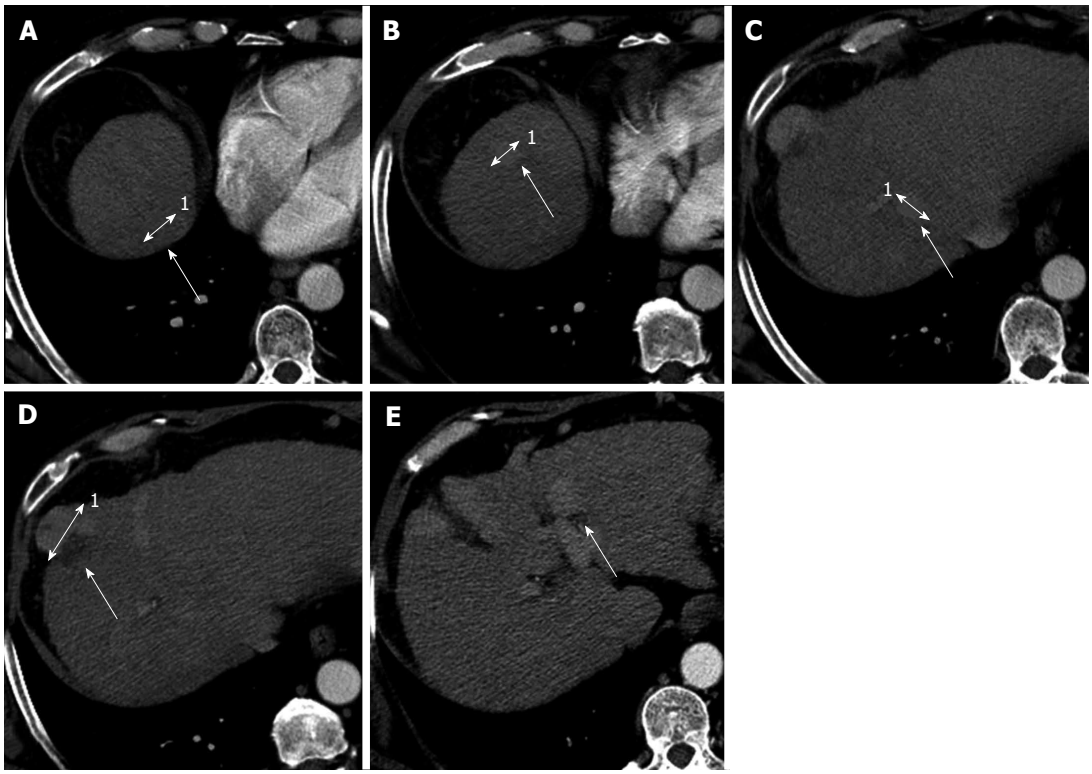

Figure 1 Contrast enhanced computed tomography in patient with multinodular hepatocellular carcinoma before the degradable starch microspheres transcatheter arterial chemoembolization not in agreement with new-Milan-criteria. The enhanced computed tomography images show 5 hypervascular lesions, in the patients with a score of 8 using the new-Milan-criteria. A: Hepatocellular carcinoma (HCC) on Ⅶ segment with maximum diameter of 14 mm[1]; B: HCC on Ⅷ segment with maximum diameter of 10 mm[1]; C: HCC on Ⅶ segment with maximum diameter of 12 mm[1]; D: HCC on Ⅷ segment with maximum diameter of 31 mm[1]; E: HCC on Ⅳ-Ⅱ segment with maximum diameter of 14 mm.

toxicity.

Anyhow this procedure is well tolerated by the patients and it could be safe also in those with a severe hepatic disease, even with a Child-Pugh score C.

The aim of our work is to assess downstaging rates in patients with HCC using DSM TACE and doxorubicin, in order to meet new Milan criteria and to create conditions allowing LT.

MATERIALS AND METHODS

The herein described study was approved by the Ethics Committee of our institution. The Child-Pugh score, the Model for End Stage Liver Disease (MELD score), radiological and pathological tumor classification according to nMC, demographics (age, sex), etiology of cirrhosis, laboratory tests, imaging studies and pathology reports were recorded for each patient.

Exclusion criteria were an active peptic ulcer, unmanageable ascites or pleural effusion, hepatic encephalopathy, or any other preexisting medical condition of sufficient severity to prevent full compliance with the study. Moreover, patients who received any other type of treatment such as thermoablative treatment, or percutaneous ethanol injection were excluded.

All patients in our study were selected during the follow-up for liver cirrhosis and the opportunity of locoregional ablative treatment was carefully evaluated by a multidisciplinary team of hepatologists, surgeons, radiologists and interventional radiologists[15].

We have selected patients with a multinodular HCC that did not meet, at baseline, Milan criteria. The diagnosis of HCC was set according to the guidelines of the European Association for the Study of the Liver[16].

All patients underwent, within two weeks before treatment, a dynamic multislice computed tomography (CT) (Light Speed 64 CT, GE Medical Systems, Milwaukee, United States) (Figure 1).

A dynamic CT evaluation following the DSM-TACE treatment was performed to assess whether HCC patients were downstaged to meet the nMC.

Embolization technique

Under local anesthesia the right common femoral artery was punctured using the Seldinger approach and a 4-French (4-Fr) sheath introducer was placed to secure the access site. Selective hepatic angiography was then performed with a 4-Fr catheter. The tip of the catheter was placed in the proper hepatic artery or right and/or left hepatic artery to inject embolic agents (Figure 2).

A combination of DSM, at a concentration of 225 mg per 6 mL of contrast medium, and doxorubicin at a dose of 50 mg/m^2, was injected until embolization of peripheral hepatic arteries was confirmed; the aforementioned procedure was repeated for three times every 4 to 6 wk.

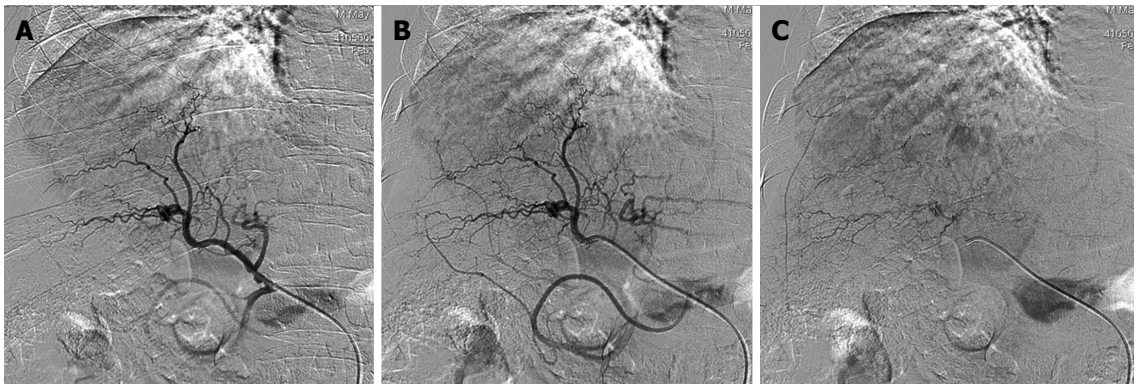

Figure 2 Hepatic intraprocedural angiograms show staining of multiple tumors, which are diagnosed as hepatocellular carcinoma. A and B: Early arterial digital subtraction angiography (DSA) phase; C: Late parenchymal DSA phase.

Table 1 Demographics and clinical characteristics of the series

Characteristics	
Gender (male), *n*	5
Mean age (yr) ± SD	59 ± 6
Mean aspartate aminotransferase levels (IU/L) ± SD	68.8 ± 53
Mean alanine transaminase levels (IU/L) ± SD	62.5 ± 65
Mean gamma-glutamyl-transpeptidase levels (IU/L) ± SD	156 ± 211
Mean creatinine levels (mg/dL)	0.86 ± 0.25
Mean international normalized ratio	1.2 ± 0.1
Mean platelet levels (10^3/μL) ± SD	975000 ± 57000
Mean alfa fetoprotein (ng/mL) ± SD	14.9 ± 21.4

Imaging evaluations

All patients were scanned by contrast-enhanced CT (ceCT), at the baseline, within two weeks before the first DSM-TACE. Thirty days after each DSM-TACE, patients were re-assessed by ceCT.

CeCT protocol

To assess any changes in the size of the nodule and/or appearance of new lesions, multidetector ceCT scan was obtained with triphasic technique (30, 65 and 180 s) after intravenous administration of 125-175 mL of iodinate contrast medium (350 mg iodine/mL) depending on the body mass index of the patient, followed by administration of saline solution (20-30 mL) using an automatic injector at flow rates of 3-4 mL/s.

CT was performed with the following parameters: rotation time of 0.6 s; 2.5 to 5 mm thick sections with the possibility of back-reconstructions up to 0.6 mm; automatic milliamperage (mA) (min 300 mA, max 450 mA), 120 kV (Figure 2).

Assessment of treatment effectiveness

Therapeutic response was determined on a lesion-by-lesion basis by evaluating morphological and vascularization changes in tumor between baseline and subsequent ratings based on ceCT.

The treatment response was evaluated according to modified Response Evaluation Criteria in Solid Tumors criteria[17]. Based on CT results, tumor response to treatment, were defined complete [complete response (CR)] when there was disappearance of all signs of the lesion and no pathological enhancement was detectable on the edges of the area treated and partial or incomplete [partial response (PR)] in case of a limited destruction of the lesion, with at least a 30% reduction in volume. A lesion not presenting such dimensional decrease or increase was considered stable disease (SD).

Statistical analysis

A descriptive statistics for baseline demographics and staging criteria were calculated.

Continuous variables were reported as the mean ± SD or the median (25%-75% interquartile range), depending on the variable distribution whereas categorical variables were reported as numbers and percentages.

Recurrence-free survival was calculated from the day of the transplantation to the first evidence of tumor relapse during follow-up or, in patients without recurrence, to the most recent follow-up visit. Follow-up of those patients who died without evidence of recurrence was censored at the time of death.

RESULTS

From September 2013 to March 2014 eight patients (5 men and 3 women) with liver cirrhosis and multinodular HCC were enrolled in this study.

Demographics and clinical characteristic are reported in Table 1.

The age of patients ranged from 56-64 years. Four patients were in Child-Pugh class B and four patients were in Child-Pugh class C. All of them had hepatitis C virus (HCV)-related cirrhosis, 2 patients had a coinfection with HBV and one had a combined HCV and alcohol related cirrhosis.

The α-fetoprotein (AFP) levels, registered before the procedure, were on average 14.9 ± 21.4 ng/mL.

A total of 35 HCC nodules were treated, 25 of which were located in the right lobe (segments Ⅴ-Ⅷ) and 10 in the left lobe (segments Ⅰ-Ⅳ). No major complications were observed after DSM-TACE. The post-

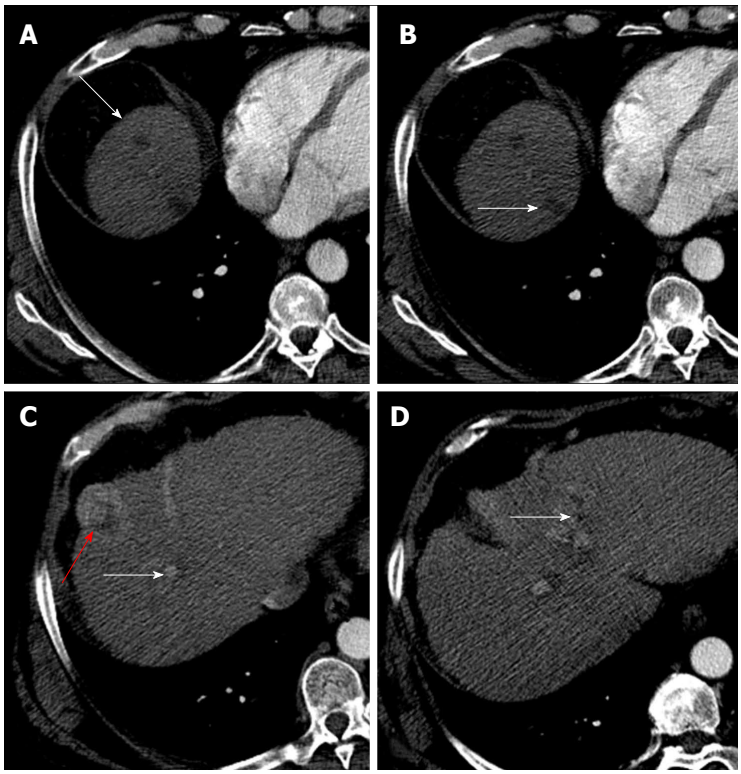

Figure 3 Enhanced computed tomography control after degradable starch microspheres transcatheter arterial chemoembolization shows a complete response of 4/5 lesions in well downstaged patient. After degradable starch microspheres transcatheter arterial chemoembolization patients had a new-Milan-criteria score of 4. A: Complete response of hepatocellular carcinoma (HCC) nodule on Ⅷ segment; B: On Ⅶ segment (white arrows); C and D: Partial response of HCC nodules (red and white arrows).

embolization syndrome (transient fever, abdominal pain, nausea) was the most common complication following chemoembolization; all side effects were successfully treated with medical therapy.

Six patients met the nMC and had downstaging of their disease (Figure 3). Two patients were considered non-downstaged. Baseline age (P = 0.25), Model for End-stage Liver Disease score (P = 0.77), and AFP level (P = 1.00) were similar between patients with and without downstaged HCC.

Complete necrosis of tumor with no evidence of viable tumor on pathologic examination was observed in 4 patients. After the treatments 18/35 lesions were classified as CR, 12 as PR and 5 as SD. Microscopically, in all lesions classified as CR, tumor necrosis was mixed, both colliquative and coagulative. The median time spent on the waiting list was 3.3 mo in patients downstaged with DSM-TACE. All six patients downstaged were successfully transplanted and after three months from LT did not experience tumor recurrence.

DISCUSSION

One of the main drawbacks of LT in patients with HCC is the downtime on the waiting list while the risk of tumor progression increases, resulting in a cumulative probability of dropout from the waiting list of 7.2% for a 6-mo waiting time, which rises to 37.8% and 55.1% for 12 and 18 mo of waiting time, respectively[18].

Thirteen years after the Milan criteria were developed, the Mazzaferro group developed more indulgent criteria called the up-to-seven criteria (nMC). In their study, the 5-year overall survival rate for Milan group patients was 73.3% and overall survival rate was 71.2%[5].

Afterwards, numerous studies have appraised the effectiveness of adopting the up-to-seven criteria as inclusion criteria for HCC LT. Similar post LT survival rates among patients were demonstrated in a study by de Ataide *et al*[19]. They directly compared the long-term outcomes of an up-to-seven criteria group, whose survival rates of 87.7%, 74.5% and 65.3% at 1, 3, and 5 years were similar to those in patients meeting the Milan criteria.

In our preliminary experience we achieved a downstaging disease within the nMC in 75% of patient treated with DSM-TACE. Moreover these patients were successfully transplanted without any complication.

In a study by Green *et al*[10] with DEB-TACE in patient with T3N0M0 HCC, were obtained survival rates quite comparable with our results with an high likelihood (77%) of downstaging the disease to meet Milan criteria.

In another recent work by Nicolini *et al*[20] on re-currence-free survival after LT in patients with HCC, were assessed the possible effect of two different types of preoperative TACE. Additionally, the effects of TACE on tumor were histologically analyzed. The authors used conventional Lipiodol TACE and DEB-TACE gaining better result with the latter treatment.

DEB-TACE could effectively promote tumor necrosis and improving recurrence-free survival after LT in HCC. In the authors' opinion the best result carried out with DEB TACE was mostly due to the increase of intra-tumor drug delivery. Indeed there are 3 main pharmacokinetic advantages associated with DEB-TACE: a long lasting elution of the drug, an higher concentration distributed locally into the tumor and a lower systemic exposure to the drug in comparison with conventional-TACE[21].

DSM were developed to avoid a permanent occlusion of blood flow to the tumor, a condition which could permit or even encourage angiogenesis.

The microspheres are 45 ± 7 × 10^{-6} m in diameter and descend until the arteriole/capillary level, where they lodge[22]. The duration of occlusion in the hepatic arteries by DSM may range between 30 and 80 min. An anticancer drug coadministered with DSM is selectively trapped with DSM in small arteries, and is concentrated in areas of liver tumor. Consequently, the activity of the drug is expected to increase in effectiveness and durability. Thus, in light of the expected reduction in toxicity levels and adverse events, it could be used safety in patients with Child C or a high MELD score.

In this respect, Niessen *et al*[23] shown that patients with unresectable intermediate-stage HCC had a lower increase in aminotransferase when they were treated with DSM-TACE compared to patients treated with doxorubicin and ethidiol TACE. In our experience the procedure with DSM-TACE was well tolerated by patients and no major complications were seen.

However, the present study results have to be viewed with caution because this is a preliminary experienced with a relative new type of chemoembolization agent and because of the small number of patients and the relative short time of follow-up after the LT.

These concerns were also expressed by Sotiropoulos *et al*[24] who, in a letter to Mazzaferro *et al*[6], stated that although the up-to-seven criteria are based on objective tumor characteristics such as tumor size, tumor number and microvascular invasion, these aspects represent pathology findings and not preoperative objective tumor characteristics. Hence, they conclude that the up-to-seven criteria are illusive and not applicable in clinical practice[24].

Anyhow, in our experience, DSM-TACE represents an effective treatment option with a similar safety and efficacy as a conventional TACE (Lipiodol or DEB), and could be safety and successfully used in patients without nMC for downstaging disease.

COMMENTS

Background

The paper trys to evaluate the possible down-staging rate using degradable starch microspheres transcatheter arterial chemoembolization (DSM-TACE) in hepatocellular carcinoma (HCC) patients without new-Milan-criteria (nMC). Liver transplantation remains the ideal treatment for small HCC and several locoregional treatment for downstaging may be used when patients were out from transplant criteria. DSMs are new materials for embolization which permit to obtain a good results also in patients with an unpaired liver function.

Research frontiers

This new technique permits to dowsntage HCC patients without nMC saftely and with a good results in term of tumor response and tolerability.

Innovations and breakthroughs

This is the first study where DSM TACE was used to obtain a down staging disease in patients with multi-nodular HCC. Previous study evaluated the efficacy of conventional TACE and drug eluting beads-TACE.

Applications

DSM-TACE represents an effective simple treatment option with a similar safety and efficacy as a conventional TACE. Transient occlusion of tumor feeding arteries permits to obtain good results in terms of tumor response without significant worsening liver function. It can be used safety in multifocal and multi-nodular HCC even when HCC has a bilobar localization.

Terminology

DSM: Degradable starch microspheres. The starch microspheres consist of a three-dimensional, cross linked hydrophilic starch matrix, which swells heavily in a water suspension environment (the specific diameter refers to its swollen state) and are completely degradable by amylase.

Peer-review

It is very interesting research work.

REFERENCES

1 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

2 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**: 277-300 [PMID: 20610543 DOI: 10.3322/caac.20073]

3 **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]

4 **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]

5 **Marsh JW**, Dvorchik I. Liver organ allocation for hepatocellular carcinoma: are we sure? *Liver Transpl* 2003; **9**: 693-696 [PMID: 12827554 DOI: 10.1053/jlts.2003.50086]

6 **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]

7 **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]

8 **Chan SC**, Fan ST, Chok KS, Cheung TT, Chan AC, Fung JY, Poon RT, Lo CM. Survival advantage of primary liver transplantation for hepatocellular carcinoma within the up-to-7 criteria with microvascular invasion. *Hepatol Int* 2012; **6**: 646-656 [PMID: 22016140]

9 **Yu CY**, Ou HY, Huang TL, Chen TY, Tsang LL, Chen CL, Cheng YF. Hepatocellular carcinoma downstaging in liver transplantation. *Transplant Proc* 2012; **44**: 412-414 [PMID: 22410030 DOI: 10.1016/j.transproceed]

10 **Green TJ**, Rochon PJ, Chang S, Ray CE, Winston H, Ruef R, Kreidler SM, Glueck DH, Shulman BC, Brown AC, Durham J. Downstaging disease in patients with hepatocellular carcinoma outside of Milan criteria: strategies using drug-eluting bead chemoembolization. *J Vasc Interv Radiol* 2013; **24**: 1613-1622

[PMID: 24060436 DOI: 10.1016/j.jvir.2013.07.024]
11 **Lencioni R**. Management of hepatocellular carcinoma with transarterial chemoembolization in the era of systemic targeted therapy. *Crit Rev Oncol Hematol* 2012; **83**: 216-224 [PMID: 22142656 DOI: 10.1016/j.critrevonc.2011.10.008]
12 **Pieper CC**, Meyer C, Vollmar B, Hauenstein K, Schild HH, Wilhelm KE. Temporary arterial embolization of liver parenchyma with degradable starch microspheres (EmboCept®S) in a swine model. *Cardiovasc Intervent Radiol* 2015; **38**: 435-441 [PMID: 25138140]
13 **Lindell B**, Aronsen KF, Rothman U. Repeated arterial embolization of rat livers by degradable microspheres. *Eur Surg Res* 1977; **9**: 347-356 [PMID: 923601 DOI: 10.1159/000127954]
14 **Furuse J**, Ishii H, Satake M, Onaya H, Nose H, Mikami S, Sakai H, Mera K, Maru Y, Yoshino M. Pilot study of transcatheter arterial chemoembolization with degradable starch microspheres in patients with hepatocellular carcinoma. *Am J Clin Oncol* 2003; **26**: 159-164 [PMID: 12714888 DOI: 10.1097/00000421-200304000-00012]
15 **Gish RG**, Lencioni R, Di Bisceglie AM, Raoul JL, Mazzaferro V. Role of the multidisciplinary team in the diagnosis and treatment of hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol* 2012; **6**: 173-185 [PMID: 22375523 DOI: 10.1586/egh.11.105]
16 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]
17 **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]
18 **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]
19 **de Ataide 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 DOI: 10.1016/j.transproceed.2012.07.006]
20 **Nicolini D**, Svegliati-Baroni G, Candelari R, Mincarelli C, Mandolesi A, Bearzi I, Mocchegiani 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]
21 **Varela M**, Real MI, Burrel 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]
22 **Sigurdson ER**, Ridge JA, Daly JM. Intra-arterial infusion of doxorubicin with degradable starch microspheres. Improvement of hepatic tumor drug uptake. *Arch Surg* 1986; **121**: 1277-1281 [PMID: 3778200 DOI: 10.1001/archsurg.1986.01400110067011]
23 **Niessen C**, Unterpaintner E, Goessmann H, Schlitt HJ, Mueller-Schilling M, Wohlgemuth WA, Stroszczynski C, Wiggermann P. Degradable starch microspheres versus ethiodol and doxorubicin in transarterial chemoembolization of hepatocellular carcinoma. *J Vasc Interv Radiol* 2014; **25**: 240-247 [PMID: 24291001 DOI: 10.1016/j.jvir.2013.10.007]
24 **Sotiropoulos GC**, Molmenti EP, Lang H. Milan criteria, up-to-seven criteria, and the illusion of a rescue package for patients with liver cancer. *Lancet Oncol* 2009; **10**: 207-208; author reply 208-209 [PMID: 19261253 DOI: 10.1016/S1470-2045(09)70053-1]

P- Reviewer: Lorenzo-Zuniga V, Ozenirler S, Pan WS
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1701

World J Hepatol 2015 June 28; 7(12): 1701-1707
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Randomized Clinical Trial

^{1}H nuclear magnetic resonance spectroscopy-based metabonomic study in patients with cirrhosis and hepatic encephalopathy

Konstantinos John Dabos, John Andrew Parkinson, Ian Howard Sadler, John Nicholas Plevris, Peter Clive Hayes

Konstantinos John Dabos, John Nicholas Plevris, Peter Clive Hayes, Centre of Liver and Digestive Disorders, Royal Infirmary of Edinburgh, Edinburgh EH16 4SA, Scotland, United Kingdom
John Andrew Parkinson, Department of Chemistry, University of Strathclyde, Glasgow G1 1XW, United Kingdom
Ian Howard Sadler, Department of Chemistry, University of Edinburgh, Edinburgh EH16 4SA, Scotland, United Kingdom

Author contributions: Plevris JN and Hayes PC conceived the study; Dabos KJ and Plevris JN designed the study; Dabos KJ and Parkinson JA performed the data collection; Sadler IH helped with the data collection; Dabos KJ wrote the manuscript; all authors critically reviewed the manuscript and approved it.

Ethics approval: The study was reviewed and approved by the Lothian Research Ethics Committee Institutional Review Board.
Clinical trial registration: As this trial recruited before 1999 it was not registered with a clinical trials registry.
Informed consent: All study participants or their next of kin provided written informed consent prior to study enrolment.
Conflict-of-interest: The authors do not disclose any conflicts of interest.
Data sharing: No additional data are available.

Correspondence to: Konstantinos John Dabos, MD, PhD, Centre of Liver and Digestive Disorders, Royal Infirmary of Edinburgh, 49 Little France Crescent, Edinburgh EH16 4SA, Scotland,
United Kingdom. konstantinos.dabos@nhslothian.scot.nhs.uk
Telephone: +44-131-2421627

Received: March 12, 2015
Peer-review started: March 12, 2015
First decision: April 10, 2015
Revised: May 9, 2015
Accepted: June 4, 2015
Article in press: June 8, 2015
Published online: June 28, 2015

Abstract

AIM: To identify plasma metabolites used as biomarkers in order to distinguish cirrhotics from controls and encephalopathics.

METHODS: A clinical study involving stable cirrhotic patients with and without overt hepatic encephalopathy was designed. A control group of healthy volunteers was used. Plasma from those patients was analysed using ^{1}H - nuclear magnetic resonance spectroscopy. We used the Carr Purcell Meiboom Gill sequence to process the sample spectra at ambient probe temperature. We used a gated secondary irradiation field for water signal suppression. Samples were calibrated and referenced using the sodium trimethyl silyl propionate peak at 0.00 ppm. For each sample 128 transients (FID's) were acquired into 32 K complex data points over a spectral width of 6 KHz. 30 degree pulses were applied with an acquisition time of 4.0 s in order to achieve better resolution, followed by a recovery delay of 12 s, to allow for complete relaxation and recovery of the magnetisation. A metabolic profile was created for stable cirrhotic patients without signs of overt hepatic encephalopathy and encephalopathic patients as well as healthy controls. Stepwise discriminant analysis was then used and discriminant factors were created to differentiate between the three groups.

RESULTS: Eighteen stabled cirrhotic patients, eighteen patients with overt hepatic encephalopathy and seventeen healthy volunteers were recruited. Patients with cirrhosis had significantly impaired ketone body metabolism, urea synthesis and gluconeogenesis. This was demonstrated by higher concentrations of acetoacetate (0.23 ± 0.02 *vs* 0.05 ± 0.00, $P < 0.01$), and b-hydroxybutarate (0.58 ± 0.14 *vs* 0.08 ± 0.00, $P < 0.01$), lower concentrations of glutamine (0.44 ± 0.08 *vs* 0.63 ± 0.03, $P < 0.05$), histidine (0.16 ± 0.01 *vs* 0.36 ± 0.04, $P < 0.01$) and arginine (0.08 ± 0.01 *vs* 0.14 ± 0.02, $P < 0.03$) and higher concentrations of glutamate (1.36 ± 0.25 *vs* 0.58 ± 0.04, $P < 0.01$), lactate (1.53 ± 0.11 *vs* 0.42 ± 0.05, $P < 0.01$), pyruvate (0.11 ± 0.02 *vs* 0.03 ± 0.00, $P < 0.01$) threonine (0.39 ± 0.02 *vs* 0.08 ± 0.01, $P < 0.01$) and aspartate (0.37 ± 0.03 *vs* 0.03 ± 0.01). A five metabolite signature by stepwise discriminant analysis could separate between controls and cirrhotic patients with an accuracy of 98%. In patients with encephalopathy we observed further derangement of ketone body metabolism, impaired production of glycerol and myoinositol, reversal of Fischer's ratio and impaired glutamine production as demonstrated by lower b-hydroxybutyrate (0.58 ± 0.14 *vs* 0.16 ± 0.02, $P < 0.0002$), higher acetoacetate (0.23 ± 0.02 *vs* 0.41 ± 0.16, $P < 0.05$), leucine (0.33 ± 0.02 *vs* 0.49 ± 0.05, $P < 0.005$) and isoleucine (0.12 ± 0.02 *vs* 0.27 ± 0.02, $P < 0.0004$) and lower glutamine (0.44 ± 0.08 *vs* 0.36 ± 0.04, $P < 0.013$), glycerol (0.53 ± 0.03 *vs* 0.19 ± 0.02, $P < 0.000$) and myoinositol (0.36 ± 0.04 *vs* 0.18 ± 0.02, $P < 0.010$) concentrations. A four metabolite signature by stepwise discriminant analysis could separate between encephalopathic and cirrhotic patients with an accuracy of 87%.

CONCLUSION: Patients with cirrhosis and patients with hepatic encephalopathy exhibit distinct metabolic abnormalities and the use of metabonomics can select biomarkers for these diseases.

Key words: Ketone bodies; Branch chain amino acids; Glutamine; Glycolysis

Core tip: Few studies have approached the metabolic abnormalities of liver cirrhosis and its complication hepatic encephalopathy. This study provides evidence that in stable cirrhosis key metabolic pathways are impaired and confirms the fact that there is impaired gluconeogensis, impaired ketogensis and ketone bodies break down as well as impaired urea cycle. In encephalopathy there is a reversal in the pattern of branch chain amino acids concentrations towards normal. By using stepwise discriminating analysis we were able to separate with remarkable accuracy metabolic phenotypes of cirrhotic patients from controls and also those who suffered from encephalopathy from those cirrhotics who did not.

Dabos KJ, Parkinson JA, Sadler IH, Plevris JN, Hayes PC. ^{1}H nuclear magnetic resonance spectroscopy-based metabonomic study in patients with cirrhosis and hepatic encephalopathy. *World J Hepatol* 2015; 7(12): 1701-1707 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1701.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1701

INTRODUCTION

Insults on the liver parenchyma could result in fat accumulation, inflammation and fibrosis. Most chronic liver injuries could result in cirrhosis, which is a combination of hepatic fibrosis and nodular regenerative hyperplasia. Hepatitis B infection is the most common cause in Asia and Africa whereas alcohol is the most common cause in the developed world. Currently worldwide, the proportion of cirrhosis due to chronic Hepatitis C infection is on the increase[1,2].

One of the major complications of cirrhosis is hepatic encephalopathy. This is a complex neuropsychiatric syndrome which has a potential for full reversibility. It is characterised by global depression of the central nervous system (CNS) and has different degrees of severity. The syndrome is usually episodic and relapsing but some patients exhibit a chronic protracted course[3].

Cirrhosis is associated with alterations in proteins and amino acids metabolism, including diminished urea formation and hyperammonaemia[4,5]. Most studies that have looked at amino acid metabolism in cirrhosis would agree that there seems to be a recognisable pattern in the plasma amino acid profile with an elevation of aromatic amino acids (AAA) and methionine and reduced levels of branch chain amino acids (BCAA)[6,7]. Although this pattern has been used as the basis for the false neurotransmitter theory in the pathogenesis of hepatic encephalopathy[8], not many studies have proved that the pattern observed in cirrhosis is valid for encepahlopathy too[6,9,10]. Furthermore it is now well accepted that encephalopathy due to acute hepatic failure is a different entity and the mechanisms contributing to the CNS dysfunction in the two diseases might be different.

Apart from disturbances in the BCAA to AAA ratio, other biochemical abnormalities are present in cirrhotics. Gluconeogensis is impaired and hyperammonaemia and diminished urea production make it necessary for the body to find other pathways for nitrogen elimination[5,11,12].

In the last few years, the emerging field of metabonomics, which examines global metabolic profiles using various data collection techniques, offered the possibility to identify biomarkers in evolving diseases[13]. Lately, this technique has been used in delineating disease phenotypes in humans suffering from chronic liver disease[14,15] and animal models of liver failure[16,17]. Metabonomics makes use of multivariate statistical approaches to analyse complex data sets, such as those obtained by ^{1}H NMR spectroscopy[16]. It is particularly useful if there are few samples to analyze and many variables to consider.

The aim of our study was to apply ^{1}H NMR spec-

troscopy in controls, cirrhotics and cirrhotics with hepatic encephalopathy and analyse the data to identify metabolic patterns that could distinguish between the three groups.

MATERIALS AND METHODS

Patients

The Local Research Ethics committee had approved the study protocol (373/1997). The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki. Patients were recruited at the Centre of Liver and Digestive Disorders and at the Day Case Unit at the Royal Infirmary of Edinburgh. All patients signed an informed consent. For those unable to consent due to encephalopathy, consent from the next of kin was sought. Encephalopathy was defined using the West Haven Criteria[8]. The diagnosis of cirrhosis was based on a combination of clinical, histopathological and imaging criteria. Patients with grade Ⅲ or Ⅳ hepatic encephalopathy were excluded from the study as they usually had an acute precipitating episode and were not stable cirrhotics. Patients with coagulation abnormalities (International Normalized Ratio < 2.0, platelets < 80000) were excluded from the study as the ethics committee did not approve internal jugular vein puncture in patients with impaired coagulation. Patients with an upper gastrointestinal bleeding episode in the previous two weeks were also excluded. Patients with hepatocellular carcinoma, pregnant and lactating women were also excluded from the study.

A total of 42 patients were recruited. Six of them eventually withdrew consent and were not included in the study. The study recruited for just over 16 mo.

We studied patients with stable cirrhosis (group A, 18 patients), patients with stable cirrhosis during an episode of hepatic encephalopathy (group B, 18 patients) and sex and age matched normal controls (group C, 17 subjects).

All patients were fasted overnight before blood collection. Blood was collected from the internal jugular vein, from all three groups two to three hours following a main meal as differences in the concentration of amino acids between sexes are less pronounced postprandially, in lithium heparin tubes. It was immediately centrifuged at 2000 g for 15 min at 4 ℃. The supernatant was then aliquoted in 2.5 mL vials. The vials were then stored at -40 ℃ until NMR analysis.

Sample preparation for NMR spectroscopy

Samples were prepared by adding a D_2O solution (150 μL) to plasma (600 μL) providing an internal field frequency lock for the spectrometer, a Varian 600 MHz at 14.1T. Five millimeter probes were used for the analysis. Chemical shifts were referenced externally to the singlet methyl resonance of sodium trimethyl silyl propionate (TSP) (75 μL) at zero ppm. Plasma samples were left in room temperature for 1 h before samples for the NMR analysis were prepared.

Proton NMR spectroscopy

The CMPG sequence was applied, to acquire our data, as this sequence enabled us to observe a flat baseline in our spectra from plasma samples, by minimising the signals acquired from macromolecules present in the plasma such as proteins and lipoproteins. All spectra were acquired at ambient probe temperature (298 ± 0.2 K). For each sample 128 transients (FID's) were acquired into 32 K complex data points over a spectral width of 6 KHz. 30° pulses were applied with an acquisition time of 4.0 s in order to achieve better resolution, followed by a recovery delay of 12 s, to allow for complete relaxation and recovery of the magnetisation. Water signal suppression was achieved by applying a gated secondary irradiation field at the water resonance frequency.

Spectral processing

FID's were multiplied by an exponential function before applying Fourier transform. Transformed spectra were automatically corrected for phase and baseline distortions and calibrated using the TSP peak at 0.00 ppm. A preliminary assignment of the amino acid metabolites was performed and only the areas between 0.70 and 3.80 ppm and between 6.80 and 7.70 ppm were subjected to stepwise discriminant analysis (SDA).

To assess which peaks were significantly different between the three groups a one-way analysis of variants was used. Normality of data distribution was assessed using the Wilk's Lamda distribution.

Spectral assignments were made by reference to literature values of chemical shifts in various media and biological fluids (18) and coupling constants. Spectra were processed using the Mestre-C software (Mestrelab, Santiago de Compostela, Spain).

Variables

We measured a large array of aminoacids and products of cellular metabolism to ensure representation of the main metabolic pathways performed by the hepatocyte in our results. The following substances were measured. Lactate, pyruvate, acetoacetate, b-hydroxybutyrate, leucine, isoleucine, valine, alanine, threonine, glycine, aspartate, glutamine, glutamate, histidine, arginine, methylamine, dimethylamine, trimethylamineoxide (TMAO), glycerol, and myoinositol. Results are expressed as mmols/L unless otherwise state.

Statistical analysis

To compare between the three groups we used the three way *ANOVA* test. Where the ANOVA test was statistically significant the Tuckey test was performed to compare between groups. Values are expressed as mean (range and standard error). A P value of < 0.05 was taken as statistically significant (two-tail test of significance).

For the multivariate analysis we opted for the SDA. Data with statistical significance on ANOVA were entered into the SDA. We used SDA to extract and classify variables from different spectra. Analysis was performed

Table 1 Patients and controls were well matched for age and sex

	Controls	Cirrhosis	Encephalopathy
Age	48.8 ± 9.9	54.3 ± 8.8	56.8 ± 6.0
Sex	M: 10	M: 9	M: 12
	F: 7	F: 9	F: 6
CP score	N/A	7.8 ± 1.6	9.9 ± 2.1
Child class a	N/A	1	1
Child class b	N/A	11	6
Child class c	N/A	6	11

Patients with hepatic encephalopathy had more severe liver disease. CP: Child-Pugh; M: Male; F: Female.

Table 3 Results for glycolysis end products and gluconeogenetic precursors are shown

	Chemical shift	Cirrhosis	Encephalopathy	Controls
Lactate	1.33	1.53 ± 0.11[b]	1.41 ± 0.13[b]	0.42 ± 0.05
Pyruvate	2.38	0.11 ± 0.02[b]	0.17 ± 0.02[b]	0.03 ± 0.00
Alanine	1.48	0.77 ± 0.04[b]	0.73 ± 0.06[b]	0.61 ± 0.05
Threonine	1.34	0.39 ± 0.02[b]	0.25 ± 0.01[b]	0.08 ± 0.1
Glycine	3.57	0.31 ± 0.03[b]	0.18 ± 0.01[b]	0.09 ± 0.1
Aspartate	2.82	0.37 ± 0.03[b]	0.27 ± 0.02[b]	0.03 ± 0.1

Lactate, pyruvate, alanine, threonine, glycine and aspartate concentrations were all significantly higher in patients than controls ([b]$P < 0.01$ in all cases).

in stepwise manner entering variables with the highest statistical significance first. A discriminant function was thus established and receiver operator curves (ROC) analysis was performed. Analyses were performed using SAS 8.0 software (SAS Institute, Cary, NC, United States).

RESULTS

Patient characteristics in groups A and C are shown in Table 1. Patients were well matched for age and sex. Patients with hepatic encephalopathy had in general more severe liver failure.

Table 2 shows the results for ketone bodies, BCAA and AAA. Acetoacetate and β-hydroxybutyrate, tyrosine, phenylalanine and methionine concentrations were all significantly higher in patients than controls ($P < 0.01$ in all cases). Valine was significantly lower in patients than controls ($P < 0.01$ in both cases). Leucine was significantly higher in encephalopathics than controls ($P < 0.01$), but there was no difference between cirrhotics and controls. Isoleucine was significantly lower in controls than encephalopathics ($P < 0.01$) but there was no difference between cirrhotics and controls.

Table 3 shows the results obtained for glycolysis. Lactate and pyruvate concentrations were significantly higher in patients than controls ($P < 0.01$ in all cases). Alanine, threonine, glycine and aspartate concentrations were significantly higher in patients than controls ($P < 0.01$ in all cases).

Table 2 Results for ketone bodies, branch chain and aromatic amino acids are shown

	Chemical shift	Cirrhosis	Encephalopathy	Controls
Acetoacetate	2.29	0.23 ± 0.02[b]	0.41 ± 0.05[b]	0.05 ± 0.00
B-hydroxybutyr	2.31	0.58 ± 0.14[b]	0.16 ± 0.02[b]	0.08 ± 0.00
Leucine	0.96	0.33 ± 0.02	0.49 ± 0.05[b]	0.35 ± 0.02
Isoleucine	1.01	0.12 ± 0.02	0.27 ± 0.02[b]	0.13 ± 0.02
Valine	1.04	0.14 ± 0.01[d]	0.16 ± 0.02[d]	0.36 ± 0.03
Phenylalanine	7.38	0.08 ± 0.01[b]	0.06 ± 0.02[b]	0.02 ± 0.01
Tyrosine	6.91	0.23 ± 0.02[b]	0.25 ± 0.06[b]	0.07 ± 0.00
Methionine	2.14	0.07 ± 0.02[b]	0.08 ± 0.02[b]	0.03 ± 0.01

Acetoacetate and β-hydroxybutyrate concentrations were significantly higher in patients than controls ([b]$P < 0.01$ in all cases). Aromatic amino acids concentrations were significantly higher in patients than controls ($P < 0.01$ in all cases). Valine concentrations were significantly lower in patients than controls ([d]$P < 0.01$). Leucine was significantly higher if we compared encephalopathics with controls ([b]$P < 0.01$), but there was no difference if we compared cirrhotics and controls. Isoleucine was significantly lower if we compared encephalopathics with controls ([b]$P < 0.01$) but there was no difference between cirrhotics and controls.

Table 4 Results for urea cycle intermediates are shown

	Chemical shift	Cirrhosis	Encephalopathy	Controls
Glutamine	2.46	0.44 ± 0.08[b]	0.36 ± 0.04[b]	0.63 ± 0.03
Glutamate	2.36	1.36 ± 0.25[d]	0.84 ± 0.16[a]	0.58 ± 0.04
Histidine	7.83	0.16 ± 0.01[b]	0.18 ± 0.02[b]	0.36 ± 0.04
Arginine	1.93	0.08 ± 0.01[b]	0.1 ± 0.01[b]	0.14 ± 0.02

Glutamine, histidine and arginine concentrations were significantly lower in patients than controls ([b]$P < 0.01$ in all cases). Glutamate concentrations were significantly higher in cirrhotics ([d]$P < 0.01$) compared to controls. It was also significantly increased if we compared encephalopathics with controls ([a]$P < 0.05$).

Table 4 shows the results obtained for urea cycle end products. Glutamine, histidine and arginine concentrations were significantly lower in patients than controls ($P < 0.01$ in all cases). Glutamate concentrations were significantly higher in cirrhotics ($P < 0.01$) compared to controls. They are also significantly increased if we compared encephalopathics with controls ($P < 0.05$).

Table 5 shows the results for methylamine, dimethylamine, TMAO, glycerol and myoinositol. Methylamine, dimethylamine and TMAO concentrations were present in similar amounts in cirrhotic and encephalopathic patients but were absent in controls. Glycerol concentrations were significantly higher in patients than controls ($P < 0.01$ in both cases). Myoinositol concentrations were significantly higher in cirrhotics ($P < 0.015$) but there were no differences between encephalopathic patients and controls.

Using SDA we were able to identify five metabolites, tyrosine, phenylalanine, methionine, pyruvate and glycine that yielded the strongest segregation between groups A and C. A discriminant function (sum of concentrations of all five metabolites (tyrosine + phenylalanine + methionine + pyruvate + glycine) in mmols/L < 0.50 for controls) was created. By performing ROC analysis it had

Table 5 Results for amines, glycerol and myo-inositol are shown

	Chemical shift	Cirrhotics	Encephalopathics	Controls
Methylamine	2.54	0.17 ± 0.03	0.19 ± 0.03	0
Dimethylamine	2.72	0.29 ± 0.03	0.31 ± 0.04	0
Tmao	3.27	0.45 ± 0.07	0.51 ± 0.08	0
Glycerol	3.79	0.53 ± 0.09[b]	0.2 ± 0.02[b]	0.08 ± 0.02
Myoinositol	3.63	0.37 ± 0.06[a]	0.19 ± 0.04	0.16 ± 0.03

Methylamine, dimethylamine and TMAO were present in patients and absent in controls. Glycerol concentrations were significantly higher in patients than controls ([b]$P < 0.01$). Myo-inositol concentrations were significantly higher in cirrhotics ([a]$P < 0.015$) but there were no differences between encephalopathics and controls.

a positive predictive value (PPV) of 100%, a negative predictive value (NPV) of 94% a sensitivity of 95%, a specificity of 100% and an overall accuracy of 98%.

If we compared between patients in groups A and B, β-hydroxybutyrate was significantly lower in encephalopathics (0.58 ± 0.14 *vs* 0.16 ± 0.02, $P < 0.0002$). In contrast, acetoacetate was significantly higher in encephalopathics (0.23 ± 0.02 *vs* 0.41 ± 0.16, $P < 0.05$). The concentration of leucine (0.33 ± 0.02 *vs* 0.49 ± 0.05, $P < 0.005$) and isoleucine 0.12 ± 0.02 *vs* 0.27 ± 0.02, $P < 0.0004$) were significantly higher in encepahopathics. Glutamine concentrations were lower in encephalopathic patients (0.44 ± 0.08 *vs* 0.36 ± 0.04, $P < 0.013$). Glycerol (0.53 ± 0.03 *vs* 0.19 ± 0.02, $P < 0.000$) and myoinositol concentrations (0.36 ± 0.04 *vs* 0.18 ± 0.02, $P < 0.01$) were significantly lower in encepahlopathic patients.

The strongest segregation was observed with input from β-hydroxybutyrate, glutamine, glycerol and glutamate in the SDA. A discriminate function (sum of concentrations of all four metabolites (β-hydroxybutyrate + glutamine + glycerol + glutamate) in mmols/L < 1.5 for encephalopathics) was created. By performing ROC analysis it had a PPV of 89%, a NPV of 83%, a sensitivity of 84%, a specificity of 88% and an overall accuracy of 87%.

DISCUSSION

Our study has confirmed that significant changes occur in plasma concentrations of amino acids and other key metabolites in patients with cirrhosis in the presence or not of hepatic encephalopathy. By using the metabonomics approach we were able to pinpoint metabolites that could be used to identify a patient with or without cirrhosis and with or without hepatic encephalopathy. We will now look at some particular substances in more detail.

Lactate and the amino acids alanine, threonine, glycine and aspartate are major precursors for gluconeogenesis. Pyruvate is also a central substance in glucose metabolism. In both cirrhotics and encephalopathics we found that the concentrations of those substances were uniformly increased. It appears then that gluconeogesis is generally impaired in cirrhosis and encephalopathy. This would be in accordance with previous studies in humans[17,18] and animal models[19,20]. Pyruvate and glycine were part of the discriminate function between cirrhotics and healthy controls.

Our study showed that the concentrations of ketone bodies were significantly increased in both groups of patients compared to controls. In encephalopathics, acetoacaetate was even more increased than in cirrhotics but β-hydroxybutyrate concentrations were decreased. The fact that all ketogenic amino acids are increased in cirrhosis as well, would favour a hypothesis of impaired ketone bodies utilisation in the periphery (muscle, brain) The fact that β-hydroxybutyrate and acetate are significantly decreased in encephalopathic cirrhotics is indicative of an impaired ketogensis. We observed, however, that acetoacetate is increased in encephalopathics. Acetoacetate is the main product of ketogenesis and then by using nicotinamide adenine dinucleotide hydrogen (NADH) as co-substrate is further metabolised to acetate in the cellular mitochondria. β-hydroxybutyrate was part of the main discriminate function between cirrhotic and encephalopathic patients. We can hypothesize that, possibly, the precarious state of energy production in encephalopathy makes the availability of NADH for this further reaction minimal and it is shifted towards energy production from the Kreb's cycle, which is vital to the hepatocytes, instead of finalising a product which is destined for export to other organs like muscle and brain. This is further consolidated by the fact that encephalopathics were shown to have a significantly lower glycerol level. This is an indication that fewer triglycerides are broken down and fewer lipids are made available for oxidation which is the main pathway that would lead to ketone body production. Glycerol was part of the main discriminant function between cirrhotic and encephalopathic patients. This lends support to the recent hypothesis that the phenylacetate could be used as a treatment in hepatic encephalopathy[21] and to subsequent studies in animal models that were in accordance with that[22,23].

Typical changes in plasma amino acid patterns have been found in different studies in patients[24,25] and experimental animals in chronic liver failure[26,27]. Those changes are increased concentrations of the AAA and methionine and decreased concentrations of the BCAA. The AAA and methionine are primarily metabolised by the liver and their raised concentrations in both cirrhotics and encepahlopathic cirrhotics are probably due to impaired liver metabolism and portosystemic shunting of blood. Our findings related to AAA confirm findings by numerous previous studies[24-27] which showed an increase in AAA concentrations.

The story is more complex for the BCAA and is further complicated by the findings of this study that in encepohalopathics there was an increase in the concentrations of leucine and isoleucine. The normal liver does not play a major role in the breakdown of the BCAA which are mostly catabolised in the skeletal muscle

and kidneys. It was postulated that hyperisulinaemia which is present in cirrhosis may drive BCAA to the muscle and the kidneys where they are broken down[28]. Our results do not support this hypothesis particularly in encephalopathy as concentrations of leucine and isoleucine are increased in encephalopathy. If we look at BCAA individually we find that their metabolic fate after the initials transamination and decarboxylation can be very different from one to the other. Leucine is a ketogenic amino acid which can be oxidised to acetyl-CoA. This study provides evidence that ketogenesis is impaired in encephalopathy as is the peripheral utilisation of the ketone bodies and this might explain the increased concentrations of leucine. Valine can only be a gluconeogenic amino acid that could enter the Kreb's cycle and provide towards the production of ATP. As acetyl-CoA is in short supply Kreb's cycle can be fuelled from alternative sources such as valine. And this might explain the decreased concentrations of that amino acid.

We do not have an immediate explanation as to why the concentration of isoleucine is high in encephalopathic cirrhotics in our study population. Isoleucine is a ketogenic amino acid and as the production of acetoacetate is increased but its catabolism is not it might be an index of diminished ketogenesis in encephalopathy.

Hyperammonaemia and diminished urea production are well characterised phenomena in cirrhotic patients[29-31]. Our study showed that cirrhotics had increased levels of glutamate, histidine and arginine and decreased levels of glutamine. This is a pattern which is not in accordance with the previous studies which showed a generalised decrease in those amino acids in chronic liver failure. It is in accordance though with studies in experimental animal models of liver failure. Although other studies in patients suffering acute liver failure confirmed this pattern, our studies in acute liver failure found no differences in any of those substances between patients and controls[32,33]. Arginine is an amino acid that is an intermediary of the urea cycle. Its observed increased concentrations are in agreement with a decreased urea cycle as is the increased histidine concentrations which is a glutamate precursor.

Glutamine however, was part of the main discriminate function between cirrhotic and encephalopathic patients. Although this might seem paradoxical, there is evidence of increased ammonia production during encephalopathy, which is implicated in its pathogenesis. The fact that glutamine synthesis is impaired may provide another point for the hyperammonaemia of encephalopathy. An alternative pathway to this is the production of amines and TMAO which can assist in the ammonia detoxification in the presence of urea cycle impairment. Glutamate and glutamine were part of the discriminate function between cirrhotics and encephalopathic patients.

In conclusion, this study provides evidence that in stable cirrhosis key metabolic pathways are impaired and confirms the fact that there is impaired gluconeogensis, impaired ketogensis and ketone bodies break down and impaired urea cycle. In encephalopathy there is a reversal in the pattern of BCAA concentrations towards normal. By using SDA we were able to separate with remarkable accuracy metabolic phenotypes of cirrhotic patients from controls and also those who suffered from encephalopathy from those cirrhotics who were not.

COMMENTS

Background

The irreversible liver damage that characterises liver cirrhosis is a bad prognostic factor. The presence of hepatic encephalopathy, a complication of cirrhosis is considered a further aggravating factor.

Research frontiers

Over the last few years a plethora of studies have looked at non-invasive markers to be used in the detection of cirrhosis. At present, the presence of hepatic encephalopathy can only be assessed clinically. Few studies have approached the metabolic abnormalities of liver cirrhosis and its complication hepatic encephalopathy.

Innovations and breakthroughs

This study provides evidence that a combination of biomarkers can differentiate between healthy volunteers and cirrhotic patients. Another combination of biomarkers can predict the presence of overt hepatic encephalopathy in cirrhotics with remarkable accuracy.

Applications

If the findings are confirmed in larger studies and the biomarkers are accurate in differentiating between cirrhosis and pre-cirrhotic states they would provide objective non-invasive criteria for the presence of cirrhosis and hepatic encephalopathy.

Terminology

^{1}H-nuclear magnetic resonance spectroscopy is an analytical method that can detect metabolites in small quantities in solutions. It uses a powerful magnetic field and software that can transform magnetic signals and footprints of metabolites into concentrations.

Peer-review

The authors have performed a good study, the manuscript is interesting.

REFERENCES

1 **Garcia-Tsao G**, Lim JK. Management and treatment of patients with cirrhosis and portal hypertension: recommendations from the Department of Veterans Affairs Hepatitis C Resource Center Program and the National Hepatitis C Program. *Am J Gastroenterol* 2009; **104**: 1802-1829 [PMID: 19455106 DOI: 10.1038/ajg.2009.360]

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 **Bajaj JS**. Review article: the modern management of hepatic encephalopathy. *Aliment Pharmacol Ther* 2010; **31**: 537-547 [PMID: 20002027 DOI: 10.1111/j.1365-2036.2009.04211.x]

4 **Butterworth RF**, Giguère JF, Michaud J, Lavoie J, Layrargues GP. Ammonia: key factor in the pathogenesis of hepatic encephalopathy. *Neurochem Pathol* 1987; **6**: 1-12 [PMID: 3306479 DOI: 10.1007/BF02833598]

5 **Kramer L**, Tribl B, Gendo A, Zauner C, Schneider B, Ferenci P, Madl C. Partial pressure of ammonia versus ammonia in hepatic encephalopathy. *Hepatology* 2000; **31**: 30-34 [PMID: 10613724 DOI: 10.1002/hep.510310107]

6 **Schmidt LE**, Tofteng F, Strauss GI, Larsen FS. Effect of treatment with the Molecular Adsorbents Recirculating System on arterial amino acid levels and cerebral amino acid metabolism in patients with hepatic encephalopathy. *Scand J Gastroenterol* 2004; **39**: 974-980 [PMID: 15513337]

7 **Plauth M**, Egberts EH, Hamster W, Török M, Müller PH, Brand O, Fürst P, Dölle W. Long term treatment of latent portosystemic encephalopathy with branch chain amino acids. A double-blind, placebo-controlled trial. *J Hepatol* 1993; **17**: 308-314 [DOI:

10.1016/S0168-8278(05)80210-7]
8 **Jalan R**, Seery JP, Taylor-Robinson SD. Review article: pathogenesis and treatment of chronic hepatic encephalopathy. *Aliment Pharmacol Ther* 1996; **10**: 681-697 [PMID: 8899075]
9 **Strauss GI**, Knudsen GM, Kondrup J, Møller K, Larsen FS. Cerebral metabolism of ammonia and amino acids in patients with fulminant hepatic failure. *Gastroenterology* 2001; **121**: 1109-1119 [PMID: 11677203 DOI: 10.1053/gast.2001.29310]
10 **Bak LK**, Iversen P, Sørensen M, Keiding S, Vilstrup H, Ott P, Waagepetersen HS, Schousboe A. Metabolic fate of isoleucine in a rat model of hepatic encephalopathy and in cultured neural cells exposed to ammonia. *Metab Brain Dis* 2009; **24**: 135-145 [PMID: 19067142 DOI: 10.1007/s11011-008-9123-4]
11 **McCullough AJ**, Glamour T. Differences in amino acid kinetics in cirrhosis. *Gastroenterology* 1993; **104**: 1858-1865 [PMID: 8500747]
12 **Jacobsen EB**, Hamberg O, Quistorff B, Ott P. Reduced mitochondrial adenosine triphosphate synthesis in skeletal muscle in patients with Child-Pugh class B and C cirrhosis. *Hepatology* 2001; **34**: 7-12 [PMID: 11431727 DOI: 10.1053/jhep.2001.25451]
13 **Nicholson JK**, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica* 1999; **29**: 1181-1189 [PMID: 10598751 DOI: 10.1080/004982599238047]
14 **Gao H**, Lu Q, Liu X, Cong H, Zhao L, Wang H, Lin D. Application of 1H NMR-based metabonomics in the study of metabolic profiling of human hepatocellular carcinoma and liver cirrhosis. *Cancer Sci* 2009; **100**: 782-785 [PMID: 19469021 DOI: 10.1111/j.1349-7006.2009.01086.x]
15 **Xue R**, Dong L, Wu H, Liu T, Wang J, Shen X. Gas chromatography/mass spectrometry screening of serum metabolomic biomarkers in hepatitis B virus infected cirrhosis patients. *Clin Chem Lab Med* 2009; **47**: 305-310 [PMID: 19676142 DOI: 10.1515/CCLM.2009.083]
16 **Barba I**, Chatauret N, García-Dorado D, Córdoba J. A 1H nuclear magnetic resonance-based metabonomic approach for grading hepatic encephalopathy and monitoring the effects of therapeutic hypothermia in rats. *Liver Int* 2008; **28**: 1141-1148 [PMID: 18637065 DOI: 10.1111/j.1478-3231.2008.01801.x]
17 **Feng B**, Wu SM, Lv S, Liu F, Chen HS, Gao Y, Dong FT, Wei L. A novel scoring system for prognostic prediction in d-galactosamine/lipopolysaccharide-induced fulminant hepatic failure BALB/c mice. *BMC Gastroenterol* 2009; **9**: 99 [PMID: 20040118 DOI: 10.1186/1471-230X-9-99]
18 **Lindon JC**, Nicholson JK, Everet JR. NMR spectroscopy of biofluids. *Ann Reports NMR Spectroscopy* 1999; **38**: 1-87 [DOI: 10.1016/S0066-4103(08)60035-6]
19 **Seglen PO**. Autoregulation of glycolysis, respiration, gluconeogenesis, and glycogen synthesis in isolated parenchymal rat liver cells under aerobic and anaerobic conditions. *Biochim Biophys Acta* 1976; **338**: 317-336 [DOI: 10.1016/0304-4165(74)90292-X]
20 **de Blaauw I**, Deutz NE, Boers W, von Meyenfeldt MF. Hepatic amino acid and protein metabolism in non-anorectic, moderately cachectic tumor-bearing rats. *J Hepatol* 1997; **26**: 396-408 [PMID: 9059963]
21 **Jalan R**, Wright G, Davies NA, Hodges SJ. L-Ornithine phenylacetate (OP): a novel treatment for hyperammonemia and hepatic encephalopathy. *Med Hypotheses* 2007; **69**: 1064-1069 [PMID: 17467190 DOI: 10.1016/j.mehy.2006.12.061]
22 **Davies NA**, Wright G, Ytrebø LM, Stadlbauer V, Fuskevåg OM, Zwingmann C, Davies DC, Habtesion A, Hodges SJ, Jalan R. L-ornithine and phenylacetate synergistically produce sustained reduction in ammonia and brain water in cirrhotic rats. *Hepatology* 2009; **50**: 155-164 [PMID: 19437490 DOI: 10.1002/hep.22897]
23 **Ytrebø LM**, Kristiansen RG, Maehre H, Fuskevåg OM, Kalstad T, Revhaug A, Cobos MJ, Jalan R, Rose CF. L-ornithine phenylacetate attenuates increased arterial and extracellular brain ammonia and prevents intracranial hypertension in pigs with acute liver failure. *Hepatology* 2009; **50**: 165-174 [PMID: 19554542]
24 **Fischer JE**, Rosen HM, Ebeid AM, James JH, Keane JM, Soeters PB. The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* 1976; **80**: 77-91 [PMID: 818729]
25 **Plauth M**, Egberts EH, Abele R, Müller PH, Fürst P. Characteristic pattern of free amino acids in plasma and skeletal muscle in stable hepatic cirrhosis. *Hepatogastroenterology* 1990; **37**: 135-139 [PMID: 2312039]
26 **Cascino A**, Cangiano C, Fiaccadori F, Ghinelli F, Merli M, Pelosi G, Riggio O, Rossi Fanelli F, Sacchini D, Stortoni M, Capocaccia L. Plasma and cerebrospinal fluid amino acid patterns in hepatic encephalopathy. *Dig Dis Sci* 1982; **27**: 828-832 [PMID: 7105954 DOI: 10.1007/BF01391377]
27 **Zwingmann C**, Butterworth R. An update on the role of brain glutamine synthesis and its relation to cell-specific energy metabolism in the hyperammonemic brain: further studies using NMR spectroscopy. *Neurochem Int* 2005; **47**: 19-30 [PMID: 15916833 DOI: 10.1016/j.neuint.2005.04.003]
28 **Tessari P**, Biolo G, Inchiostro S, Orlando R, Vettore M, Sergi G. Leucine and phenylalanine kinetics in compensated liver cirrhosis: effects of insulin. *Gastroenterology* 1993; **104**: 1712-1721 [PMID: 8500731]
29 **Holmin T**, Agardh CD, Alinder G, Herlin P, Hultberg B. The influence of total hepatectomy on cerebral energy state, ammonia-related amino acids of the brain and plasma amino acids in the rat. *Eur J Clin Invest* 1983; **13**: 215-220 [PMID: 6135613 DOI: 10.1111/j.1365-2362.1983.tb00090.x]
30 **Swain MS**, Bergeron M, Audet R, Blei AT, Butterworth RF. Monitoring of neurotransmitter amino acids by means of an indwelling cisterna magna catheter: a comparison of two rodent models of fulminant liver failure. *Hepatology* 1992; **16**: 1028-1035 [PMID: 1356903 DOI: 10.1002/hep.1840160428]
31 **Weissenborn K**, Ahl B, Fischer-Wasels D, van den Hoff J, Hecker H, Burchert W, Köstler H. Correlations between magnetic resonance spectroscopy alterations and cerebral ammonia and glucose metabolism in cirrhotic patients with and without hepatic encephalopathy. *Gut* 2007; **56**: 1736-1742 [PMID: 17660226 DOI: 10.1136/gut.2006.110569]
32 **Dabos KJ**, Newsome PN, Parkinson JA, Mohammed HH, Sadler IH, Plevris JN, Hayes PC. Biochemical prognostic markers of outcome in non-paracetamol-induced fulminant hepatic failure. *Transplantation* 2004; **77**: 200-205 [PMID: 14742981 DOI: 10.1097/01.TP.0000100466.22441.37]
33 **Dabos KJ**, Newsome PN, Parkinson JA, Davidson JS, Sadler IH, Plevris JN, Hayes PC. A biochemical prognostic model of outcome in paracetamol-induced acute liver injury. *Transplantation* 2005; **80**: 1712-1717 [PMID: 16378066 DOI: 10.1097/01.tp.0000187879.51616.e0]

P- Reviewer: Frider B, He ST, Pan JJ **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1708

World J Hepatol 2015 June 28; 7(12): 1708-1717
ISSN 1948-5182 (online)

SYSTEMATIC REVIEWS

Hepatocellular carcinoma in Asia: Prevention strategy and planning

Sara Ashtari, Mohamad Amin Pourhoseingholi, Afsaneh Sharifian, Mohamad Reza Zali

Sara Ashtari, Mohamad Amin Pourhoseingholi, Afsaneh Sharifian, Mohamad Reza Zali, Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran 1985717413, Iran

Author contributions: Sharifian A and Zali MR designed the research; Pourhoseingholi MA designed and checked an edited the paper as a correspondence; Ashtari S performed the research and write the paper.

Supported by Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences.

Conflict-of-interest: The authors declared do not have anything to disclose regarding funding of conflict-of-interest with respect to this manuscript.
Data sharing: No additional data are available.

Correspondence to: Mohamad Amin Pourhoseingholi, PhD, Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tabnak St, Yaman Ave, Velenjak, Tehran 1985717413, Iran. amin_phg@yahoo.com
Telephone: +98-21-22432515
Fax: +98-21-22432517

Received: September 17, 2014
Peer-review started: September 20, 2014
First decision: November 27, 2014
Revised: December 31, 2014
Accepted: May 26, 2015
Article in press: May 27, 2015
Published online: June 28, 2015

Abstract

AIM: To review all of epidemiological and etiological aspects of hepatocellular carcinoma (HCC) and examined the prevention of this disease in Asia.

METHODS: We conducted a systematic review according to the PRISMA guidelines. We were chosen articles that published previously, from PubMed (MEDLINE), the Cochrane database and Scopus. The key words used in this research were as follows: HCC in Asia and the way of prevention of this disease, with no language limitations. We selected those papers published before 2014 that we considered to be most important and appropriate. All relevant articles were accessed in full text and all relevant materials was evaluated and reviewed.

RESULTS: More than 70% of all new cases of liver cancer were diagnosed in Asia, a region that 75% of all those chronically infected with hepatitis B virus (HBV) in the world. Chronic HBV infection is the main cause of HCC in Asia, where the virus is endemic and vertical transmission is common. Japan, Saudi Arabia, Egypt and Pakistan are exception because of high prevalence of HCV infection in these regions. The prevalence of this cancer is high in Eastern and South-Eastern Asia, But Middle Eastern countries are characterized as moderate prevalence rate of HCC region and Central Asia and some part of Middle Eastern countries are known as low prevalence rate of HCC. In addition of HBV and HCV the other factors such as aflatoxin, alcohol, obesity, diabetes and non-alcoholic fatty liver disease (NAFLD) might be responsible for a low prevalence of HCC in Asian countries. Currently available HCC therapies, chemotherapy, surgical are inefficient, mainly due to usually late diagnosis and high recurrence rates after surgical resection, and usually end with treatment failure. Liver transplantation also remains as a difficult strategy in patients with HCC. Thus prevention of HCC by treating and prevention HBV and HCV infection, the major causative agents of HCC, and the other risk

factors such as aflatoxin, alcohol, obesity, diabetes and NAFLD is of a great medical importance.

CONCLUSION: The main challenge which still present in Asia, is the high prevalence of chronic hepatitis. So, prevention of HBV and HCV is the key strategy to reduce the incidence of HCC in Asia.

Key words: Hepatocellular carcinoma; Viral hepatitis; Prevention strategy; Asian countries

Core tip: In this current review, the burden and incidence of hepatocellular carcinoma (HCC) in Asian countries, risk factors and prevention of HCC are discussed. Infection of hepatitis B virus (HBV) is the main cause of HCC in Asia continent, where the virus is endemic and vertical transmission is common. Japan, Saudi Arabia, Egypt and Pakistan are exception due to of high prevalence of HCV infection. The main challenge which still present in Asia, is the high prevalence of chronic hepatitis. So, HBV and HCV prevention is the key strategy to decrease the incidence and burden of HCC in Asia.

Ashtari S, Pourhoseingholi MA, Sharifian A, Zali MR. Hepatocellular carcinoma in Asia: Prevention strategy and planning. *World J Hepatol* 2015; 7(12): 1708-1717 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1708.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1708

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. HCC is the fifth most common cancer in men (554000 cases, 7.5% of the total) and the ninth in women (228000 cases, 3.4% of the total), with 782000 new cases occurring in 2012 and approximately 746000 persons die each year from this[1-3]. The rate of incidence and mortality are similar because most HCCs are diagnosed at an advanced stage[4]. HCC is the second most common cause of death from cancer in the world[1,5]. Its distribution geographically related to the hepatitis B virus (HBV) and/or HCV prevalence, which are HCC's main risk factors[6]. Its burden is the highest in the South-East Asia and Sub-Saharan Africa due to HBV infection's endemic[7] and most new cases (up to 80%) occur in theses area with the age-standardized incidence (> 20 per 100000), compared to low-incidence areas with (< 5 per 100000) in South and Central America, and the some part of Europe[6,8,9]. For men, high incidence regions are Eastern and South-Eastern Asia (> 20 per 100000). Intermediate rates occur in Southern Europe and Northern America (5-10 per 100000) and the lowest rates are in Northern Europe and South-Central Asia (< 5 per 100000)[1]. For women, the rates are much lower, the highest rate are in Eastern Asia and Western Africa (5-10 per 100000), the lowest in Northern Europe and Micronesia (< 5 per 100000)[1,3]. In Asian and African countries, HBV is the most common cause of HCC, while HCV is the most common cause in regions with a low prevalence of HBV (*e.g.,* America, Northern Europe and Australia)[10].

This cancer is generally affecting men more than women, although this difference varies across the world[4,7,11]. According to the GLOBOCON estimates for 2002, the overall male to female ratio was 2.4 and this ratio was even higher in regions with high incidence rate of HCC[9,12]. High rate of HCC in men (compare to women) may be related to higher consume of alcohol and smoking, or/and it has been related to the estrogen and androgens activities[13-15].

The age distribution of HCC varies by incidence rate, sex and region[16]. In low-risk countries (*e.g.,* United States, Canada, and United Kingdom), and also in high-risk Asian countries (*e.g.*, Hong Kong and China) the highest age-specific rates are among persons aged 75 and older[13] and this is despite the fact that, the age-specific rates occur among male aged 60 and 65 before declining in high-risk African countries (*e.g.*, Gambia, Mali), whereas the peak of female's rates are between 65 and 70 before declining[8,13].

MATERIALS AND METHODS

We conducted a systematic review according to the PRISMA guidelines. All searches for writing this review is based on the papers was found in PubMed (MEDLINE), Cochrane database and Scopus in August and September 2014 for topic of HCC in Asia and the way of prevention of this disease, with no language limitations. All relevant articles were accessed in full text and all relevant materials was evaluated and reviewed. We extracted data on epidemiology of HCC, burden and prevalence of HCC, risk factors characteristics association HCC, and prevention of HCC. All findings were reviewed and analyzed, then reported as results in the tables and text.

RESULTS

Burden of liver cancer in Asia

Asian continent covers approximately 4.3 billion people (60% of the world's current population). More than 70% (50% in China alone) of all new cases of liver cancer were diagnosed in Asia, a region that 75% of all those chronically infected with HBV in the world[17]. HBV is the main cause of HCC in Asia, where the virus is endemic and vertical transmission is common[5,18]. In Japan (68%), Saudi Arabia (39.5%), Egypt (69%) and Pakistan (45%) infected with HCV is the main risk factor for HCC[19-22].

Incidence rate of HCC is high in Eastern and South-Eastern Asia (*e.g.,* China, Hong Kong, Taiwan, South Korea, Thailand, and Philippines)[6]. It is less in the

Table 1 Incidence rate for males and females, and common cause of hepatocellular carcinoma in some Asian countries[6,10]

Country	Incidence rate (per 100000 persons)		Common cause of HCC
	Males	Females	
China	58	22	HBV
Hong Kong	29.9	8.3	HBV
India	0.9-3.4	0.2-1.8	HBV
Japan	8	6	HCV
South Korea	45	33.6	HBV
Malaysia	3.6	1.6	HBV
Philippines	13.4	4.8	HBV
Singapore	7.1	1.5	HCV
Taiwan	53	21	HBV
Thailand	33.4	12.3	HBV
Egypt	21.9	4.5	HCV
Iran	1.4	1.9	HBV
Kuwait	8.1	3.6	HBV
Oman	7.4	3.2	HBV
Saudi Arabia	5.9	2.2	HCV
Bahrain	5.3	3.1	HBV
Lebanon	3.5	2.2	HBV
Qatar	3.4	1.8	HBV
Palestine	2.6	0.7	HBV
Tunisia	2.2	0.7	HBV
Jordan	1.9	1.3	HBV

HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Middle-East countries (*e.g.,* Iran, Iraq, Kuwait, Oman, Qatar, Saudi Arabia, Bahrain, and Lebanon) compared to high incidence countries in South-East Asia, except in Egypt because of higher incidence of HCC in Middle-East region[10]. Central Asia and some part of Middle East regions (Kazakhstan, Kyrgyzstan, Tajikistan, and Turkmenistan) are characterized by a low incidence rate of HCC[1]. The summery of incidence rate of HCC for males and females and also common cause of HCC in some countries of Asia are shows in Table 1.

Risk factors of HCC: HCC is a complex disease associated with many risk factors and cofactors[12,23]. Main risk factors for HCC include HBV/HCV infection, alcohol, aflatoxin exposure, obesity, non-alcoholic fatty liver disease (NAFLD) and diabetes[12,24,25]. In developing countries, chronic HBV with or without aflatoxin exposure in most cases, is the major cause of HCC. Besides, in these countries, HBV infection is transmitted from mother to newborn and in these infected children, 90% experienced developing chronic HBV[23,26]. In developed countries, in contrast, 90% of HCC cases occur in cirrhosis patients (with chronic HCV or alcohol abuse) and in this regions HCV infection spreads mainly through sexual and other horizontal transmission an adulthood, and if that the HBV vaccination is widely effective in this countries[27-29].

Hepatitis B in Asia: In general, the 350 million people diagnosed with HBV worldwide, and it is estimated that HBV is responsible for (50% to 80%) cause of HCC[24,30]. Although HBV would be the cause HCC in the absence of cirrhosis, the majority of HCC patients (70%-80%) who infected with HBV-related, have cirrhosis too. The increased HCC risk associated with HBV. Thus, the HCC's incidence increases with the prevalence of hepatitis B surface antigen (HBsAg) in all areas, particularly in endemic HBV region[31,32]. Similar to the world distribution of HBV, Asian countries also divided in to the low (< 2%), intermediate (2%-8%) and high endemic areas (> 8%) of HBV[33]. Although the region of South-East Asia previously has classified as a high endemicity area, China is now the only country, classified as high endemic area with 8%-20% prevalence of HBV[34]. Oman, Yemen and Jordan in the Middle East are characterizes by a moderate to high prevalence rate of HBV in their own populations[34,35]. Countries with intermediate endemicity in Asia includes; India, Taiwan, Thailand, Philippines, Korea, Iraq and United Arab Emirates, and countries with low endemicity including Japan, Pakistan, Singapore, Sri Lanka, Bangladesh, Malaysia, Iran, Kuwait and Bahrain[36,37].

Because of hepatitis B prevention programs (vaccination), the epidemiological pattern of HBV prevalence changes with time in most Asian countries. In Taiwan due immunization program of HBV, the percentage of HBV-related HCC in children and adolescents decreased[38,39]. In Saudi Arabia and Malaysia, the prevalence of HBV infection in children have declined since the beginning of the vaccination[40,41]. Iran is located in low risk area and characterized as low incidence rate of HCC (< 5 per 100000)[42]. According to a recent study designed in Southern Iran, HBV was the main HCC's risk factor, accounts for of 52.1% of cases[43]. After setting the HBV National Vaccination Program for all newborns and high risk groups since 1992 in Iran, the prevalence of the virus decreased dramatically[35,44]. According to World Health's report in 2001 and Centers for Disease

Control and Prevention's in 2005, prevalence of chronic hepatitis B (CHB) infection in Iran ranges between 2%-7%[35,45]. Factors which increased the risk of HCC in persons with chronic HBV infection include; male, age, long time infected, family history of HCC, aflatoxin exposure, alcohol, tobacco, and infected with HBV genotype C[46].

Hepatitis C in Asia: 170-200 million people are infected with HCV worldwide and its play an important role in HCC especially in regions where CHB is less common[47,48]. In contrast, HCC in HCV patients almost occurs in people with cirrhosis[49,50]. The estimated risk of HCC in patients with HCV is 15 to 20 times higher in healthy persons, and also increased the risk of HCC in patients with advanced hepatic fibrosis or cirrhosis[51]. Prior to anti-HCV screening tests for blood donors (in 1990/1991 in Europe and United States), blood transfusion and injection drug use (IDU) were recognized as the leading cause of HCV, but after the implementation of routine blood donor screening, IDU is the main risk factor (only in developed countries)[10,52]. According to World Health Organization's (WHO) Global Database for Blood Safety. It is calculated that 43% of blood donors (in developing countries) are not properly screened for prevent the transfer of infections, including HCV[53]. Therefore, in developing countries blood transfusion is a highly main risk for HCV transmission. Sexual and maternal-infant HCV transmission can occur but it is rare[54]. Generally, the population at risk for HCV infections who are exposed to infected blood, hemodialysis, IDU, prisoners, tattooing, and during medical and dental care[55].

The most high prevalence rate of HCV occurs in African and Asian countries (5.3% in Africa and 2.15%-3.9% in Asia)[56,57]. The prevalence of HCV infection in Asian countries varies geographically, In Japan, Saudi Arabia, Egypt and Pakistan, HCV is the cause of HCC. The markers of Hepatitis C infection (positive anti-HCV) are found in 80%-90% HCC patients in Japan, 70% in Egypt, 40%-50% in the Pakistan and 35%-40% in Saudi Arabia[10,58-61].

According to the population-based study of Merat *et al*[62] the prevalence of HCV in Iran was 0.3%, 1.6% and 1.0% in Tehran, Hormozgan, and Golestan provinces, respectively. After HBV, HCV infection is the main risk factor of HCC in Iran with an incidence of 8.5%[43].

Factors which increased the risk of HCC in persons with chronic HCV include; male, elderly, co-infected with human immunodeficiency virus and HBV infection, heavy alcohol intake, diabetes and obesity[63-66].

Concept of carcinogens in HBV and HCV

HBV is members of hepadnaviruses that can cause transient or chronic infections. And finally chronic infections can lead to liver failure with cirrhosis and HCC[67]. Multiple factors are involved in the hepatocarcinogenesis of HBV infection. A main factor is chronic necroinflammation and subsequent fibrosis/liver cell proliferation. In spite of that, HCC only occurs in a small proportion of HBsAg carriers. Because the hepatocarcinogenic process includes the interplay between hepatitis B and host hepatocytes, both genomes could contribute to the final pathogenic outcomes, individually or synergistically[68].

HBV contains a double stranded genomic DNA that may encode oncogenic viral proteins which is possibly contributed to hepatocarcinogenesis[69]. For example, protein HBx (which is a well-known viral non-structural gene) operates as a multifunctional regulator modulating gene transcription, cell responses to oxidative stress, protein degradation, apoptosis, and several signaling pathways[70]. Due to this fact that, the specific mechanism is still unknown, its role in liver malignant transformation has been clearly demonstrated by HBx[71]. In addition to viral oncogenic proteins, several viral factors, including genotype, BCP mutation, and viral load have been confirmed to be associated with hepatocarcinogensis. In Asia, it is revealed that, genotype C is more commonly associated with liver cirrhosis and HCC compared with genotype B[23,72].

Hepatitis C is member of the flaviviruses, which it forms its own genus, hepacivirus. HCV is a small, enveloped positive-sense, single stranded RNA virus, and its life cycle is predominantly cytoplasmic[73]. Therefore, this virus is likely to predispose to cancer by alteration of cell signaling and metabolism as similar as by inducing immune responses[74]. Modulation of cellular immunity and metabolism are processes that establish a liver microenvironment which characterized by chronic inflammation, oxidative stress and repair processes that lead to liver fibrosis, cirrhosis and HCC[75-78].

Other environmental and genetic risk factors of HCC

HBV and HCV infections are the major causes of more than 75% of the HCC in the world, with an even more in developing countries[16]. HBV infection is most common in Asia, except in Japan, Saudi Arabia, Egypt and Pakistan, where HCV instead, is the main cause of primary HCC. In addition, exposure to aflatoxin in Asia is a significant risk factor, especially in China and Taiwan[18,79]. On the other hand, other factors such as alcohol, obesity, diabetes and NAFLD might be responsible for a low prevalence of HCC in Asian countries[6,10].

Aflatoxin: Aflatoxin is a mycotoxin produced by the Aspergillus fungus. This fungus grows easily on foodstuffs including; peanuts, corn, pistachio, *etc*., which stored in warm and damp conditions[80]. Studies have been done in sub-Saharan Africa and South-East Asia revealed the association between aflatoxin and HCC[81]. Also, some studies in Asia, Shanghai and Taiwan, indicated the interaction between aflatoxin exposure and hepatitis B infection and a study in Taiwan reported that in HBsAg carriers, who were susceptible to aflatoxin, were more likely to develop HCC[82-86]. Besides, in most regions where aflatoxin exposure is high, HBV infection also is highly prevalent[13]. A recent study in Taiwan[87]

reported the relationship between aflatoxin and HCV with advanced liver disease. Unfortunately we don't have any study in Middle East countries that worked on association between of aflatoxin and HCC.

Alcohol: Alcohol generally contributed to 15% to 45% of HCC cases in developed countries due to its significant role in cirrhosis[13,88]. Many studies have shown the association of heavy alcohol intake (> 50-70 g/d for several years) and HCC[89-91]. Men tend to consume more alcohol than women[10]. The annual incidence of HCC due to alcohol cirrhosis is 1%-4%[92]. Alcohol consumption in Asian countries, in contrast to American and European countries, plays a minor role for HCC development. Especially in Middle Eastern countries, rather than to south Eastern countries in Asia the consumption of alcohol is very low[6,10,43].

Obesity, diabetes and non-alcoholic fatty liver: Epidemiological studies have shown that obesity is a risk factor for HCC. Similar studies further indicate that type 2 of diabetes milieus (T2DM) is also a major risk factor. Both obesity and T2DM are often related to NAFLD. Case reports have shown progression of NAFLD to cirrhosis and HCC[93,94]. A Danish study indicated that, the chance of HCC is more in obese people than general population (RR = 1.9)[65]. The risk of HCC in obese Patients (with body mass index greater than 30) is increasing more than cirrhotic patients[95]. The prevalence of obesity in Asian countries varies geographically. This prevalence is 19.4% in Iran, 33.3% in Saudi Arabia, 33.2% in Qatar, 33.1% in Egypt, 32.9% in Bahrain, 5.7% in China, 5.0% in Japan, 14.0% in Malaysia[96]. The highest prevalence belongs to Kuwait with 42.0% and lowest prevalence belongs to Bangladesh with 1.1%[96] (data adjusted for 2008 for comparability). Prevalence of overweight and obese people based on several national health surveys in Asia has increased[97]. The prevalence of obesity in adults in South-East Asian countries is usually low, compared to developed countries like as the United State, but in contrast to South-East Asian countries, the prevalence of obesity in Middle-East countries is high and almost is equal to developed countries. In the future, obesity may be play as an important role of HCC because of the high prevalence in Middle-East countries[98].

Comparative Prevalence of diabetes in Asian countries also, varies geographically. This prevalence is 9.94% in Iran, 23.09% in Kuwait, 22.87% in Qatar, 16.80% in Egypt, 17.30% in Bahrain, 9.02% in China, 12.28% in Singapore, 10.85% in Malaysia[99]. The highest prevalence belongs to Saudi Arabia with 23.87% and lowest prevalence belongs to Japan with 5.12%[99]. The prevalence of diabetes in countries located in South-east Asia is quite low but, in contrast, this prevalence is high in Middle-East countries. In the future, the high incidence rate of diabetes in countries of Middle-East might become it as the major risk factor for HCC in this region.

According to community-based cohort studies in the United States, Scandinavia, Taiwan, and Japan[64,65,93], the occurrences of HCC was 1.5 to 2.0 times higher in obese persons than in people with normal weight. Also some case-control studies and a few cohort studies indicated that, occurrence of HCC in persons with T2DM than in non-diabetics persons is double[100,101]. NAFLD is clearly linked with obesity and T2DM, that is way it is recognizes as a possible risk factor for HCC[102]. NAFLD may started as simple steatosis (NAFLD), to non-alcoholic steatohepatitis (NASH) or cirrhosis and HCC (due to obesity), T2DM associated to metabolic derangements[103]. NASH is a more advanced stage of NAFLD, so that about 20% of NASH patients usually progress to liver cirrhosis or even some patients with NASH show HCC with or without liver cirrhosis[104,105]. NASH is the first damage caused by a buildup of fat in the liver (NAFLD), NASH can progress and get worse with scar and severe inflammation and fibrosis. With 5-year follow-up of patients with NASH, observed that the progression of fibrosis can lead to cirrhosis[104]. Some factors such as abnormal glucose regulation, obesity, T2DM and triglyceride can increase the risk of NASH[106]. Generally the whole fibrogenesis develops of NASH from NAFLD due to multiple factors, including; oxidative stress, insulin resistance, lipotoxicity, pro-inflammatory cytokine and hepatic stem cells[107].

DISCUSSION

The main challenge which still present in Asia, is the high prevalence of chronic hepatitis. So, prevention of infection with hepatitis B and hepatitis C is the key to reduce the burden of HCC in Asia[108,109].

Prevention of HBV

HBV vaccination is the most effective methods to prevent HBV in both newborn and adult infections with HBV[110]. National HBV vaccination program reduces the prevalence of HBV and also the incidence of HCC dramatically[24]. However, more time is needed to reach the final results, because this program were introduced between 1982 and 1990 in the world and most cases of HCC occur after the age of 40 years[111,112].

Antiviral treatment of HBV

The results of many studies suggested that antiviral therapy is very effective to controls HBV infection. In a study has been done in China, cirrhosis and fibrosis HBV patients randomly assigned in two groups; first received 100 mg of lamivudine per day and second received placebo for up to 5 years. According to the results, the incidence of HCC was significantly reduced in the lamivudine group (3.9% *vs* 7.4%; HR = 0.49; *P* = 0.047)[113].

Prevention of HCV

HCV's prevention, in absence of an effective vaccine, is more challenging than the HBV's and requires a fundamental and comprehensive strategy, including;

blood donations screening, safe injection and systematic avoidance of unnecessary injections[22].

Antiviral treatment of HCV

Combination antiviral therapy helps to prevent the HCV and followed by HCC. Combination therapy decreases the risk of HCC in patients with HCV-related cirrhosis, even without complete biochemical and virological clearing[60]. The current treatments for HCV are combination therapy of pegylated interferon with ribavirin[114-117].

Other strategy and remaining challenge to prevent HBV and HCV infection: In most Asian countries, HBV is usually transmitted from mother to newborn[118]. In order to avoid of maternal-child transmission, WHO is recommending HBV vaccination at birth, but unfortunately less than half of member states have policy to provide HBV vaccination at birth and only 27% of newborns globally received this vaccine[119,120].

Raising awareness and knowledge about the viral hepatitis B and C infection help reduce transmission in the community, also increasing awareness among policy-makers, health professionals and decision- makers in society can help to make better decision and planning to prevent viral hepatitis[120]. Implementation of blood safety strategies is one of the best ways to prevent transmission of hepatitis C infection[24,108], screening blood donation is really effective but in low-income countries where data available, only 35% of donated blood samples were screened in a quality assured manner in 2008[120].

Early detection of HBV and HCV cases provides the best opportunity for effective medical support and prevention of further spread[22,108].

Most new cases of HCV and HBV infections in Asia (or elsewhere) are due to IDU. Needle and syringe sharing practices between Injecting drug users, largely increase the risk of HCV and HBV. Generally, about 60%-80% (about 10 million people) of injecting drug users is positive for HCV and 5%-10% positive for HBV. Controlling this social problem is important in prevention of HCV and HBV cirrhosis related to HCC[121-123].

Prevention of HCC associated with other risk factors: The proportion of HCC cases due to other causes (except HBV and HCV) is usually between 10% and 20% in Asia[108]. Such cases include aflatoxin, alcohol consumption, obesity, type 2 diabetes and NAFLD. Therefore abstaining from alcohol and toxin exposure is very effective for reducing the risk of HCC.

NAFLD in synergy with other risk factors such as obesity, diabetes and metabolic syndrome, is becoming one of the other risk factors for HCC. Due to the lack of understanding of the pathogenesis of the disease, the prevention of NAFLD remains as a difficult problem. So prevention of the risk factors of NAFLD such as obesity, insulin resistance, diabetes and metabolic syndrome is the key strategy to reduce the incidence of NAFLD in the world[124]. Therefore, changing the life style such as weight loss and regular physical activity is directed towards reducing HCC risk factors. Based on the epidemiologic evidence, obesity and T2DM are associated to NAFLD and they are independent risk factors of HCC. In addition, early detection and treatment of diabetes and hyperinsulinemia are very essential and critical to prevent of HCC associated with diabetes and NAFLD. Several studies showed that the use of insulin-sensitizing (metformin and thiazolidinediones) agents in diabetes could reduce the risk of HCC[125-127]. Insulin-sensitizing drugs and avoiding from treatments contributing to hyperinsulinemia would be helpful to prevent HCC and to improve disease outcomes[103].

COMMENTS

Background

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and the second cause of cancer death. Hepatitis B virus (HBV) and HCV infections are the major cause of HCC in the Asian countries, where the virus is endemic and vertical transmission is common. In addition of HBV and HCV the other factors such as aflatoxin, alcohol, obesity, diabetes and non-alcoholic fatty liver disease (NAFLD) might be responsible for a low prevalence of HCC in Asian countries.

Research frontiers

The objective of this study was to review systematically all of aspects of HCC in Asia, provides updated epidemiological data on HCC and its etiology and also this study have examined the current and future possibilities of prevention of this disease in Asian countries.

Innovations and breakthroughs

Unfortunately, most previous studies only focused on South-East countries on Asia. However, in this study the authors have tried to consider all the countries is located in Asia. And generally the authors collected useful information.

Applications

Based on this systematic review obesity, diabetes and NAFLD is growing in Asian countries, which can increase the risk of HCC. An also aflatoxin should be more considered.

Terminology

HCC, also called malignant hepatoma, is the most common type of liver cancer. Most cases of HCC are due to HBV, HCV or cirrhosis.

Peer-review

This is a well-written comprehensive review of the epidemiology of HCC in Asia.

REFERENCES

1 **IARC**. Liver Cancer: Estimated Incidence, Mortality, Prevalence Worldwide in 2012. [Accessed 2013 Dec 12]. Available from: URL: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx 2012

2 **Flores A**, Marrero JA. Emerging trends in hepatocellular carcinoma: focus on diagnosis and therapeutics. *Clin Med Insights Oncol* 2014; **8**: 71-76 [PMID: 24899827 DOI: 10.4137/CMO.S9926]

3 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

4 **Yang JD**, Roberts LR. Hepatocellular carcinoma: A global view. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 448-458 [PMID: 20628345 DOI: 10.1038/nrgastro.2010.100]

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

6 **Yuen MF**, Hou JL, Chutaputti A. Hepatocellular carcinoma in the

Asia pacific region. *J Gastroenterol Hepatol* 2009; **24**: 346-353 [PMID: 19220670 DOI: 10.1111/j.1440-1746.2009.05784.x]
7 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]
8 **Nordenstedt H**, White DL, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma. *Dig Liver Dis* 2010; **42** Suppl 3: S206-S214 [PMID: 20547305 DOI: 10.1016/S1590-8658(10)60507-5]
9 **Ferlay J**, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. Lyon: IARC Press, 2001
10 **Poustchi H**, Sepanlou S, Esmaili S, Mehrabi N, Ansarymoghadam A. Hepatocellular carcinoma in the world and the middle East. *Middle East J Dig Dis* 2010; **2**: 31-41 [PMID: 25197510]
11 **McGlynn KA**, Tsao L, Hsing AW, Devesa SS, Fraumeni JF. International trends and patterns of primary liver cancer. *Int J Cancer* 2001; **94**: 290-296 [PMID: 11668511]
12 **Venook AP**, Papandreou C, Furuse J, de Guevara LL. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *Oncologist* 2010; **15** Suppl 4: 5-13 [PMID: 21115576 DOI: 10.1634/theoncologist.2010-S4-05]
13 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576 [PMID: 17570226 DOI: 10.1053/j.gastro.2007.04.061]
14 **Naugler WE**, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007; **317**: 121-124 [PMID: 17615358 DOI: 10.1126/science.1140485]
15 **Nagasue N**, Ogawa Y, Yukaya H, Ohta N, Ito A. Serum levels of estrogens and testosterone in cirrhotic men with and without hepatocellular carcinoma. *Gastroenterology* 1985; **88**: 768-772 [PMID: 2981753]
16 Cancer incidence in five continents. Volume VIII. *IARC Sci Publ* 2002; (**155**): 1-781 [PMID: 12812229]
17 **Lai CL**, Ratziu V, Yuen MF, Poynard T. Viral hepatitis B. *Lancet* 2003; **362**: 2089-2094 [PMID: 14697813 DOI: 10.1016/S0140-6736(03)15108-2]
18 **Bosch FX**, Ribes J, Cléries R, Díaz M. Epidemiology of hepatocellular carcinoma. *Clin Liver Dis* 2005; **9**: 191-211, v [PMID: 15831268 DOI: 10.1016/j.cld.2004.12.009]
19 **Salim EI**, Moore MA, Al-Lawati JA, Al-Sayyad J, Bazawir A, Bener A, Corbex M, El-Saghir N, Habib OS, Maziak W, Mokhtar HC, Seif-Eldrin IA, Sobue T. Cancer epidemiology and control in the arab world - past, present and future. *Asian Pac J Cancer Prev* 2009; **10**: 3-16 [PMID: 19469617]
20 **Umemura T**, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. *J Gastroenterol* 2009; **44** Suppl 19: 102-107 [PMID: 19148802 DOI: 10.1007/s00535-008-2251-0]
21 **But DY**, Lai CL, Yuen MF. Natural history of hepatitis-related hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1652-1656 [PMID: 18350595 DOI: 10.3748/wjg.14.1652]
22 **Franceschi S**, Raza SA. Epidemiology and prevention of hepatocellular carcinoma. *Cancer Lett* 2009; **286**: 5-8 [PMID: 19070421 DOI: 10.1016/j.canlet.2008.10.046]
23 **Gomaa AI**, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. *World J Gastroenterol* 2008; **14**: 4300-4308 [PMID: 18666317 DOI: 10.3748/wjg.14.4300]
24 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]
25 **Kim do Y**, Han KH. Epidemiology and surveillance of hepatocellular carcinoma. *Liver Cancer* 2012; **1**: 2-14 [PMID: 24159567 DOI: 10.1159/000339016]
26 **Parikh A**, Taouli B. Imaging of hepatocellular carcinoma: current concepts. *Recent Results Cancer Res* 2013; **190**: 33-55 [PMID: 22941012 DOI: 10.1007/978-3-642-16037-0_3]
27 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750 DOI: 10.1016/S0140-6736(03)14964-1]
28 **Montalto G**, Cervello M, Giannitrapani L, Dantona F, Terranova A, Castagnetta LA. Epidemiology, risk factors, and natural history of hepatocellular carcinoma. *Ann N Y Acad Sci* 2002; **963**: 13-20 [PMID: 12095924 DOI: 10.1111/j.1749-6632.2002.tb04090.x]
29 **Seeff LB**, Hoofnagle JH. Epidemiology of hepatocellular carcinoma in areas of low hepatitis B and hepatitis C endemicity. *Oncogene* 2006; **25**: 3771-3777 [PMID: 16799618 DOI: 10.1038/sj.onc.1209560]
30 **El-Serag HB**. Epidemiology of hepatocellular carcinoma in USA. *Hepatol Res* 2007; **37** Suppl 2: S88-S94 [PMID: 17877502 DOI: 10.1111/j.1872-034X.2007.00168.x]
31 **Simonetti RG**, Cammà C, Fiorello F, Politi F, D'Amico G, Pagliaro L. Hepatocellular carcinoma. A worldwide problem and the major risk factors. *Dig Dis Sci* 1991; **36**: 962-972 [PMID: 1649041 DOI: 10.1007/BF01297149]
32 **Sherman M**. Hepatocellular carcinoma: epidemiology, risk factors, and screening. *Semin Liver Dis* 2005; **25**: 143-154 [PMID: 15918143 DOI: 10.1055/s-2005-871194]
33 **World Health Organization**. Global Alert and Response (GAR): Hepatitis B. Available from: URL: http://www.who.int/csr/disease/hepatitis/whocdscsrlyo20022/en/index1.html
34 **Zidan A**, Scheuerlein H, Schüle S, Settmacher U, Rauchfuss F. Epidemiological pattern of hepatitis B and hepatitis C as etiological agents for hepatocellular carcinoma in iran and worldwide. *Hepat Mon* 2012; **12**: e6894 [PMID: 23233864 DOI: 10.5812/hepatmon.6894]
35 **Smolle E**, Zöhrer E, Bettermann K, Haybaeck J. Viral hepatitis induces hepatocellular cancer: what can we learn from epidemiology comparing iran and worldwide findings? *Hepat Mon* 2012; **12**: e7879 [PMID: 23233866 DOI: 10.5812/hepatmon.7879]
36 **Qirbi N**, Hall AJ. Epidemiology of hepatitis B virus infection in the Middle East. *East Mediterr Health J* 2001; **7**: 1034-1045 [PMID: 15332746]
37 **André F**. Hepatitis B epidemiology in Asia, the Middle East and Africa. *Vaccine* 2000; **18** Suppl 1: S20-S22 [PMID: 10683538 DOI: 10.1016/S0264-410X(99)00456-9]
38 **Lu SN**, Su WW, Yang SS, Chang TT, Cheng KS, Wu JC, Lin HH, Wu SS, Lee CM, Changchien CS, Chen CJ, Sheu JC, Chen DS, Chen CH. Secular trends and geographic variations of hepatitis B virus and hepatitis C virus-associated hepatocellular carcinoma in Taiwan. *Int J Cancer* 2006; **119**: 1946-1952 [PMID: 16708389 DOI: 10.1002/ijc.22045]
39 **Chen CH**, Su WW, Yang SS, Chang TT, Cheng KS, Lin HH, Wu SS, Lee CM, Changchien CS, Chen CJ, Sheu JC, Chen DS, Lu SN. Long-term trends and geographic variations in the survival of patients with hepatocellular carcinoma: analysis of 11,312 patients in Taiwan. *J Gastroenterol Hepatol* 2006; **21**: 1561-1566 [PMID: 16928217 DOI: 10.1111/j.1440-1746.2006.04425.x]
40 **Al-Faleh FZ**, Ayoola EA, Al-Jeffry M, Arif M, Al-Rashed RS, Ramia S. Integration of hepatitis B vaccine into the expanded program on immunization: The Saudi Arabian experience. *Ann Saudi Med* 1993; **13**: 231-236 [PMID: 17590667]
41 **Ng KP**, Saw TL, Baki A, Rozainah K, Pang KW, Ramanathan M. Impact of the Expanded Program of Immunization against hepatitis B infection in school children in Malaysia. *Med Microbiol Immunol* 2005; **194**: 163-168 [PMID: 15834754 DOI: 10.1007/s00430-004-0231-4]
42 **Pourhoseingholi MA**, Fazeli Z, Zali MR, Alavian SM. Burden of hepatocellular carcinoma in Iran; Bayesian projection and trend analysis. *Asian Pac J Cancer Prev* 2010; **11**: 859-862 [PMID: 21133591]
43 **Hajiani E**, Masjedizadeh R, Hashemi J, Azmi M, Rajabi T. Risk factors for hepatocellular carcinoma in Southern Iran. *Saudi Med J* 2005; **26**: 974-977 [PMID: 15983686]
44 **Alavian SM**, Fallahian F, Lankarani KB. The changing epidemiology of viral hepatitis B in Iran. *J Gastrointestin Liver Dis* 2007; **16**: 403-406 [PMID: 18193122]
45 **Poorolajal J**, Majdzadeh R. Prevalence of chronic hepatitis B infection in Iran: a review article. *J Res Med Sci* 2009; **14**: 249-258

[PMID: 21772891]
46 **Yang HI**, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, Liaw YF, Chen CJ. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 1134-1143 [PMID: 18695135 DOI: 10.1093/jnci/djn243]
47 **Bruix J**, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, Ventura M, Vall M, Bruguera M, Bru C. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989; **2**: 1004-1006 [PMID: 2572739 DOI: 10.1016/S0140-6736(89)91015-5]
48 **Chen DS**. Hepatitis C virus in chronic liver disease and hepatocellular carcinoma in Taiwan. *Princess Takamatsu Symp* 1995; **25**: 27-32 [PMID: 8875606]
49 **Fattovich G**, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, Nevens F, Solinas A, Mura D, Brouwer JT, Thomas H, Njapoum C, Casarin C, Bonetti P, Fuschi P, Basho J, Tocco A, Bhalla A, Galassini R, Noventa F, Schalm SW, Realdi G. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997; **112**: 463-472 [PMID: 9024300 DOI: 10.1053/gast.1997.v112.pm9024300]
50 **Tan A**, Yeh SH, Liu CJ, Cheung C, Chen PJ. Viral hepatocarcinogenesis: from infection to cancer. *Liver Int* 2008; **28**: 175-188 [PMID: 18251977 DOI: 10.1111/j.1478-3231.2007.01652.x]
51 **Donato F**, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, Decarli A, Trevisi P, Ribero ML, Martelli C, Porru S, Nardi G. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol* 2002; **155**: 323-331 [PMID: 11836196 DOI: 10.1093/aje/155.4.323]
52 **Touzet S**, Kraemer L, Colin C, Pradat P, Lanoir D, Bailly F, Coppola RC, Sauleda S, Thursz MR, Tillmann H, Alberti A, Braconier JH, Esteban JI, Hadziyannis SJ, Manns MP, Saracco G, Thomas HC, Trépo C. Epidemiology of hepatitis C virus infection in seven European Union countries: a critical analysis of the literature. HENCORE Group. (Hepatitis C European Network for Co-operative Research. *Eur J Gastroenterol Hepatol* 2000; **12**: 667-678 [PMID: 10912488 DOI: 10.1097/00042737-200012060-00017]
53 **World Health Organization**. Global database on blood safety summary report: 1998-1999. Available from: URL: http://www.who.int/bloodsafety/global_database/en/
54 **Ohto H**, Terazawa S, Sasaki N, Sasaki N, Hino K, Ishiwata C, Kako M, Ujiie N, Endo C, Matsui A. Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Collaborative Study Group. *N Engl J Med* 1994; **330**: 744-750 [PMID: 8107740 DOI: 10.1056/NEJM199403173301103]
55 **Sarkari B**, Eilami O, Khosravani A, Sharifi A, Tabatabaee M, Fararouei M. High prevalence of hepatitis C infection among high risk groups in Kohgiloyeh and Boyerahmad Province, Southwest Iran. *Arch Iran Med* 2012; **15**: 271-274 [PMID: 22519374]
56 **Shepard CW**, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]
57 **Sy T**, Jamal MM. Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006; **3**: 41-46 [PMID: 16614741 DOI: 10.7150/ijms.3.41]
58 **Miyakawa Y**, Iino S. Toward prevention of hepatocellular carcinoma developing in chronic hepatitis C. *J Gastroenterol Hepatol* 2001; **16**: 711-714 [PMID: 11446875 DOI: 10.1046/j.1440-1746.2001.02543.x]
59 **Rahman El-Zayadi A**, Abaza H, Shawky S, Mohamed MK, Selim OE, Badran HM. Prevalence and epidemiological features of hepatocellular carcinoma in Egypt-a single center experience. *Hepatol Res* 2001; **19**: 170-179 [PMID: 11164741]
60 **Sievert W**, Altraif I, Razavi HA, Abdo A, Ahmed EA, Alomair A, Amarapurkar D, Chen CH, Dou X, El Khayat H, Elshazly M, Esmat G, Guan R, Han KH, Koike K, Largen A, McCaughan G, Mogawer S, Monis A, Nawaz A, Piratvisuth T, Sanai FM, Sharara AI, Sibbel S, Sood A, Suh DJ, Wallace C, Young K, Negro F. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. *Liver Int* 2011; **31** Suppl 2: 61-80 [PMID: 21651703 DOI: 10.1111/j.1478-3231.2011.02540.x]
61 **Fakeeh M**, Zaki AM. Hepatitis C: prevalence and common genotypes among ethnic groups in Jeddah, Saudi Arabia. *Am J Trop Med Hyg* 1999; **61**: 889-892 [PMID: 10674665]
62 **Merat S**, Rezvan H, Nouraie M, Jafari E, Abolghasemi H, Radmard AR, Zaer-rezaii H, Amini-Kafiabad S, Maghsudlu M, Pourshams A, Malekzadeh R, Esmaili S. Seroprevalence of hepatitis C virus: the first population-based study from Iran. *Int J Infect Dis* 2010; **14** Suppl 3: e113-e116 [PMID: 20362479 DOI: 10.1016/j.ijid.2009.11.032]
63 **Davis GL**, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010; **138**: 513-521, 521.e1-6 [PMID: 19861128 DOI: 10.1053/j.gastro.2009.09.067]
64 **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638 [PMID: 12711737 DOI: 10.1056/NEJMoa021423]
65 **Møller H**, Mellemgaard A, Lindvig K, Olsen JH. Obesity and cancer risk: a Danish record-linkage study. *Eur J Cancer* 1994; **30A**: 344-350 [PMID: 8204357 DOI: 10.1016/0959-8049(94)90254-2]
66 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101 DOI: 10.1053/j.gastro.2004.09.014]
67 **Bréchot C**. Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. *Gastroenterology* 2004; **127**: S56-S61 [PMID: 15508104 DOI: 10.1053/j.gastro.2004.09.016]
68 **Amit S**, Jorge A M. Screening for hepatocellular carcinoma. *Gastroenterol Hepatol* (N Y) 2008; **4**: 201-208 [PMID: 21904498]
69 **Miller JC**, Hahn PF, Chung RT, Thrall JH, Lee SI. Screening for hepatocellular carcinoma in cirrhotic patients. *J Am Coll Radiol* 2008; **5**: 1012-1014 [PMID: 18755444 DOI: 10.1016/j.jacr.2008.01.017]
70 **Murakami S**. Hepatitis B virus X protein: a multifunctional viral regulator. *J Gastroenterol* 2001; **36**: 651-660 [PMID: 11686474 DOI: 10.1007/s005350170027]
71 **Liu CJ**, Kao JH. Hepatitis B virus-related hepatocellular carcinoma: epidemiology and pathogenic role of viral factors. *J Chin Med Assoc* 2007; **70**: 141-145 [PMID: 17475593 DOI: 10.1016/S1726-4901(09)70346-6]
72 **Shlomai A**, de Jong YP, Rice CM. Virus associated malignancies: the role of viral hepatitis in hepatocellular carcinoma. *Semin Cancer Biol* 2014; **26**: 78-88 [PMID: 24457013 DOI: 10.1016/j.semcancer.2014.01.004]
73 **Moradpour D**, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007; **5**: 453-463 [PMID: 17487147 DOI: 10.1038/nrmicro1645]
74 **Bartosch B**, Thimme R, Blum HE, Zoulim F. Hepatitis C virus-induced hepatocarcinogenesis. *J Hepatol* 2009; **51**: 810-820 [PMID: 19545926 DOI: 10.1016/j.jhep.2009.05.008]
75 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]
76 **McGivern DR**, Lemon SM. Tumor suppressors, chromosomal instability, and hepatitis C virus-associated liver cancer. *Annu Rev Pathol* 2009; **4**: 399-415 [PMID: 18928409 DOI: 10.1146/annurev.pathol.4.110807.092202]
77 **Koike K**. Pathogenesis of HCV-associated HCC: Dual-pass carcinogenesis through activation of oxidative stress and intracellular signaling. *Hepatol Res* 2007; **37** Suppl 2: S115-S120 [PMID: 17877471 DOI: 10.1111/j.1872-034X.2007.00173.x]
78 **Bartosch B**. Hepatitis B and C viruses and hepatocellular carcinoma. *Viruses* 2010; **2**: 1504-1509 [PMID: 21994691 DOI: 10.3390/v2081504]

79 **Ananthakrishnan A**, Gogineni V, Saeian K. Epidemiology of primary and secondary liver cancers. *Semin Intervent Radiol* 2006; **23**: 47-63 [PMID: 21326720 DOI: 10.1055/s-2006-939841]

80 **Wild CP**, Montesano R. A model of interaction: aflatoxins and hepatitis viruses in liver cancer aetiology and prevention. *Cancer Lett* 2009; **286**: 22-28 [PMID: 19345001 DOI: 10.1016/j.canlet.2009.02.053]

81 **IARC**. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Lyon: IARC Press, 1993

82 **Qian GS**, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, Wogan GN, Groopman JD. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 3-10 [PMID: 8118382]

83 **Wu W**. Occupational cancer epidemiology in the People's Republic of China. *J Occup Med* 1988; **30**: 968-974 [PMID: 3068337 DOI: 10.1097/00043764-198812000-00017]

84 **Wang JS**, Huang T, Su J, Liang F, Wei Z, Liang Y, Luo H, Kuang SY, Qian GS, Sun G, He X, Kensler TW, Groopman JD. Hepatocellular carcinoma and aflatoxin exposure in Zhuqing Village, Fusui County, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 143-146 [PMID: 11219772]

85 **Wang LY**, Hatch M, Chen CJ, Levin B, You SL, Lu SN, Wu MH, Wu WP, Wang LW, Wang Q, Huang GT, Yang PM, Lee HS, Santella RM. Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. *Int J Cancer* 1996; **67**: 620-625 [PMID: 8782648 DOI: 10.1002/(SICI)1097-0215(19960904)67:5<620::AID-IJC5>3.0.CO;2-W]

86 **Wu HC**, Wang Q, Yang HI, Ahsan H, Tsai WY, Wang LY, Chen SY, Chen CJ, Santella RM. Aflatoxin B1 exposure, hepatitis B virus infection, and hepatocellular carcinoma in Taiwan. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 846-853 [PMID: 19273485 DOI: 10.1158/1055-9965.epi-08-0697]

87 **Chen CH**, Wang MH, Wang JH, Hung CH, Hu TH, Lee SC, Tung HD, Lee CM, Changchien CS, Chen PF, Hsu MC, Lu SN. Aflatoxin exposure and hepatitis C virus in advanced liver disease in a hepatitis C virus endemic area in Taiwan. *Am J Trop Med Hyg* 2007; **77**: 747-752 [PMID: 17978082]

88 **Abdel-Hamid NM**. Recent insights on risk factors of hepatocellular carcinoma. *World J Hepatol* 2009; **1**: 3-7 [PMID: 21160959 DOI: 10.4254/wjh.v1.i1.3]

89 **Hassan MM**, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002; **36**: 1206-1213 [PMID: 12395331 DOI: 10.1053/jhep.2002.36780]

90 **Braga C**, La Vecchia C, Negri E, Franceschi S. Attributable risks for hepatocellular carcinoma in northern Italy. *Eur J Cancer* 1997; **33**: 629-634 [PMID: 9274446 DOI: 10.1016/S0959-8049(96)00500-X]

91 **Donato F**, Tagger A, Chiesa R, Ribero ML, Tomasoni V, Fasola M, Gelatti U, Portera G, Boffetta P, Nardi G. Hepatitis B and C virus infection, alcohol drinking, and hepatocellular carcinoma: a case-control study in Italy. Brescia HCC Study. *Hepatology* 1997; **26**: 579-584 [PMID: 9303486 DOI: 10.1002/hep.510260308]

92 **Jepsen P**, Ott P, Andersen PK, Sørensen HT, Vilstrup H. Risk for hepatocellular carcinoma in patients with alcoholic cirrhosis: a Danish nationwide cohort study. *Ann Intern Med* 2012; **156**: 841-847, W295 [PMID: 22711076 DOI: 10.7326/0003-4819-156-12-201206190-00004]

93 **Wolk A**, Gridley G, Svensson M, Nyrén O, McLaughlin JK, Fraumeni JF, Adam HO. A prospective study of obesity and cancer risk (Sweden). *Cancer Causes Control* 2001; **12**: 13-21 [PMID: 11227921]

94 **Caldwell SH**, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S97-S103 [PMID: 15508109 DOI: 10.1053/j.gastro.2004.09.021]

95 **Marrero JA**, Fontana RJ, Fu S, Conjeevaram HS, Su GL, Lok AS. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. *J Hepatol* 2005; **42**: 218-224 [PMID: 15664247 DOI: 10.1016/j.jhep.2004.10.005]

96 **World Health Organization**. Global status report on non-communicable diseases 2010. [Accessed 2011 April]. Available from: URL: http://www.who.int/nmh/publications/ncd_report_full_en.pdf

97 **Ramachandran A**, Snehalatha C. Rising burden of obesity in Asia. *J Obes* 2010; **2010**: pii: 868573 [PMID: 20871654 DOI: 10.1155/2010/868573]

98 **Yoon KH**, Lee JH, Kim JW, Cho JH, Choi YH, Ko SH, Zimmet P, Son HY. Epidemic obesity and type 2 diabetes in Asia. *Lancet* 2006; **368**: 1681-1688 [PMID: 17098087 DOI: 10.1016/s0140-6736(06)69703-1]

99 **International Diabetes Federation**. IDF Diabetes Atlas. 6th ed. Brussles, Belgium: International Diabetes Federation, 2013

100 **El-Serag HB**, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460-468 [PMID: 14762783 DOI: 10.1053/j.gastro.2003.10.065]

101 **El-Serag HB**, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; **4**: 369-380 [PMID: 16527702 DOI: 10.1016/j.cgh.2005.12.007]

102 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219 [PMID: 12717402 DOI: 10.1053/jhep.2003.50193]

103 **Baffy G**, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012; **56**: 1384-1391 [PMID: 22326465 DOI: 10.1016/j.jhep.2011.10.027]

104 **Starley BQ**, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; **51**: 1820-1832 [PMID: 20432259 DOI: 10.1002/hep.23594]

105 **Ertle J**, Dechêne A, Sowa JP, Penndorf V, Herzer K, Kaiser G, Schlaak JF, Gerken G, Syn WK, Canbay A. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011; **128**: 2436-2443 [PMID: 21128245 DOI: 10.1002/ijc.25797]

106 **van der Poorten D**, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, Peduto T, Chisholm DJ, George J. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008; **48**: 449-457 [PMID: 18627003 DOI: 10.1002/hep.22350]

107 **Okazaki I**, Noro T, Tsutsui N, Yamanouchi E, Kuroda H, Nakano M, Yokomori H, Inagaki Y. Fibrogenesis and Carcinogenesis in Nonalcoholic Steatohepatitis (NASH): Involvement of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinase (TIMPs). *Cancers* (Basel) 2014; **6**: 1220-1255 [PMID: 24978432 DOI: 10.3390/cancers6031220]

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

109 **Fazeli Z**, Pourhoseingholi MA, Vahedi M, Zali MR. Burden of Hepatocellular Carcinoma in Aisa. *Asian Pacific J Cancer Prevention* 2012; **13**: 5955-5958 [DOI: 10.7314/APJCP.2012.13.12.5955]

110 **Zanetti AR**, Van Damme P, Shouval D. The global impact of vaccination against hepatitis B: a historical overview. *Vaccine* 2008; **26**: 6266-6273 [PMID: 18848855 DOI: 10.1016/j.vaccine.2008.09.056]

111 **Chen DS**. Toward elimination and eradication of hepatitis B. *J Gastroenterol Hepatol* 2010; **25**: 19-25 [PMID: 20136972 DOI: 10.1111/j.1440-1746.2009.06165.x]

112 **Lim SG**, Mohammed R, Yuen MF, Kao JH. Prevention of hepatocellular carcinoma in hepatitis B virus infection. *J Gastroenterol Hepatol* 2009; **24**: 1352-1357 [PMID: 19702903 DOI: 10.1111/j.1440-1746.2009.05985.x]

113 **Liaw YF**, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and

advanced liver disease. *N Engl J Med* 2004; **351**: 1521-1531 [PMID: 15470215 DOI: 10.1056/NEJMoa033364]

114 **Ashtari S**, Vahedi M, Pourhoseingholi MA, Karkhane M, Kimiia Z, Pourhoseingholi A, Safaee A, Moghimi-Dehkordi B, Zali MR, Alavian SM. Direct medical care costs associated with patients diagnosed with chronic HCV. *Hepat Mon* 2013; **13**: e8415 [PMID: 23930132 DOI: 10.5812/hepatmon.8415]

115 **Coppola N**, Pisaturo M, Tonziello G, Sagnelli C, Sagnelli E, Angelillo IF. Efficacy of Pegylated interferon α-2a and α-2b in patients with genotype 1 chronic hepatitis C: a meta-analysis. *BMC Infect Dis* 2012; **12**: 357 [PMID: 23245594 DOI: 10.1186/1471-2334-12-357]

116 **Singal AK**, Singh A, Jaganmohan S, Guturu P, Mummadi R, Kuo YF, Sood GK. Antiviral therapy reduces risk of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. *Clin Gastroenterol Hepatol* 2010; **8**: 192-199 [PMID: 19879972 DOI: 10.1016/j.cgh.2009.10.026]

117 **Ashtari S**, Vahedi M, Pourhoseingholi MA, Pourhoseingholi A, Safaee A, Moghimi-Dehkordi B, Zali MR. Estimation of average diagnosis and treatment costs of hepatitis C. *Gastroenterol Hepatol Bed Bench* 2012; **5**: 139-145 [PMID: 24834215]

118 **McMahon BJ**, Alberts SR, Wainwright RB, Bulkow L, Lanier AP. Hepatitis B-related sequelae. Prospective study in 1400 hepatitis B surface antigen-positive Alaska native carriers. *Arch Intern Med* 1990; **150**: 1051-1054 [PMID: 2158773 DOI: 10.1001/archinte.150.5.1051]

119 **World Health Organization**. Global Immunization Data. [Accessed 2012 March]. Available from: URL: http://www.who.int/immunization/monitoring_surveillance/global_immunization_data.pdf

120 **World Health Organization**. Prevention and control of viral hepatitis infection: framework for global action. [Accessed 2012]. Available from: URL: http://www.who.int/csr/disease/hepatitis/GHP_Framework_En.pdf

121 **McCaughan GW**, Omata M, Amarapurkar D, Bowden S, Chow WC, Chutaputti A, Dore G, Gane E, Guan R, Hamid SS, Hardikar W, Hui CK, Jafri W, Jia JD, Lai MY, Wei L, Leung N, Piratvisuth T, Sarin S, Sollano J, Tateishi R. Asian Pacific Association for the Study of the Liver consensus statements on the diagnosis, management and treatment of hepatitis C virus infection. *J Gastroenterol Hepatol* 2007; **22**: 615-633 [PMID: 17444847 DOI: 10.1111/j.1440-1746.2007.04883.x]

122 **Farrell GC**. New hepatitis C guidelines for the Asia-Pacific region: APASL consensus statements on the diagnosis, management and treatment of hepatitis C virus infection. *J Gastroenterol Hepatol* 2007; **22**: 607-610 [PMID: 17444844 DOI: 10.1111/j.1440-1746.2007.04969.x]

123 **Li L**, Fan HB, Yang DL. [An introduction of the consensus statements on the diagnosis, management and treatment of hepatitis C virus infection from the Asian Pacific Association for the Study of the Liver]. *Zhonghua Gan Zang Bing Za Zhi* 2007; **15**: 955-957 [PMID: 18171543]

124 **Zelber-Sagi S**, Lotan R, Shlomai A, Webb M, Harrari G, Buch A, Nitzan Kaluski D, Halpern Z, Oren R. Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. *J Hepatol* 2012; **56**: 1145-1151 [PMID: 22245895 DOI: 10.1016/j.jhep.2011.12.011]

125 **Donadon V**, Balbi M, Mas MD, Casarin P, Zanette G. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. *Liver Int* 2010; **30**: 750-758 [PMID: 20331505 DOI: 10.1111/j.1478-3231.2010.02223.x]

126 **Nkontchou G**, Cosson E, Aout M, Mahmoudi A, Bourcier V, Charif I, Ganne-Carrie N, Grando-Lemaire V, Vicaut E, Trinchet JC, Beaugrand M. Impact of metformin on the prognosis of cirrhosis induced by viral hepatitis C in diabetic patients. *J Clin Endocrinol Metab* 2011; **96**: 2601-2608 [PMID: 21752887 DOI: 10.1210/jc.2010-2415]

127 **Hassan MM**, Curley SA, Li D, Kaseb A, Davila M, Abdalla EK, Javle M, Moghazy DM, Lozano RD, Abbruzzese JL, Vauthey JN. Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. *Cancer* 2010; **116**: 1938-1946 [PMID: 20166205 DOI: 10.1002/cncr.24982]

P- Reviewer: Fujino Y, Mihaila RG, Tomizawa M **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1718

World J Hepatol 2015 June 28; 7(12): 1718-1722
ISSN 1948-5182 (online)

CASE REPORT

Severe immune thrombocytopenia after peg-interferon-alpha2a, ribavirin and telaprevir treatment completion: A case report and systematic review of literature

Rosario Arena, Paolo Cecinato, Andrea Lisotti, Federica Buonfiglioli, Claudio Calvanese, Giuseppe Grande, Marco Montagnani, Francesco Azzaroli, Giuseppe Mazzella

Rosario Arena, Paolo Cecinato, Andrea Lisotti, Federica Buonfiglioli, Claudio Calvanese, Giuseppe Grande, Marco Montagnani, Francesco Azzaroli, Giuseppe Mazzella, Department of Medical and Surgical Science - DIMEC, University of Bologna, S.Orsola-Malpighi Hospital, 40138 Bologna, Italy

Author contributions: Arena R and Mazzella G wrote the paper; Cecinato P, Lisotti A, Buonfiglioli F, Calvanese C and Grande G performed literature search; Montagnani M and Azzaroli F reviewed the paper for important intellectual content.

Ethics approval: The study was reviewed and approved by the Institutional Review Board (IRB) of the S.Orsola-Malpighi Hospital of Bologna; the IRB confirmed the retrospective design of the study (case report) which did not required any further documentation.

Informed consent: The patient provided written informed consent for antiviral treatment and for anonymous review of clinical data for research purpose.

Conflict-of-interest: All Authors declare no conflict of interest for this study and for the preparation of this manuscript.

Correspondence to: Dr. Andrea Lisotti, MD, Department of Medical and Surgical Sciences - DIMEC, University of Bologna, S.Orsola-Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy. lisotti.andrea@gmail.com
Telephone: +39-051-2144120
Fax: +39-051-2086001

Received: March 21, 2015
Peer-review started: March 22, 2015
First decision: April 10, 2015
Revised: May 1, 2015
Accepted: May 27, 2015
Article in press: May 28, 2015
Published online: June 28, 2015

Abstract

Mild to moderate autoimmune thrombocytopenia (AITP) is a common finding in patients receiving interferon-based antiviral treatment, due to bone marrow suppression. Here we report the case of a patient with chronic genotype 1b hepatitis C virus (HCV) infection treated with pegylated-interferon alpha-2a, ribavirin and telaprevir for 24 wk; the patient developed severe AITP three weeks after treatment withdrawal. We performed a systematic literature search in order to review all published cases of AITP related to HCV antiviral treatment. To our knowledge, this is the second case of AITP observed after antiviral treatment withdrawal. In most published cases AITP occurred during treatment; in fact, among 24 cases of AITP related to interferon-based antiviral treatment, only one occurred after discontinuation. Early diagnosis of AITP is a key factor in order to achieve an early interferon discontinuation; in the era of new direct antiviral agents those patients have to be considered for interferon-free treatment regimens. Prompt prescription of immuno-suppressant treatment (*i.e.*, corticosteroids, immunoglobulin infusion and even rituximab for unresponsive cases) leads to favourable prognosis in most of cases. Physicians using interferon-based treatments should be aware that AITP can occur both during and after treatment, specially in the new era of interferon-free antiviral treatment. Finally, in the case of suspected AITP, presence of anti-platelet antibodies should be checked not only during treatment but also

after discontinuation.

Key words: Autoimmune thrombocytopenia; Pegylated interferon; Chronic hepatitis C; Viral hepatitis; Anti-platelet antibody

Core tip: This is the second case report of autoimmune thrombocytopenia (AITP) occurred after peg-interferon/ribavirin treatment completion: generally, AITP was observed in course of interferon treatment. To our knowledge, among 24 interferon-related AITP cases reported in literature, in 23 cases the side effect occurred during treatment while in only one after treatment completion. Physicians using interferon-based antiviral therapy should be aware that acute AITP can occur both during and after treatment; in the case of suspected AITP, presence of anti-platelet antibodies should be checked not only during treatment but also after discontinuation.

Arena R, Cecinato P, Lisotti A, Buonfiglioli F, Calvanese C, Grande G, Montagnani M, Azzaroli F, Mazzella G. Severe immune thrombocytopenia after peg-interferon-alpha2a, ribavirin and telaprevir treatment completion: A case report and systematic review of literature. *World J Hepatol* 2015; 7(12): 1718-1722 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1718.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1718

INTRODUCTION

Presence of autoimmune thrombocytopenia (AITP) could be directly related to hepatitis C virus (HCV) infection, even in early stages of the disease. To date, AITP was not considered an absolute contraindication for interferon-based antiviral treatment and usually resolves after virus clearance[1,2]; however, with the registration of interferon-free regimen, patients with severe thrombocytopenia should be considered for all-oral new direct antiviral agents therapy.

On the other side, mild to moderate thrombocytopenia could be observed in patients receiving antiviral treatment with interferon alpha, due to bone marrow suppression[3]. However, severe life-threatening immune thrombocytopenia has rarely been associated with interferon treatment[4-26]. Finally, thrombocytopenia is a common finding in liver cirrhosis, usually related to congestive splenomegaly and to inadequate liver thrombopoietin synthesis[27].

We present a case of a chronic HCV infected patient in which severe AITP occurred 3 wk after pegylated-interferon (Peg-IFN)α-2a, ribavirin (RBV) and telaprevir treatment completion.

CASE REPORT

We describe the case of a 68-year-old female patient affected by genotype 1b chronic HCV infection (on histology, fibrosis staging F3 according to Metavir); the patient reported a partial response (fall of 4 $\log_{10}$ HCV-RNA) to a previous course of therapy with Peg-IFNα-2a plus RBV and, in February 2012, started treatment with Peg-INFα-2a (180 mcg/wk) plus RBV (1200 mg/d) and telaprevir (2250 mg/d during the first 12 wk).

Before starting treatment the patient had normal laboratory tests, in particular: haemoglobin 14 g/dL, white blood cell count 6.840/mmc, platelet count 155.000/microl, aspartate aminotransferase (AST) 32 IU/L, alanine aminotransferase (ALT) 52 IU/L, γ-glutamyl transpeptidase 48 IU/L, total bilirubin 0.89 mg/dL, albumin 4.1 g/dL, gamma-globulin 1550 mg/dL, HCV-RNA 256665 IU/mL, thyroid stimulating hormone 2.08 microU/mL; anti-mitochondrial antibodies, anti-smooth muscle antibodies, anti-thyroid autoantibodies were negative while anti-nuclear antibodies were found positive at a title of 1:80 with speckled pattern. Anti-platelet antibodies were not assessed. HCV-RNA level decreased rapidly and became undetectable after 2 wk of treatment (-5.4 log UI/mL HCV-RNA in 2 wk). After completing the 12-wk course of triple therapy, the patient continued Peg-INFα-2a and RBV for other 36 wk.

During treatment, the patient developed severe anemia without clinical signs of blood loss that required two blood transfusions and then therapy with erythropoietin 40000 U/wk for 14 wk.

At the end of the treatment, laboratory values showed: HCV RNA not detectable, normal ALT and AST, hemoglobin 11 g/dL, platelets 115.000/mmc, white blood 2.560/mmc, gamma-globulins 1280 mg/dL.

Three weeks after treatment withdrawal, an episode of gingival bleeding occurred; laboratory finding showed severe thrombocytopenia (1.000/microl) with normal white and red blood cells count; liver and kidney function and coagulation tests were normal. Anti-platelets auto-antibodies, both immunoglobulin M (IgM) and IgG, were found positive, while direct Coomb's test and irregular antibodies against erythrocytes were negative. Anti-nuclear antibodies were still positive with a speckled pattern. Antibodies anti-*Helicobacter pylori* were negative.

Clinical and laboratory findings were consistent with the diagnosis of AITP; therefore, intravenous methyl-prednisolone 60 mg/d was started for one week, followed by 30 mg/d of oral prednisone, gradually tapered for three months. No further bleeding and a gradual increase in platelet count were observed; after one week of treatment, platelet count was 40.000/microl. After eight weeks, platelet count was within normal range (211.000/microl), anti-platelet as well as antinuclear antibodies became negative and HCV-RNA was persistently undetectable (the patient achieved a sustained virological response).

DISCUSSION

This is the second case of AITP occurred after Peg-IFN and RBV treatment completion: generally, this side

Table 1 Literature review and summary of all published cases of interferon-induced autoimmune thrombocytopenia for hepatitis C virus infection

Ref.	Sex	Age	HCV genotype	Antiviral therapy	Occurrence of AITP	Clinical outcome
de Manuel Moreno *et al*[5]	F	46	1b	Peg-IFNα-2b + RBV	12 wk	Corticosteroids responsive
Kim *et al*[6]	F	72	NR	Peg-IFNα-2a	120 wk	Corticosteroids responsive
Li *et al*[26]	F	54	1b	Peg-IFNα-2a + RBV	12 wk	CR
Elefsiniotis *et al*[16]	M	27	NR	Peg-IFNα-2b + RBV	24 wk after therapy discontinuation	CR
Huang *et al*[7]	F	48	2	Peg-IFNα-2a + RBV	1 wk	Corticosteroids responsive
Naz *et al*[8]	F	60	NR	Peg-IFNα-2b + RBV	7 wk	CR (treated with ursodeoxycholic acid)
Enomoto *et al*[9]	F	69	1b	Peg-IFNα-2b + RBV	12 wk	Corticosteroids responsive
Carnero-Fernández *et al*[10]	M	20	NR	Peg-IFNα-2b + RBV	20 wk	CR (treated with *iv* immunoglobulin)
Alves Couto *et al*[11]	M	44	NR	Peg-IFNα-2b + RBV	16 wk	CR
Weitz *et al*[12]	F	43	1b	Peg-INF + RBV	48 wk	CR (treated with Rituximab)
Lambotte *et al*[24]	F	73	1b	Peg-IFNα-2a + RBV	8 wk	CR
Nakajima *et al*[23]	M	47	1b	IFNα-2a + RBV	8 wk	Incomplete response
Medeiros *et al*[13]	M	40	NR	IFN-α + RBV (for 24 wk) and Peg-IFNα-2a + RBV	36 wk	CR
Dimitroulopoulos *et al*[22]	F	20	3	IFNα	28 wk	CR
Sevastianos *et al*[14]	F	38	4	Peg-IFNα-2b	4 wk	CR
Fujii *et al*[21]	F	24	NR	IFNα	4 wk	CR
Sagir *et al*[15]	M	45	NR	Peg-IFNα-2b + RBV	10 wk	CR
Pockros *et al*[20]	M	61	1b	IFNα	16 wk	CR
Jiménez-Sáenz *et al*[19]	M	46	NR	IFNα-2b	144 wk	CR
Tappero *et al*[18]	F	NR	NR	IFNα-2a	8 wk	CR
Dourakis *et al*[25]	M	39	NR	IFNα	32 wk	CR
Dourakis *et al*[25]	F	64	NR	IFNα	24 wk	CR
Shrestha *et al*[17]	M	41	NR	IFNα	NR	CR
Demirturk *et al*[27]	NR	NR	NR	Peg-IFN + RBV	NR	CR

CR: Completely resolved; NR: Not reported; HCV: Hepatitis C virus; IFN: Interferon; Peg-IFN: Pegylated-IFN; AITP: Autoimmune thrombocytopenia; RBV: Ribavirin; *iv*: Intravenous.

effect is observed in course of interferon treatment. Thrombocytopenia is usually defined as a platelet count less than 100000/microl. Severe thrombocytopenia (< 50000/microl) is associated with increased bleeding risk during invasive procedures, while spontaneous and even severe bleeding could be observed in patients with platelet count less than 1000/microl.

Thrombocytopenia is a frequently observed in patients with haematological disorders, with HCV infection, with hypersplenism associated to liver cirrhosis or related to drug assumption.

Drug-induced thrombocytopenia develop through two main mechanisms: (1) bone marrow toxicity (*i.e.,* cytotoxic drugs) resulting in reduced production of all blood cells (red cells, white cells and platelets); and (2) increased destruction of normal platelets (both immune-mediated or not)[28].

The incidence of drug induced AITP in the general population is approximately 10 cases per million inhabitants per year, and its pathogenesis is not completely understood yet; however, IgG-type antibodies against platelet glycoprotein (GP) Ⅱb/Ⅲa, GP Ⅰa/Ⅱa, and/or GP Ⅰb/Ⅸ seem to play an important role[29].

In literature, we found several cases of PegIFN-induced autoimmune cytopenias; among those, severe life-threatening AITP, although rare, is a well-documented and recognized adverse event.

To our knowledge, 24 cases of AITP are related to interferon-based treatment for HCV; among those, only one occurred after treatment discontinuation[16] (Table 1).

In the reported case, the patient experienced an episode of severe thrombocytopenia three weeks after antiviral therapy completion, despite negative HCV-RNA (the patient achieved a sustained virological response): the concomitant presence of spontaneous bleeding, anti-platelets antibodies positivity, the exclusion of all other possible causes (*i.e.*, viral and *Helicobacter pylori* infection, haematological or autoimmune disorders and other concomitant therapies) led to the diagnosis of Peg-IFN-induced AITP.

The relationship between AITP and discontinuation of the drug, in our case, could be explained by the long half-life of Peg-IFNα-2a ($T_{1/2}$ = approximately 160 h); since five plasma half-lives are necessary to eliminate about 99% of administered drug, 33 d are necessary for a 99% clearance of Peg-IFNα-2a[30].

Peg-IFN-induced autoimmune diseases may appear even after treatment withdrawal; however, we encourage the investigation all other causes of autoimmunity, especially when a tight time-correlation with the event is absent.

Based on the latest guidelines on the management of AITP[31], we prescribed a first-line long course of corticosteroids (*e.g.*, prednisone 1 mg/kg orally for 21 d then tapered off); in this case, the patient presented a rapid improvement in platelet count after first-line treatment (Figure 1); therefore the administration of

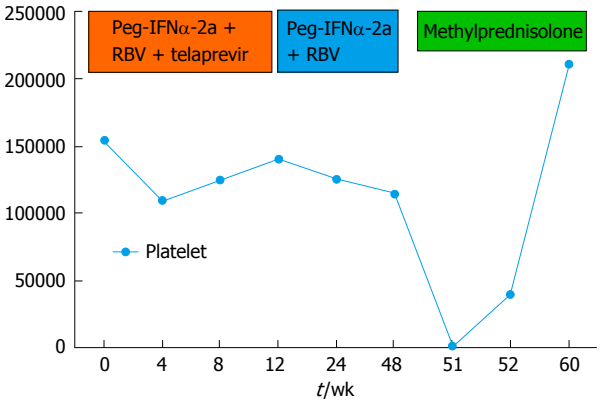

Figure 1 Total platelet count during antiviral treatment, after discontinuation and during steroid therapy. IFN: Interferon; Peg-IFN: Pegylated-IFN; RBV: Ribavirin.

Ⅳ immunoglobulin was not necessary. In fact, immunoglobulin infusion should be prescribed, together with corticosteroids, when a rapid increase in platelet count is clinically required[30]. In non-responsive cases, rituximab may be an option. Platelets infusion should be considered only in cases of severe thrombocytopenia associated with active bleeding. In cases of AITP survival time of infused platelets is reduced to a few hours. However, this practice can help to control acute bleeding[32].

Literature review shows a higher incidence of AITP in patients treated with Peg-IFNα-2b compared to those treated with α-2a; however, published data are insufficient to assume a greater immunogenicity of Peg-IFNα-2b over Peg-IFNα-2a[30].

In conclusion, physicians prescribing pegylated interferon should be aware that acute AITP can occur both during and after treatment. Consequently, it seems logical to us that anti-platelet antibodies dosage should be determined in all patients presenting with thrombocytopenia both during treatment and after discontinuation. A deep knowledge and prompt recognition of interferon-related adverse events is even more important with the availability of interferon-sparing treatment regimens.

COMMENTS

Clinical diagnosis

Gingival spontaneous bleeding was the first finding in this patient; severe thrombocytopenia coupled with positive anti-platelet antibodies led to the diagnosis.

Differential diagnosis

All other causes of autoimmune thrombocytopenia (AITP) had been evaluated; moreover, the not-tight time-correlation required an accurate evaluation of possible hematological or autoimmune disorders, assumption of concomitant drugs.

Laboratory diagnosis

Severe thrombocytopenia (1000/mmc) and positive anti-platelet antibodies suggested the diagnosis of AITP.

Treatment

Treatment with intravenous corticosteroids led to prompt total platelet count increase within one week; after 8 wk of treatment, normal platelet count and negative anti-platelet antibodies were observed.

Related reports

All related cases of interferon-related AITP were reviewed and summarized in Table 1.

Term explanation

IFN: Interferon; Peg-IFN: Pegylated-IFN; AITP: Autoimmune thrombocytopenia; RBV: Ribavirin.

Experiences and lessons

AITP can occur even after interferon withdrawal; physicians must be aware of this unusual clinical manifestation and consider interferon-related AITP also in this setting.

Peer-review

Authors report a rare case of AITP in a very particular population.

REFERENCES

1 **Durand JM**. Extrahepatic manifestations of hepatitis C virus infection. *Am J Gastroenterol* 1997; **92**: 1402 [PMID: 9260829]

2 **Hernández F**, Blanquer A, Linares M, López A, Tarín F, Cerveró A. Autoimmune thrombocytopenia associated with hepatitis C virus infection. *Acta Haematol* 1998; **99**: 217-220 [PMID: 9644300 DOI: 10.1159/000040842]

3 **Raanani P**, Ben-Bassat I. Immune-mediated complications during interferon therapy in hematological patients. *Acta Haematol* 2002; **107**: 133-144 [PMID: 11978934 DOI: 10.1159/000057631]

4 **Peck-Radosavljevic M**, Zacherl J, Meng YG, Pidlich J, Lipinski E, Längle F, Steininger R, Mühlbacher F, Gangl A. Is inadequate thrombopoietin production a major cause of thrombocytopenia in cirrhosis of the liver? *J Hepatol* 1997; **27**: 127-131 [PMID: 9252085]

5 **de Manuel Moreno J**, Costero Pastor B, Sandoval Martínez A, Villafruela Cives M, Bejarano Redondo L, del Pozo Prieto D, Becerro Pedreño I, Borrego Rodríguez G, Poves Martínez E. [Idiopathic thrombocytopenic purpura induced by PEG interferon]. *Rev Esp Enferm Dig* 2010; **102**: 400-401 [PMID: 20575609]

6 **Kim SR**, Imoto S, Kudo M, Nakajima T, Ando K, Mita K, Fukuda K, Hong HS, Lee YH, Nakashima K, Shoji I, Nagano-Fujii M, Hotta H, Hayashi Y. Autoimmune thrombocytopenic purpura during pegylated interferon α treatment for chronic hepatitis C. *Intern Med* 2010; **49**: 1119-1122 [PMID: 20558927]

7 **Huang HH**, Lin CF, Shih YL, Chang WK, Hsieh TY. Rapid development of severe thrombocytopenia in a female with chronic hepatitis C after single-dose pegylated interferon therapy. *Dig Liver Dis* 2010; **42**: 233-234 [PMID: 19592316]

8 **Naz H**, Aslan V. Treatment of autoimmune thrombocytopenia in a case of chronic hepatitis C with ursodeoxycholic acid. *J Infect Dev Ctries* 2009; **3**: 644-646 [PMID: 19801810 DOI: 10.3855/jidc.559]

9 **Enomoto M**, Yamane T, Hino M, Ohnishi M, Tamori A, Kawada N. Platelet-associated IgG for the diagnosis of immune thrombocytopaenic purpura during peginterferon alpha and ribavirin treatment for chronic hepatitis C. *Liver Int* 2008; **28**: 1314-1315 [PMID: 18662271 DOI: 10.1111/j.1478-3231.2008.01747.x]

10 **Carnero-Fernández M**, Pineda JR, Lite-Alvarez JM. [Severe thrombocytopenia with probable autoimmune mechanism associated with interferon therapy in a patient with chronic hepatitis C]. *Gastroenterol Hepatol* 2006; **29**: 297-298 [PMID: 16733036]

11 **Alves Couto C**, Costa Faria L, Dias Ribeiro D, de Paula Farah K, de Melo Couto OF, de Abreu Ferrari TC. Life-threatening thrombocytopenia and nephrotic syndrome due to focal segmental glomerulosclerosis associated with pegylated interferon alpha-2b and ribavirin treatment for hepatitis C. *Liver Int* 2006; **26**: 1294-1297 [PMID: 17105597 DOI: 10.1111/j.1478-3231.2006.01361.x]

12 **Weitz IC**. Treatment of immune thrombocytopenia associated with interferon therapy of hepatitis C with the anti-CD20 monoclonal antibody, rituximab. *Am J Hematol* 2005; **78**: 138-141 [PMID: 15682410 DOI: 10.1002/ajh.20270.x]

13 **Medeiros BC**, Seligman PA, Everson GT, Forman LM. Possible autoimmune thrombocytopenia associated with pegylated interferon-alpha2a plus ribavarin treatment for hepatitis C. *J Clin Gastroenterol* 2004; **38**: 84-86 [PMID: 14679335]

14 **Sevastianos VA**, Deutsch M, Dourakis SP, Manesis EK. Pegylated

interferon-2b-associated autoimmune thrombocytopenia in a patient with chronic hepatitis C. *Am J Gastroenterol* 2003; **98**: 706-707 [PMID: 12650821]

15 **Sagir A**, Wettstein M, Heintges T, Häussinger D. Autoimmune thrombocytopenia induced by PEG-IFN-alpha2b plus ribavirin in hepatitis C. *Dig Dis Sci* 2002; **47**: 562-563 [PMID: 11911342]

16 **Elefsiniotis IS**, Pantazis KD, Fotos NV, Moulakakis A, Mavrogiannis C. Late onset autoimmune thrombocytopenia associated with pegylated interferon-alpha-2b plus ribavirin treatment for chronic hepatitis C. *J Gastroenterol Hepatol* 2006; **21**: 622-623 [PMID: 16638114 DOI: 10.1111/j.1440-1746.2006.03418.x]

17 **Shrestha R**, McKinley C, Bilir BM, Everson GT. Possible idiopathic thrombocytopenic purpura associated with natural alpha interferon therapy for chronic hepatitis C infection. *Am J Gastroenterol* 1995; **90**: 1146-1147 [PMID: 7611214]

18 **Tappero G**, Negro F, Farina M, Gallo M, Angeli A, Hadengue A. Safe switch to beta-interferon treatment of chronic hepatitis C after alpha-interferon-induced autoimmune thrombocytopenia. *J Hepatol* 1996; **25**: 270 [PMID: 8878792]

19 **Jiménez-Sáenz M**, Rojas M, Piñar A, Salas E, Rebollo J, Carmona I, Herrerías-Esteban JM, Herrerías-Gutiérrez JM. Sustained response to combination therapy in a patient with chronic hepatitis C and thrombocytopenia secondary to alpha-interferon. *J Gastroenterol Hepatol* 2000; **15**: 567-569 [PMID: 10847447 DOI: 10.1046/j.1440-1746.2000.02155.x]

20 **Pockros PJ**, Duchini A, McMillan R, Nyberg LM, McHutchison J, Viernes E. Immune thrombocytopenic purpura in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 2002; **97**: 2040-2045 [PMID: 12190174]

21 **Fujii H**, Kitada T, Yamada T, Sakaguchi H, Seki S, Hino M. Life-threatening severe immune thrombocytopenia during alpha-interferon therapy for chronic hepatitis C. *Hepatogastroenterology* 2003; **50**: 841-842 [PMID: 12828100]

22 **Dimitroulopoulos D**, Dourakis SP, Xinopoulos D, Tsamakidis K, Paraskevas E. Immune thrombocytopenic purpura in a patient treated with interferon alfacon-1. *J Viral Hepat* 2004; **11**: 477-478 [PMID: 15357656 DOI: 10.1111/j.1365-2893.2004.00536.x]

23 **Nakajima H**, Takagi H, Yamazaki Y, Toyoda M, Takezawa J, Nagamine T, Mori M. Immune thrombocytopenic purpura in patients with hepatitis C virus infection. *Hepatogastroenterology* 2005; **52**: 1197-1200 [PMID: 16001660]

24 **Lambotte O**, Gelu-Simeon M, Maigne G, Kotb R, Buffet C, Delfraissy JF, Goujard C. Pegylated interferon alpha-2a-associated life-threatening Evans' syndrome in a patient with chronic hepatitis C. *J Infect* 2005; **51**: e113-e115 [PMID: 16230187 DOI: 10.1016/j.jinf.2004.10.010]

25 **Dourakis SP**, Deutsch M, Hadziyannis SJ. Immune thrombocytopenia and alpha-interferon therapy. *J Hepatol* 1996; **25**: 972-975 [PMID: 9007728]

26 **Li L**, Han DK, Lu J. Interferon-alpha induced severe thrombocytopenia: a case report and review of the literature. *World J Gastroenterol* 2010; **16**: 1414-1417 [PMID: 20238410 DOI: 10.3748/wjg.v16.i11.1414]

27 **Demirturk N**, Cevik F, Demirdal T, Aykin N, Aslan V. Autoimmune thrombocytopenia induced by PEG-IFN-alpha plus ribavirin in hepatitis C. *Platelets* 2006; **17**: 340-343 [PMID: 16928608 DOI: 10.1080/09537100600757935]

28 **van den Bemt PM**, Meyboom RH, Egberts AC. Drug-induced immune thrombocytopenia. *Drug Saf* 2004; **27**: 1243-1252 [PMID: 15588119]

29 **McCarthy LJ**, Dzik W. Immune thrombocytopenic purpura. *N Engl J Med* 2002; **347**: 449-450; author reply 449-450 [PMID: 12168645 DOI: 10.1056/NEJM200208083470617]

30 **Foster GR**. Pegylated interferons for the treatment of chronic hepatitis C: pharmacological and clinical differences between peginterferon-alpha-2a and peginterferon-alpha-2b. *Drugs* 2010; **70**: 147-165 [PMID: 20108989 DOI: 10.2165/11531990-000000000-00000]

31 **Neunert C**, Lim W, Crowther M, Cohen A, Solberg L Jr, Crowther MA. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood* 2011; **117**: 4190-4207 [PMID: 21325604 DOI: 10.1182/blood-2010-08-302984]

32 **Wazny LD**, Ariano RE. Evaluation and management of drug-induced thrombocytopenia in the acutely ill patient. *Pharmacotherapy* 2000; **20**: 292-307 [PMID: 10730685 DOI: 10.1592/phco.20.4.292.34883]

P- Reviewer: Imashuku S, Lesmana CRA, Redondo PC, Zhu X
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i12.1723

World J Hepatol 2015 June 28; 7(12): 1723-1724
ISSN 1948-5182 (online)

LETTERS TO THE EDITOR

Non-alcoholic fatty liver disease and beneficial effects of dietary supplements

Ludovico Abenavoli

Ludovico Abenavoli, Department of Health Sciences, University Magna Graecia, 88100 Catanzaro, Italy

Author contributions: Abenavoli L designed the paper, performed research of literature data and wrote the paper.

Conflict-of-interest: No conflicts of interest are reported.

Correspondence to: Ludovico Abenavoli, MD, PhD, Department of Health Sciences, University Magna Graecia, Campus Germaneto, Viale Europa, 88100 Catanzaro, Italy. l.abenavoli@unicz.it
Telephone: +39-0961-3694387
Fax: +39-0961-754220

Received: March 31, 2015
Peer-review started: April 1, 2015
First decision: April 27, 2015
Revised: May 6, 2015
Accepted: May 16, 2015
Article in press: May 18, 2015
Published online: June 28, 2015

Abstract

I read with great interest the review published by Eslamparast *et al*, on the dietary supplements with hepato-protective properties, and their proposed mechanisms to protect against non-alcoholic fatty liver disease. In this way, recently, our study group reported the efficacy of the Mediterranean diet associated to an antioxidant complex, to improve in overweight patients not only anthropometric parameters, but also insulin-resistance, lipid serum levels, and intra-hepatic fat accumulation.

Key words: Non-alcoholic fatty liver disease; Metabolic syndrome; Mediterranean diet; Milk thistle; Antioxidant

Core tip: The prescription of an antioxidant rich dietary regimen by the physicians and nutritionists, may represent an appropriate approach on non-alcoholic fatty liver disease, in clinical practice.

Abenavoli L. Non-alcoholic fatty liver disease and beneficial effects of dietary supplements. *World J Hepatol* 2015; 7(12): 1723-1724 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i12/1723.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i12.1723

TO THE EDITOR

I read with a great interest the review published by Eslamparast *et al*[1], on the dietary supplements with hepato-protective properties, and their proposed mechanisms to protect against non-alcoholic fatty liver disease (NAFLD). Actually, NAFLD is emerging as one of the most common chronic liver diseases worldwide, and represents one of main cause of hepatology referral in some centers. NAFLD is a clinical syndrome that ranges from simple fatty liver, to non-alcoholic steatohepatitis, to advanced fibrosis, and cirrhosis[2]. It is associated with insulin-resistance, obesity, and dyslipidemia, which are the main features of the metabolic syndrome (MS). NAFLD and MS are often seen in the same individual, and it has been reported that nearly 90% of the subjects affected by NAFLD, have more than one component of MS. In fact, the NAFLD patients are inclined to a

higher energy intake, and in particular to a greater carbohydrate intake when compared with the healthy subjects[3].

Currently dietary modifications and physical exercise, should be recommended in clinical practice for the management of NAFLD. The general recommendations for the diet are individualized, depending on the body weight of the subject, and one should aim to achieve energy restriction of 500-1000 kcal/d. Total fat and reduced saturated fat, should constitute less than 30% of the total energy input, with an increase in soluble fibre intake and the decrease of refined sugars consumption. The recommended physical activity is 60 min/d for at least 3 d/wk, and the physical exercise should be progressively increased to 5 d/wk[4]. The reduction of hepatic fat deposition is directly related to the lifestyle intervention, and requires a weight loss of 5% to 10%.

The traditional Mediterranean diet, is a dietary pattern that has been associated with a favourable health profile, mainly in relation to cardiovascular diseases, cancers, and in the treatment of MS[5]. It has been hypothesized that carotenoids, fibres and folic acid, characteristic components of this diet, can play a central role in preventing or slowing oxidative stress phenomena. In addition the vegetables, important elements of the Mediterranean diet, are the main source of phytosterols and a natural cholesterol-lowering agent, that reduce cardiovascular risk. Finally, Mediterranean diet can improve the serum level of adiponectin, a soluble matrix protein expressed by adipoctyes and hepatocytes, reduced in insulin resistance, type-2 diabetes, and obesity, and linked with development of liver steatosis. Recently, our study group reported the efficacy of the Mediterranean diet associated to an antioxidant complex with silybin phytosome complex (silybin plus phosphatidylcholine) and with vitamin E in improving in overweight patients not only anthropometric parameters, but also insulin-resistance, lipid serum levels, and intrahepatic fat accumulation[6]. In this way, the prescription of an antioxidant rich dietary regimen by the physicians and nutritionists, may present an appropriate approach in clinical practice. It can play a main role in the prevention and in the treatment of several chronic diseases, and in particular in the management of NAFLD.

REFERENCES

1 **Eslamparast T**, Eghtesad S, Poustchi H, Hekmatdoost A. Recent advances in dietary supplementation, in treating non-alcoholic fatty liver disease. *World J Hepatol* 2015; **7**: 204-212 [PMID: 25729475 DOI: 10.4254/wjh.v7.i2.204]

2 **Nascimbeni F**, Pais R, Bellentani S, Day CP, Ratziu V, Loria P, Lonardo A. From NAFLD in clinical practice to answers from guidelines. *J Hepatol* 2013; **59**: 859-871 [PMID: 23751754 DOI: 10.1016/j.jhep.2013.05.044]

3 **Tarantino G**, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; **19**: 3375-3384 [PMID: 23801829 DOI: 10.3748/wjg.v19.i22.3375]

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

5 **Abenavoli L**, Milic N, Peta V, Alfieri F, De Lorenzo A, Bellentani S. Alimentary regimen in non-alcoholic fatty liver disease: Mediterranean diet. *World J Gastroenterol* 2014; **20**: 16831-16840 [PMID: 25492997 DOI: 10.3748/wjg.v20.i45.16831]

6 **Abenavoli L**, Greco M, Nazionale I, Peta V, Milic N, Accattato F, Foti D, Gulletta E, Luzza F. Effects of Mediterranean diet supplemented with silybin-vitamin E-phospholipid complex in overweight patients with non-alcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 519-527 [PMID: 25617046 DOI: 10.1586/17474124.2015.1004312]

P- Reviewer: Dirchwolf M, Gwak GY, Sinakos E, Zhang SJ
S- Editor: Ji FF **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