ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2015 December 18; 7(29): 2859-2926

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 29 December 18, 2015

Contents

World Journal of Hepatology
Volume 7 Number 29 December 18, 2015

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)

EDITORIAL OFFICE
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director
World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: editorialoffice@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
December 18, 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.i29.2859

World J Hepatol 2015 December 18; 7(29): 2859-2870
ISSN 1948-5182 (online)

EDITORIAL

Wilson's disease: A review of what we have learned

Kryssia Isabel Rodriguez-Castro, Francisco Javier Hevia-Urrutia, Giacomo Carlo Sturniolo

Kryssia Isabel Rodriguez-Castro, Gastroenterology and Endoscopy, Policlinico Abano Terme, 35031 Abano Terme, Padua, Italy

Kryssia Isabel Rodriguez-Castro, Francisco Javier Hevia-Urrutia, Gastroenterology, Hospital San Juan de Dios, Apdo Postal 10138-1000, San José, Costa Rica

Kryssia Isabel Rodriguez-Castro, Giacomo Carlo Sturniolo, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, 35120 Padua, Italy

Francisco Javier Hevia-Urrutia, Hospital CIMA, Apdo Postal 10201, San José, Costa Rica

Author contributions: All authors contributed to this manuscript.

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

Correspondence to: Kryssia Isabel Rodriguez-Castro, MD, PhD, Gastroenterology and Endoscopy, Policlinico Abano Terme, Piazza Cristoforo Colombo, 1, 35031 Abano Terme, Padua, Italy. kryssiarodriguez@gmail.com
Telephone: +39-33-36167592
Fax: +39-04-98221211

Received: June 14, 2015
Peer-review started: June 15, 2015
First decision: August 4, 2015
Revised: November 5, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: December 18, 2015

Abstract

Wilson's disease (WD), which results from the defective ATP7B protein product, is characterized by impaired copper metabolism and its clinical consequences vary from an asymptomatic state to fulminant hepatic failure, chronic liver disease with or without cirrhosis, neurological, and psychiatric manifestations. A high grade of suspicion is warranted to not miss cases of WD, especially less florid cases with only mild elevation of transaminases, or isolated neuropsychiatric involvement. Screening in first and second relatives of index cases is mandatory, and treatment must commence upon establishment of diagnosis. Treatment strategies include chelators such as D-penicillamine and trientine, while zinc salts act as inductors of methallothioneins, which favor a negative copper balance and a reduction of free plasmatic copper. As an orphan disease, research is lacking in this field, especially regarding therapeutic strategies which are associated with better patient compliance and which could eventually also reverse established injury.

Key words: Wilson's disease; Wilson disease; Chelating agents; Penicillamine; Zinc; Copper; Orphan disease; Liver transplantation

Core tip: A century after its initial description by Kinnear Wilson in 1912, knowledge on diagnosis and management of Wilson's disease reflect its prevalence as a rare disease, largely deriving from experts' opinions and the use of pharmacological agents without the rigorous randomized clinical trials that are the mainstay. Prompt recognition and treatment are paramount and life-saving.

Rodriguez-Castro KI, Hevia-Urrutia FJ, Sturniolo GC. Wilson's

disease: A review of what we have learned. *World J Hepatol* 2015; 7(29): 2859-2870 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2859.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2859

INTRODUCTION

Initially described by Kinnear Wilson[1] in 1912, Wilson's disease (WD, or Wilson disease), is the clinical condition resulting from mutations in the chromosome 13q14 in the region coding for the protein product *ATP7B*, and occurs in a sporadic fashion as well as inherited as an autosomal recessive disease. Homozygous, or, more commonly, compound heterozygous mutations lead to defective incorporation of copper into apo-ceruloplasmin and the subsequent formation of holoceruloplasmin, hampering the normal excretion of copper into bile. Consequences of this defect are the impaired copper metabolism and consequent copper intoxication. With a shorter half-life than that of holoceruloplasmin, circulating apoceruloplasmin (ceruloplasmin) are abnormally low, albeit the gene responsible for this protein, localized on chromosome 3, is intact[2], providing one of the most important clinical diagnostic tools for WD. Copper overload, and actually free copper as the main acting element, exerts its toxicity through two main mechanisms: Direct oxidative stress, with lipid peroxidation of membranes, DNA, and mitochondria, as well as due to unregulated apoptosis leading to cell death from copper-induced changes in the anti-apoptotic protein, X-linked inhibitor of apoptosis, and its loss of inhibitory control of caspase-3[3]. It is now known that it is not the accumulation of copper itself what is deleterious to the organism, but rather free copper in the blood, which determines copper intoxication, as opposed to ceruloplasmin-bound copper. Thus, the old paradigm of eliminating copper stores as the therapeutic objective has given way to the concept of normalizing free copper concentrations in the bloodstream[4].

It should be stated that much of the knowledge that has accumulated in the decades following the first description of the disease, as well as the mainstays of treatment, derive greatly from experts' opinions and some from anecdotal experiences, and not on adequately designed randomized comparative studies.

EPIDEMIOLOGY

The prevalence of WD, a rare disease, is similar in most world regions, corresponding to approximately 0.5 cases per 100000 inhabitants[5,6], or the most common figure 30 cases per million, with a gene frequency of 0.56% and a carrier frequency of 1 in 90[7]. Nevertheless, the disease is much less uncommon in certain areas/countries, with certain mutations being described more frequently in specific populations. Over 500 mutations have been found so far[8], and the lower number of actual clinically manifest cases with respect to the frequency of allele carriers in the population, probably reflect the reduced penetrance of mutations. The most common mutations include His1069Glu (H1069Q) in Europe and North America[9], Arg778Leu in South Korea[10], Japan[11] and China[12], 2007del7 in Iceland[13], and Met645Arg in Spain[14]. The disease is most frequent in Germany (2.5/100000 inhabitants), Japan (3.3/100000 inhabitants)[11] and Austria (3.0/100000) inhabitants[15]. The country with the highest incidence in the world, however, is Costa Rica (4.9/100000 inhabitants; see below section on perspectives from a high-incidence country), possibly due to elevated degree of consanguinity and a possible founder effect, the most frequent mutant beingAsn 1270 Ser[16-18], previously described only in Sicilian, Lebanese and Turkish populations. The other region of the world with a very high incidence (estimated 1/10000-1/7000) is Sardinia[19,20], where a well-documented founder mutation (-441/-427del) is highly prevalent (67%) and all other mutations are present with a relative frequency below 10%[19,21,22].

CLINICAL MANIFESTATIONS

Although the form of the disease initially described was predominantly neurological[1], the disease manifestations can be pleomorphic, and although the correlation mutation-predominant manifestation has been elusive[23,24], clinical forms of the disease tend to cluster and wide geographical differences exist[25]. Thus, WD may be predominantly hepatic, neurological or psychiatric, and manifestations of disease may range from an asymptomatic state to life-threatening fulminant hepatic failure[26-30]. In Costa Rica, the majority of WD patients exhibit a liver-predominant disease, with more than 5% presenting as fulminant Wilson disease (FW)[17].

Liver involvement spans from asymptomatic disease with transaminase elevation, to acute hepatitis, acute-on-chronic liver failure, and cirrhosis. Liberation of copper into the bloodstream causes Coomb's negative hemolytic anemia, with transient episodes of low-grade hemolysis and jaundice[31,32]. Neurological manifestations can be categorized as: (1) an akinetic-rigid syndrome similar to Parkinson's disease; (2) pseudosclerosis dominated by tremor; (3) ataxia; and (4) a dystonic syndrome, which often leads to severe contractures[33,34]. Neuropsychiatric symptoms and signs, including decrease in scholastic performance, hand-eye discoordination, and behavioral changes may foretell a more florid neurological presentation[35]. Other findings include drooling, spasticity, chorea, athetosis, myoclonus, micrographia, dyslalia, hypomimia, and dysarthria[35,36]. Ocular manifestations include the Kayser-Fleischer ring and sunflower cataracts in the lens, deposition of copper in the Descemet's membrane in the first case, and in the anterior and posterior capsule of the lens, sparing epithelial and cortical cells, in the latter[37,38]. Although Kayser-Fleishcer rings are very common in WD, especially in patients with neurological forms of the disease, sunflower

cataracts are more rarely observed. Sunflower cataracts associated with Kayser-Fleischer rings in WD patients were first described in 1922 by Seimerling and Oloff, who observed a striking similarity between these lesions and those induced by a copper-containing foreign body lodged in the eye. Both manifestations may resolve with continued therapy[39]. Small fiber peripheral neuropathy involving the corneal nerve plexus[40], as well as neuronal degeneration involving the retina have also been described[41], using novel techniques such as corneal confocal microscopy and spectral domain optical coherence tomography, respectively. Psychiatric abnormalities, which may be present before hepatic or neurological signs in up to one third of patients[42], include decreased academic performance or personality changes, sexual exhibitionism, impulsiveness, labile mood, inappropriate behavior, depression, paranoia, and schizophrenia, leading also to suicide in a discrete number of cases[15,33,43,44]. Other manifestations of disease may include renal abnormalities such as hypercalciuria, nephrocalcinosis, nephrolithiasis, and aminoaciduria, cardiomyopathy with arrhythmias, autonomous nervous alterations, gigantism, hyoparathyroidism, osteoarthritis, pathological fractures and pancreatitis[42,45].

DIAGNOSIS

The working party at the 8th International Meeting on Wilson disease held in Leipzig (2001)[26,42] proposed a diagnostic score for diagnosing WD. The fundamental diagnostic elements include: (1) serum ceruloplasmin, which is typically decreased by 50% of the lower normal value, but may be elevated - and thus lead to a false negative result in inflammatory states, as an acute phase reactant; (2) twenty-four hours urinary copper excretion, which is typically greater than 100 mcg/24 h in adults and greater than 40 mcg/24 h in children; (3) serum free copper, which is typically greater than 200 mcg/L; (4) hepatic copper, which is typically greater than 250 mcg/g dry weight; and (5) the presence of Kayser-Fleischer rings on slit-lamp examination, which however may be absent in up to 50% of patients with hepatic WD, may be absent in most asymptomatic siblings, and may be present in other hepatic diseases such as primary biliary cirrhosis. In contrast, Kayser-Fleischer rings are present almost invariably in neurological WD[37]. Although oftentimes most criteria of the Leipzig score are met[26], the alteration of at least one copper metabolism test with low ceruloplasm in levels in the presence of clinical manifestations is enough to establish the diagnosis of WD. The advent of genetic testing did not provide the expected diagnostic yield, due to the existence of more than 500 mutants, and the laborious and expensive nature of genetic testing[46]. Moreover, most patients are mixed (compound) heterozygotes, and even more so, no mutation has been identified in approximately 17% of confirmed WD cases[47]. However, genetic testing might prove to be very useful, and molecular techniques allow for a rapid diagnosis in a substantial part of patients with prevalent mutations. Furthermore, genetic testing is especially useful in screening relatives of the index patient, upon determination of his/her mutant.

Other simple diagnostic options include the relative exchangeable copper, defined by the ratio of serum exchangeable copper (CuEXC)/total serum copper (CuT) > 18.5%[48]. The simplest test, however, remains the serum copper/ceruloplasmin ratio (μg/dL), which if is greater than 2 determines the existence of WD, and if < 1 determines a healthy subject or a heterozygote[49]. Although the 24 h urine test is used widely, it is not practical and may be difficult to collect for patients. A 6 h copper urine test after challenge with D-penicillamine is being proposed by our group, which provides diagnostic rapidity, and good accuracy with the cutoff level of 118 mcg Cu/6 h as diagnostic for WD[50]. This test has not yet been universally validated yet, however, and has not been standardized for routine diagnosis, but rather represents a valid tool under certain circumstances, especially in patients with acute on chronic presentation with rapid clinical deterioration. Moreover, the future holds promising new techniques such as tandem mass spectrometry as a diagnostic tool, which although not yet widely available, may be used as a confirmatory test to diagnose WD in newborns, allowing a very fast measurement of several metabolites in different biological specimens[51].

As prompt diagnosis is crucial in order to initiate treatment hopefully in the early, asymptomatic stage of the disease and not when liver decompensation or advanced neurological irreversible damage have already ensued, family screening is warranted. In this scenario, the best approach is to complete copper studies in first- and second-degree relatives of the index case.

TREATMENT

Treatment should ideally commence upon diagnosis in pre-symptomatic subjects (when testing is performed as part of screening for affected family members), or in symptomatic subjects, immediately after prompt diagnosis. If treatment is initiated opportunely, deterioration can be prevented and life expectancy can be comparable to subjects without the disease[52,53]. Prognosis for WD patients is excellent provided compliance to therapy is adequate. On the contrary, the natural course of the disease is characterized almost inevitably by progressive, relentless deterioration leading to death due to liver or neurological disease[54]. Discontinuation of treatment may be catastrophic, placing the patient at high risk of FW, and with a high toll on mortality, as in a study where 8 of 11 patients who suspended treatment died an average of 2.6 years after treatment cessation[55].

The objectives of treatment, therefore, are to prevent appearance of symptoms in asymptomatic subjects, prevent clinical deterioration in affected subjects, and can also be life-saving in cases o acute-on-chronic hepatitis. Treatment principles in WD include the esta-

blishment of a certain diagnosis, since the treatment is lifelong, as well as the monitoring of compliance, early detection of complications, and integral management including early neuropsychiatric screening/evaluation and physiotherapy, as required.

It is recommended that asymptomatic patients be treated with zinc salts or with chelators at a lower dosage than that used for symptomatic disease. In contrast, symptomatic patients should be treated with chelators or a combination of chelators plus zinc, while patients with acute-on-chronic liver failure or those with end-stage liver disease unresponsive to medical therapy should be considered urgently for liver transplantation. In order to determine the adherence to therapy, its effectiveness, and the eventual development of side effects, lifelong monitoring is warranted.

Treatment is based on the removal of copper excess by chelating agents such as penicillamine, trientine[55], or tetrathiomolybdate[56,57] or by blocking the intestinal copper absorption with zinc salts[58], with the ultimate goal of normalizing free plasmatic copper. Being WD a rare disease[59], pharmacological agents to treat it belong to the group of orphan drugs[60], and have not been developed through a rigorous process like most drugs, for which information on pharmacokinetics and pharmacodynamics is available before their establishment as treatment and their marketing. Rather, pharmacological agents in WD originated from the desperate need to treat an otherwise lethal disease and even derive from knowledge in other fields, like chemistry and veterinary medicine, as in the case of tetrathiomolybdate. An exception is represented by zinc acetate, for which formal preclinical and clinical trials were conducted prior to its release as a treatment agent for WD, as requested by the American Food and Drug Administration for approval of an alternative zinc salt[61]. The continued use of these agents through the years, however, has permitted a body of evidence and experience to be accumulated regarding these drugs' effectiveness and adverse effects. The orphan drugs presently used for the treatment of WD, with no registered active clinical trials, research projects or networks[60] are penicillamine D (or D-penicillamine) (Cuprimine, Cupripen, Cilamin, Trolovol, Orpha number ORPHA34567), zinc acetate (Galzin, Wilzin, Orpha number ORPHA56897), Trientinedihydrochloride (Metalite, Syprine, Orpha number ORPHA24924), and Ammonium tetrathiomolybdate (Orpha number ORPHA137334), designation granted to the last agent by the European Commission in 2008[62].

D-penicillamine

D-penicillamine, introduced in 1956 as the first oral agent for treating WD[63], chelates not only copper, but other metals as well. In fact, the initial racemic mixture that was available required the co-administration with pyridoxine[32], to avoid deficit of this vitamin, although supplementation with pyridoxine (25-50 mg daily) is still recommended. This drug favors urinary excretion of copper, but it also induces the endogenous intracellular chelatormetallothionein[64,65], favoring reduced absorption by elimination in feces. As D-penicillamine has some immunosuppressant properties, it was in fact initially used to treat rheumatoid arthritis[66]. Moreover, this agent's interference with collagen cross-linking has several consequences[67,68], including impaired or delayed wound healing, but also potential benefic effects in preventing, delaying, or ameliorating hepatic fibrosis. This last effect was not demonstrated in a histopathological study in which fibrosis progression occurred in the same proportion in patients treated with zinc or with D-penicillamine[69], although larger studies and longer follow-up is warranted to evaluate this aspect optimally. Although initial worsening of neurologic symptoms may occur in 10%-50% of patients[53,70], it is widely used due to its low cost and considerable efficacy, although no comparative data exist to support its superiority as opposed to zinc therapy[71].

Improvement in hepatic function may be observed as early as 2-6 mo after initiation of treatment, with improvement in hepatic synthetic function, ascites, and jaundice[32,72]. Initial recommended dose is 250-500 mg/d, with 250 mg increments every 4-7 d to a maximum of 1-1.5 g/d in two to four divided dosages, in cases of symptomatic disease. Maintenance dose is lower: 750-1000 mg/d administered in two daily doses. In the pediatric population, the recommended dose is 20 mg/kg per day rounded off to the nearest 250 mg and distributed in two or three doses daily, reducing by 25%-30% in maintenance therapy. As food inhibits the absorption of D-penicillamine, this drug should be administered one hour before or two hours after meals[73,74]. Adverse effects are unfortunately relatively common with the use of D-penicillamine and determine the need for discontinuation (and switching to another pharmacological treatment) in approximately 20%-30% of patients[75]. Early adverse effects include sensitivity reactions characterized by fever and cutaneous eruptions, neutropenia or thrombocytopenia, lymphadenopathy, and proteinuria. Although in the past desensitization measures were used due do the absence of pharmacological alternatives, the occurrence of these adverse reactions warrants immediate drug suspension and switching to either trientene or zinc. Another late adverse effect of D-penicillamine which requires immediate suspension is nephrotoxicity, which is often preceded by proteinuria or the appearance of other celluar elements in the urine. Other late-onset undesirable effects of the drug include a lupus-like syndrome characterized by hematuria, proteinuria, arthralgia, and appearance of antinuclear antibodies; bone marrow toxicity with severe thrombocytopenia and even aplasia may occur, while dermatologic alterations such as elastosis perforans serpiginosa, progeria, pemphigus, lichen planus, and apthous stomatitis may occur[67,76]. Hepatic iron accumulation has been demonstrated to occur with prolonged use of D-penicillamine, as opposed to therapy with zinc, which avoids copper aborption, and hepcidin might play a role in altered iron metabolism in WD[77].

Neurological damage, in some cases irreversible, may be induced by massive and sudden free copper elevation following therapy with D-penicillamine and other potent chelators[78]; neurological worsening has in fact been linked with spikes in free copper, induced by chelators including D-penicillamine[79,80]. The mechanism behind neurological worsening is the mobilization of important amounts of free copper, which together with an increase in malanodialdehyde and a reduction in glutathione, lead to cellular damage[81]. Moreover, due to its effects on collagen formation and thus on wound healing, D-penicillamine must be suspended (and therapy changed to zinc or trientine) between 2 to 3 mo before planned surgery.

Trientine

A chelator of several metals including copper, zinc, and iron, trientine was developed and introduced in 1969 as an alternative for patients intolerant to D-penicillamine and favors urinary excretion of copper[82,83]. Although its elevated cost might hamper its use as an initial medication, and although clinical information available is limited only to uncontrolled studies, this agent possesses a good safety profile and is efficacious, offering the possibility of use as alternative to other pharmacological agents or as initial therapy, even in cases of decompensated liver disease[84]. Albeit worsening of neurological symptoms has been reported to occur, this phenomenon seems to occur less frequently than with D-penicillamine. Cases of neurotoxicity have been reported[85], however, and a clinical trial found that trientine led to initial neurological deterioration in approximately 26% of treated patients[86]. Co-administration with iron should be avoided, since the resulting complex may induce toxicity. Copper deficiency induced by trientine through overtreatment can result in reversible side roblastic anemia due to marrow copper deficiency[87] and iron overload in livers of patients with WD, similar to that observed for D-penicillamine. Recommended dose is 750-1500 mg/d in two or three divided doses, while maintenance dose is 750-1000 mg/d in two or three divided doses. In the pediatric population, dosing is 20 mg/kg per day, rounded off to the nearest 250 mg, and should be administered in two or three divided doses. Similar to D-penicillamine, trientine should be administered either one hour before or two hours after meals, as food inhibits its absorption. Another particular aspect is that trientine must be kept refrigerated. Adverse effects include dyspepsia, anemia caused by iron deficiency, muscle cramps and spasms, and dystonia, the last being difficult to exclude as manifestations of the disease itself[88].

Zinc

Initially its chloride salt, followed by its sulfate salt, zinc was first used in the early 1960s to treat WD but was kept unrecognized until 1978[89]. Zinc acetate is regarded to have a better gastric tolerance. However, in terms of efficacy, there is no difference between zinc salts[90]. Its mechanism of action is different from the above mentioned agents, in that it induces enterocyte metallothionein, an endogenous chelator of metals, thus favoring copper entrapment into enterocytes and its elimination in the feces with the normal shedding of intestinal cells[61,91]. Furthermore, zinc may also act beneficially by inducing intra-hepatic metallothionein, potentially providing further hepato-protection[92]. Another possible mechanism of action of zinc is the inhibition of lipid peroxidation and the increase of available glutathione within hepatocytes, reducing oxidative damage[93]. This drug has demonstrated to be efficacious in slowly creating a negative copper balance, and although initially it was favored only as maintenance therapy or in asymptomatic subjects[94], it is increasingly and successfully being used as first-line therapy as well[4,95,96]. As the deleterious effect of copper are associated with its free form in blood, induction of metallothionein and its binding of free copper by zinc results in an efficacious therapy, achieving normalization of free copper levels. Recommended dosing in milligrams of elemental zinc is 150 mg/d divided in three doses. In the pediatric population dosing is 75 mg/d divided in three doses. As well as with the other pharmacological drugs in the armamentarium for treating WD, food interferes with absorption, which is why this drug must be administered at least one hour before or two hours after meals. Whenever the therapeutic decision to switch from chelators to zinc is made, it should be noted that since the maximum induction of intestinal metalloproteins occurs three weeks after the initiation of zinc, the chelator should be continued for this period of time, administered at least one hour before or after zinc. Adverse effects of zinc are fortunately few and not life-threatening, including gastric irritation, which can improve with time, alcohol intolerance, headaches, increase in perspiration, transient elevation of plasmatic lipase, amylase and alkaline phosphatase, and sideroblastic anemia, the latter of which can indicate excessive copper removal, with copper deficiency[97].

Since neurological worsening might be induced with the use of potent chelators, it has been proposed[98], and it has been the experience of our groups, that the optimal therapeutic approach in patients with severe neurological impairment is to commence treatment with zinc, which acts not by rapid mobilization of copper, but by blockage of copper absorption both from food as well as from endogenously secreted copper, creating a consistent negative copper balance without inducing an intense and sudden copper redistribution[78]. Not only as initial therapy in cases of severe neurologic involvement, but also as an alternative agent after discontinuing chelators, zinc therapy has been proven to favor improvement and even resolution of neurological damage in WD[99].

Although not standardized, combined therapy, with the administration of either D-penicillamine and zinc, or trientine and zinc, at widely spaced intervals during the day, is a valid strategy that has yielded good results[100]. Both the EASL and the AASLD guidelines recommend including a chelating agent in the initial treatment of

symptomatic patients (D-penicillamine or trientine), although trientine may be better tolerated[32,42]. Likewise, according to these guidelines, maintenance therapy can be ensued with reduced doses of either chelating agent or with zinc.

Diet

Elevated amounts of copper are naturally found in numerous food products, including chocolate, nuts, mushrooms, crustaceans, soy, and gelatin, and although dietary restriction of copper-rich foodstuffs is by no means sufficient therapy for WD[101], its importance should not be overlooked as part of WD management. Moreover, the use of cooking utensils containing copper is discouraged, and in order for tap water coming from copper pipes to be sufficiently safe for consumption, it must be left running for a few minutes[97].

PHARMACOLOGICAL TREATMENT MONITORING

Adequate compliance to therapy is key in the management of patients with WD[94]. Being a lifelong disease, frequently diagnosed in the pediatric age, and requirement daily intake of one or more pharmacological agents, oftentimes two or three times daily, adherence can sometimes be an issue, and can lead to life-threatening deterioration of clinical conditions. Monitoring should be performed at least every six months, especially in adolescents, in whom non-adherent behavior has been documented in spite monitoring[102]. Monitoring intervals should be shorter for patients who initiate therapy, patients who are switched to another type of medication, cases in whom non-compliance is suspected, or patients in whom worsening occurs in spite of treatment.

Monitoring of treatment with all agents includes liver function tests, which should tend to normalize within a variable period of several months. With either D-penicillamine or trientine, plasmatic non-ceruloplasmin bound copper values should exceed 25 μg/dL at the start of therapy and should lie between 15-25 μg/dL during maintenance therapy. At the start of therapy, 24 h urinary copper excretion should be between 500 and 1000 μg/24 h (or from 300 to 1000 with trientine), and should lie between 200-500 μg/24 h during maintenance therapy. Conversely, monitoring of patients on zinc therapy must ensure a reduction in urinary copper excretion (initially below 100 μg/24, and from 30-80 μg/24 h on maintenance therapy), with a normalization off non-ceruloplasmin-bound copper (initially above 25 μg/dL, but 15-25 μg/dL on maintenance therapy). Additionally, urinary zinc excretion (which should be above 1.5-2 g/d) indicates adequate compliance to therapy[3,32].

It is important to look out for overtreatment, which presents with neutropenia and anemia, due to failed iron mobilization, with transaminase elevation due to increased hepatic iron, accompanied by increased ferritin. In these cases, non-ceruloplasmin copper is typically lower than 15 μg/dL, and urinary copper is reduced with respect to the patient's previous values[3]. Temporary discontinuation of therapy, or switching from a chelating agent to zinc, with close observation, is warranted, followed by reintroduction of therapy at a reduced dose[32].

LIVER TRANSPLANTATION

Liver transplantation is the recommended therapy for patients with fulminant hepatitis, or in those with relentless progression of hepatic dysfunction despite drug therapy, and survival rates are only very slightly inferior to those after transplant for other indications[103-105]. Liver transplantation corrects the underlying hepatic metabolic defect in WD[106], and is one of the few indications for liver transplantation in which there is no risk of recurrence, unless in the unfortunate and improbable hypothesis of receiving a graft from an undiagnosed WD. With the availability of effective treatment, liver transplantation is clearly not indicated to treat the metabolic defect, but rather as a life-saving procedure in cases of advanced cirrhosis or fulminant hepatic failure. In a study analyzing UNOS results on 170 children and 400 adults who underwent liver transplantation for FW or end-stage liver disease in the United States between 1987 and 2008, Arnon *et al*[102] found that both one- and five-year survival rates were similar between children and adults (90.1% and 89% *vs* 88.3% and 86%, respectively, P = 0.53, 0.34). Moreover, both adults and children transplanted for chronic liver disease had better long term survival than patients transplanted for FW, although the difference was not statistically significant[107]. Neurological, and especially psychiatric[108] involvement may show little improvement with transplantation, however[27]. In a multicenter Italian study, 37 cases of patients who underwent liver transplantation, 8 for FW and 29 for WD-related chronic liver disease, were analyzed, demonstrating decreased survival in patients who previous to transplant had neuropsychiatric manifestations, in spite of neurological improvement after transplantation[103]. Improvement in neurological symptoms, without a negative effect on survival, has been reported however, in smaller series[109,110]. Thus, caution must be employed in decision making regarding listing for liver transplantation in patients with severe neuropsychiatric involvement. Moreover, liver transplantation for sole neuropsychiatric disease is currently not recommended.

ALTERNATIVE TREATMENTS

Ammonium tetrathiomolybdate

Tetrathiomolybdate, another copper-chelating drug with antiangiogenic properties[111], derives from experience in the veterinary field, where this agent has been used to treat copper-poisoning[106,112]. Its mechanism of action is chelating copper and also inducing intestinal

metalloproteins, increasing both copper elimination in the urine and in the feces[86]. Administered with meals, this agent forms a complex with copper and protein in the gut, inhibiting copper uptake; when administered between meals, it binds plasmatic copper[113-115]. Athough not yet approved by the American Food and Drug Association, it has been approved for use in WD in Europe since 2008. In spite of the reduced clinical experience available, it seems this agent is safe and efficacious, especially in patients with severe neurological manifestations[54,57,111,115]. In a randomized, double-blind study analyzing patients with neurologic WD, treatment with trientine and tetrathiomolybdate were compared against each other. The authors found that neurological deterioration occurred in 6 of 23 patients in the trientine treatment arm *vs* 1 of 25 patients in the tetrathiomolybdate arm[86]. Adverse effects include elevation of transaminases and bone marrow suppression[116].

Vitamin E

Vitamin E, a powerful antioxidant, may be used as adjunctive treatment, especially in the scenario of liver failure. Moreover, low levels of this vitamin have been demonstrated in patients with WD, providing further rationale for its supplementation[117-119]. Nevertheless, no randomized controlled studies offer solid evidence for its use.

TREATMENT IN SPECIAL CONDITIONS

Fulminant hepatic failure

Prompt recognition and establishment of diagnosis is the first, crucial step in the management of acute liver failure; timely diagnosis in these circumstances is especially critical, and high suspicion for this entity must be raised in the presence of a fulminant hepatitis (in most cases in the absence of previous symptoms), Coombs negative hemolytic anemia, transaminase elevation (AST/ALT > 2.2), alkaline phosphatase/total bilirubin < 4, and an increase in serum total copper levels. The diagnosis of WD is even likelier if this presentation occurs in young females between 11 and 25 years of age, coinciding with puberty, as FW has been described more frequently in this subgroup of patients.

Treatment in this setting is life-saving, and the best option is offered by liver transplantation[120,121]. Determination of which patients will likely not survive without a liver transplant is key to deciding urgent placement with high priority in the waiting list for liver transplant in countries/regions were this resource is available. The prognostic score developed by Nazer *et al*[122] incorporates serum bilirubin, aspartate aminotransferase, and prothrombin time, and patients with a score of 7 or more had fatal outcome. Dhawan *et al*[123] recently modified this score (revised King's score), with the addition of leucocyte count and INR instead of prothrombin time, with a newly established cutoff of 10 points which determines a breaking point in survival without liver transplantation. The specific prognostic index revised Wilson prognostic index represents a valid tool for assessing these critically ill patients[121,122].

As bridging therapies to liver transplantation, or as an alternative altogether in regions were liver transplantation is not possible, strategies such as rapid plasma exchange[124,125] *via* any method such as plasmapheresis[126], hemofiltration[127,128], albumin dyalisis[129], or exchange transfusion, may be successfully used to lower circulating copper levels, renal protection from copper-mediated tubular damage, and reduce hemolysis[130]. Albeit some degree of improvement has been reported, the need for liver transplantation has not been obviated in numerous cases, although successful treatment without transplantation has been reported[131]. The molecular adsorbent recycling system ultrafiltration device, which combines ion exchange with albumin dialysis might provide some therapeutic efficacy in this setting, achieving copper removal and clinical stabilization, that might constitute a bridge for liver transplantation[132-135].

Pregnancy

Treatment must be maintained during all the duration of pregnancy; acute liver failure has been reported as a result of therapy discontinuation during pregnancy[136]. Penicillamine, trientine, and zinc salts have been successfully used to treat pregnant WD patients, with satisfactory outcomes for both mother and fetus. However, there are reports of teratogenicity associated with D-penicillamine in both animals[137] as well as human beings[138], and although it is not clear if trientine is effectively teratogenic in humans, its teratogenicity has been reported in animals[139]. Although there have been reports of birth defects during treatment for WD, the rarity of this disease makes it difficult to establish a true increased risk in this population. The risk of decompensation in cases of discontinuation of therapy clearly outweighs any possible risk to the fetus. Thus, the best treatment option during pregnancy and breastfeeding is zinc, with adequate protection of the mother's as well as the fetus' health[140]. Dosing should be unaltered for zinc salts, but reduction (20%-50%) is warranted for D-penicillamine and trientine. Monitoring of liver function tests during each trimester is recommended.

PERSPECTIVES FROM TWO HIGH-INCIDENCE COUNTRIES: ITALY AND COSTA RICA

In an Italian study analyzing 35 patients with WD, with a mean follow-up of 15 years, hepatic presentation was the dominant clinical manifestation, with 65.7% of patients with hepatic form and 34.3% a combination of neurologic and hepatic involvement. After initial treatment with D-penicillamine (23/35 patients) or zinc sulphate (12/35 patients), neurological symptoms worsened or remained stationary in 75% in patients treated with d-penicillamine, while 90% of patients

treated with zinc showed improvement in neurological symptoms, while hepatic disease improved in both treatment groups. Four patients underwent liver transplantation, and while 3 patients survived a mean of 4.6 years, one patient, who previous to the transplant had severe neurological impairment, died shortly after transplantation due to central pontinemyelinolysis[141].

United by a possible founder effect that has been traced to Italian origins[16,17], WD is very frequent in Costa Rica, favored by the high degree of consanguinity. In a cohort of 55 WD patients in Costa Rica, with a mean follow up of 11.59 years, for a total of 633 patient-years, mean age at diagnosis was 22.1 years, with the youngest patient being diagnosed at 3 years of age and the oldest patient at 72 years of age. Interestingly, women tended to be diagnosed at a later age than men (25.8 years *vs* 17.9 years, $P = 0.04$), and 41.8% of patients had at least one relative who had been diagnosed with the disease. Notably, 21 patients were asymptomatic at diagnosis (diagnosed by screening of family members), 21/55 had predominantly hepatic disease, 5/55 predominantly neurological disease, 3/55 both hepatic and psychiatric disease, 3/55 both hepatic and neurological disease, and 2/55 patients had all three possible predominant manifestations of WD: Hepatic, neurologic and psychiatric. Approximately 60% of these patients is on treatment with D-penicillamine, 8.5% on combined treatment D-penicillamine + zinc, and the remainder of patients receive either trientenemonotherapy, trientene + zinc, or zinc monotherapy (10.6%, respectively). Interestingly, survival was significantly different according to age at diagnosis (and start of therapy), with 97% *vs* 66.7% survival at 15 years for patients diagnosed before or after 30 years of age, respectively ($P < 0.01$). During the follow-up period, 2 patients underwent liver transplantation, one for FW and the other for end-stage liver disease, with excellent outcome post-transplantation (13 and 7 years' survival, respectively). Before the institution of the National Liver Transplantation program in Costa Rica, however, management of FW was successful with the use of prostaglandins (misoprostol) and high dose vitamin E in four cases[97]. This strategy, together with copper filtering, may be life-saving and might represent a valid strategy either as a bridge to transplant or as salvage therapy in regions where liver transplantation is not an option.

CONCLUSION

Much has been learnt since the initial description of the disease, and certainly advances in the pharmacological and transplantation fields have allowed better management of patients affected by WD. However, the pharmacological armamentarium is still rudimentary, with side effects, a non-specific mechanism of action, and which constrain patients to take medication several times a day life-long. Gene therapy and hepatocyte cell transplantation are promising strategies in the treatment of WD, although there is still a long way to go until they can be used safely and readily in humans[142,143].

REFERENCES

1 **Wilson S**. Progressive lenticular degeneration: A familial nervous disease associated with cirrhosis of the liver. *Brain* 1912: 295-509
2 **Yang FM**, Friedrichs WE, Cupples RL, Bonifacio MJ, Sanford JA, Horton WA, Bowman BH. Human ceruloplasmin. Tissue-specific expression of transcripts produced by alternative splicing. *J Biol Chem* 1990; **265**: 10780-10785 [PMID: 2355023]
3 **Rosencrantz R**, Schilsky M. Wilson disease: pathogenesis and clinical considerations in diagnosis and treatment. *Semin Liver Dis* 2011; **31**: 245-259 [PMID: 21901655 DOI: 10.1055/s-0031-1286056]
4 **Hoogenraad TU**. Paradigm shift in treatment of Wilson's disease: zinc therapy now treatment of choice. *Brain Dev* 2006; **28**: 141-146 [PMID: 16466879 DOI: 10.1016/j.braindev.2005.08.008]
5 **Ala A**, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. *Lancet* 2007; **369**: 397-408 [PMID: 17276780 DOI: 10.1016/S0140-6736(07)60196-2]
6 **Scheinberg IH**, Sternlieb I. Wilson's disease. In: Smith JL, editor. Major problems in internal medicine. Philadelphia: WB Saunders, 1984: 25-35
7 **Scheinberg I**, Sternlieb I. Wilson Disease. In: Lloyd H, Smith J, editors. Major Problems in Internal Medicine. Saunders: Philadelphia, 1984: 23
8 University of Alberta Wilson Disease Mutation Database [Internet]. Available from: URL: http://www.wilsondisease.med.ualberta.ca/database
9 **Caca K**, Ferenci P, Kühn HJ, Polli C, Willgerodt H, Kunath B, Hermann W, Mössner J, Berr F. High prevalence of the H1069Q mutation in East German patients with Wilson disease: rapid detection of mutations by limited sequencing and phenotype-genotype analysis. *J Hepatol* 2001; **35**: 575-581 [PMID: 11690702]
10 **Kim EK**, Yoo OJ, Song KY, Yoo HW, Choi SY, Cho SW, Hahn SH. Identification of three novel mutations and a high frequency of the Arg778Leu mutation in Korean patients with Wilson disease. *Hum Mutat* 1998; **11**: 275-278 [PMID: 9554743]
11 **Nanji MS**, Nguyen VT, Kawasoe JH, Inui K, Endo F, Nakajima T, Anezaki T, Cox DW. Haplotype and mutation analysis in Japanese patients with Wilson disease. *Am J Hum Genet* 1997; **60**: 1423-1429 [PMID: 9199563]
12 **Wu ZY**, Wang N, Lin MT, Fang L, Murong SX, Yu L. Mutation analysis and the correlation between genotype and phenotype of Arg778Leu mutation in chinese patients with Wilson disease. *Arch Neurol* 2001; **58**: 971-976 [PMID: 11405812]
13 **Thomas GR**, Jensson O, Gudmundsson G, Thorsteinsson L, Cox DW. Wilson disease in Iceland: a clinical and genetic study. *Am J Hum Genet* 1995; **56**: 1140-1146 [PMID: 7726170]
14 **Margarit E**, Bach V, Gómez D, Bruguera M, Jara P, Queralt R, Ballesta F. Mutation analysis of Wilson disease in the Spanish population -- identification of a prevalent substitution and eight novel mutations in the ATP7B gene. *Clin Genet* 2005; **68**: 61-68 [PMID: 15952988 DOI: 10.1111/j.1399-0004.2005.00439.x]
15 **Beinhardt S**, Leiss W, Stättermayer AF, Graziadei I, Zoller H, Stauber R, Maieron A, Datz C, Steindl-Munda P, Hofer H, Vogel W, Trauner M, Ferenci P. Long-term outcomes of patients with Wilson disease in a large Austrian cohort. *Clin Gastroenterol Hepatol* 2014; **12**: 683-689 [PMID: 24076416 DOI: 10.1016/j.cgh.2013.09.025]
16 **Shah AB**, Chernov I, Zhang HT, Ross BM, Das K, Lutsenko S, Parano E, Pavone L, Evgrafov O, Ivanova-Smolenskaya IA, Annerén G, Westermark K, Urrutia FH, Penchaszadeh GK, Sternlieb I, Scheinberg IH, Gilliam TC, Petrukhin K. Identification and analysis of mutations in the Wilson disease gene (ATP7B): population frequencies, genotype-phenotype correlation, and functional analyses. *Am J Hum Genet* 1997; **61**: 317-328 [PMID: 9311736 DOI: 10.1086/514864]
17 **Hevia FJ**, Miranda M. The Special Problem of Wilson's Disease

in Costa Rica - An Unexpected High Prevalence. *Gastroenterol Int* 1989; **1**: 228

18 **Barada K**, El-Atrache M, El-Hajj II, Rida K, El-Hajjar J, Mahfoud Z, Usta J. Homozygous mutations in the conserved ATP hinge region of the Wilson disease gene: association with liver disease. *J Clin Gastroenterol* 2010; **44**: 432-439 [PMID: 20485189 DOI: 10.1097/MCG.0b013e3181ce5138]

19 **Loudianos G**, Dessi V, Lovicu M, Angius A, Figus A, Lilliu F, De Virgiliis S, Nurchi AM, Deplano A, Moi P, Pirastu M, Cao A. Molecular characterization of wilson disease in the Sardinian population--evidence of a founder effect. *Hum Mutat* 1999; **14**: 294-303 [PMID: 10502776]

20 **Giagheddu A**, Demelia L, Puggioni G, Nurchi AM, Contu L, Pirari G, Deplano A, Rachele MG. Epidemiologic study of hepatolenticular degeneration (Wilson's disease) in Sardinia (1902-1983). *Acta Neurol Scand* 1985; **72**: 43-55 [PMID: 3876678]

21 **Gialluisi A**, Incollu S, Pippucci T, Lepori MB, Zappu A, Loudianos G, Romeo G. The homozygosity index (HI) approach reveals high allele frequency for Wilson disease in the Sardinian population. *Eur J Hum Genet* 2013; **21**: 1308-1311 [PMID: 23486543 DOI: 10.1038/ejhg.2013.43]

22 **Zappu A**, Magli O, Lepori MB, Dessì V, Diana S, Incollu S, Kanavakis E, Nicolaidou P, Manolaki N, Fretzayas A, De Virgiliis S, Cao A, Loudianos G. High incidence and allelic homogeneity of Wilson disease in 2 isolated populations: a prerequisite for efficient disease prevention programs. *J Pediatr Gastroenterol Nutr* 2008; **47**: 334-338 [PMID: 18728530 DOI: 10.1097/MPG.0b013e31817094f6]

23 **Vrabelova S**, Letocha O, Borsky M, Kozak L. Mutation analysis of the ATP7B gene and genotype/phenotype correlation in 227 patients with Wilson disease. *Mol Genet Metab* 2005; **86**: 277-285 [PMID: 15967699 DOI: 10.1016/j.ymgme.2005.05.004]

24 **Nicastro E**, Loudianos G, Zancan L, D'Antiga L, Maggiore G, Marcellini M, Barbera C, Marazzi MG, Francavilla R, Pastore M, Vajro P, D'Ambrosi M, Vegnente A, Ranucci G, Iorio R. Genotype-phenotype correlation in Italian children with Wilson's disease. *J Hepatol* 2009; **50**: 555-561 [PMID: 19118915 DOI: 10.1016/j.jhep.2008.09.020]

25 **Lee BH**, Kim JH, Lee SY, Jin HY, Kim KJ, Lee JJ, Park JY, Kim GH, Choi JH, Kim KM, Yoo HW. Distinct clinical courses according to presenting phenotypes and their correlations to ATP7B mutations in a large Wilson's disease cohort. *Liver Int* 2011; **31**: 831-839 [PMID: 21645214 DOI: 10.1111/j.1478-3231.2011.02503.x]

26 **Ferenci P**, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003; **23**: 139-142 [PMID: 12955875 DOI: 10.1034/j.1600-0676.2003.00824.x]

27 **Schilsky ML**, Scheinberg IH, Sternlieb I. Liver transplantation for Wilson's disease: indications and outcome. *Hepatology* 1994; **19**: 583-587 [PMID: 8119682]

28 **Walshe JM**. Wilson's disease presenting with features of hepatic dysfunction: a clinical analysis of eighty-seven patients. *Q J Med* 1989; **70**: 253-263 [PMID: 2602537]

29 **Scott J**, Gollan JL, Samourian S, Sherlock S. Wilson's disease, presenting as chronic active hepatitis. *Gastroenterology* 1978; **74**: 645-651 [PMID: 75819]

30 **Stremmel W**, Meyerrose KW, Niederau C, Hefter H, Kreuzpaintner G, Strohmeyer G. Wilson disease: clinical presentation, treatment, and survival. *Ann Intern Med* 1991; **115**: 720-726 [PMID: 1929042]

31 **Saito T**. Presenting symptoms and natural history of Wilson disease. *Eur J Pediatr* 1987; **146**: 261-265 [PMID: 3595645]

32 **Roberts EA**, Schilsky ML. Diagnosis and treatment of Wilson disease: an update. *Hepatology* 2008; **47**: 2089-2111 [PMID: 18506894 DOI: 10.1002/hep.22261]

33 **Svetel M**, Potrebić A, Pekmezović T, Tomić A, Kresojević N, Jesić R, Dragasević N, Kostić VS. Neuropsychiatric aspects of treated Wilson's disease. *Parkinsonism Relat Disord* 2009; **15**: 772-775 [PMID: 19559640 DOI: 10.1016/j.parkreldis.2009.01.010]

34 **Svetel M**, Kozić D, Stefanova E, Semnic R, Dragasevic N, Kostic VS. Dystonia in Wilson's disease. *Mov Disord* 2001; **16**: 719-723 [PMID: 11481698]

35 **Taly AB**, Meenakshi-Sundaram S, Sinha S, Swamy HS, Arunodaya GR. Wilson disease: description of 282 patients evaluated over 3 decades. *Medicine* (Baltimore) 2007; **86**: 112-121 [PMID: 17435591 DOI: 10.1097/MD.0b013e318045a00e]

36 **del Rosario MA**, Davis MM, Chong SK. Wobbly handwriting. *Lancet* 1998; **351**: 336 [PMID: 9652615 DOI: 10.1016/S0140-6736(97)10254-9]

37 **Walshe JM**. The eye in Wilson disease. *QJM* 2011; **104**: 451-453 [PMID: 20444834 DOI: 10.1093/qjmed/hcq065]

38 **Litwin T**, Langwińska-Wośko E, Dzieżyc K, Członkowska A. Sunflower cataract: do not forget Wilson's disease. *Pract Neurol* 2015; **15**: 385-386 [PMID: 25935927 DOI: 10.1136/practneurol-2014-001056]

39 **Deguti MM**, Tietge UJ, Barbosa ER, Cancado EL. The eye in Wilson's disease: sunflower cataract associated with Kayser-Fleischer ring. *J Hepatol* 2002; **37**: 700 [PMID: 12399241]

40 **Sturniolo GC**, Lazzarini D, Bartolo O, Berton M, Leonardi A, Fregona IA, Parrozzani R, Midena E. Small fiber peripheral neuropathy in Wilson disease: an in vivo documentation by corneal confocal microscopy. *Invest Ophthalmol Vis Sci* 2015; **56**: 1390-1395 [PMID: 25613947 DOI: 10.1167/iovs.14-15004]

41 **Albrecht P**, Müller AK, Ringelstein M, Finis D, Geerling G, Cohn E, Aktas O, Hartung HP, Hefter H, Methner A. Retinal neurodegeneration in Wilson's disease revealed by spectral domain optical coherence tomography. *PLoS One* 2012; **7**: e49825 [PMID: 23166778 DOI: 10.1371/journal.pone.0049825]

42 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol* 2012; **56**: 671-685 [PMID: 22340672 DOI: 10.1016/j.jhep.2011.11.007]

43 **Shanmugiah A**, Sinha S, Taly AB, Prashanth LK, Tomar M, Arunodaya GR, Reddy JY, Khanna S. Psychiatric manifestations in Wilson's disease: a cross-sectional analysis. *J Neuropsychiatry Clin Neurosci* 2008; **20**: 81-85 [PMID: 18305288 DOI: 10.1176/appi.neuropsych.20.1.81]

44 **Zimbrean PC**, Schilsky ML. Psychiatric aspects of Wilson disease: a review. *Gen Hosp Psychiatry* 2014; **36**: 53-62 [PMID: 24120023 DOI: 10.1016/j.genhosppsych.2013.08.007]

45 **Verma R**, Junewar V, Sahu R. Pathological fractures as an initial presentation of Wilson's disease. *BMJ Case Rep* 2013; **2013**: pii: bcr2013008857 [PMID: 23576656 DOI: 10.1136/bcr-2013-008857]

46 **Schilsky ML**, Ala A. Genetic testing for Wilson disease: availability and utility. *Curr Gastroenterol Rep* 2010; **12**: 57-61 [PMID: 20425485 DOI: 10.1007/s11894-009-0084-5]

47 **Durand F**. Wilson's disease: an old disease keeps its old secrets. *Eur J Gastroenterol Hepatol* 2007; **19**: 97-99 [PMID: 17272992 DOI: 10.1097/MEG.0b013e32800fef34]

48 **Mallet I**, Guillaud O, Dumortier J, Brunet AS, Lachaux A, Pelosse M, Broussole E, Lion-François L, Vallin M, Juget Pietu F, Petronio M, El Bakhi S, Poupon J, Bost M. Le cuivre échangeable sérique: un nouveau marqueur pour le diagnostic de la maladie de Wilson. AFEF Congress. Montpellier, 2012. Available from: URL: http://www.afef.asso.fr/rc/org/afef/htm/Article/2012/20121115-114120-394/src/htm_fullText/fr/63_fc.pdf

49 **Jimenez-Díaz M**, Schosinsky-Nevermann K. Validación de la determinación de cobre en suero empleando el acido bicinconinico: relación cobre/ceruloplasmina en pacientes con enfermedad de Wilson y pacientes sin la enfermedad. *Rev Costarric Cienc Med* 2002; **23**: 1-2

50 **Hevia FJ**, Schosinsky JK, Esquivel JM, Miranda D. Diagnosis of Wilson's disease six-hour D-penicillamine urinary test. [Abstract presented at the 7th International Symposium on Wilson's Disease]. *Ger J Gastroenterol* 1995; **8**: 486

51 **Ombrone D**, Giocaliere E, Forni G, Malvagia S, la Marca G. Expanded newborn screening by mass spectrometry: New tests, future perspectives. *Mass Spectrom Rev* 2015; Epub ahead of print [PMID: 25952022 DOI: 10.1002/mas.21463]

52 **Smolarek C**, Stremmel W. [Therapy of Wilson disease]. *Z Gastroenterol* 1999; **37**: 293-300 [PMID: 10378366]

53 **Walshe JM**, Yealland M. Chelation treatment of neurological Wilson's disease. *Q J Med* 1993; **86**: 197-204 [PMID: 8369040]
54 **Brewer GJ**. Zinc and tetrathiomolybdate for the treatment of Wilson's disease and the potential efficacy of anticopper therapy in a wide variety of diseases. *Metallomics* 2009; **1**: 199-206 [PMID: 21305118 DOI: 10.1039/b901614g]
55 **Scheinberg IH**, Jaffe ME, Sternlieb I. The use of trientine in preventing the effects of interrupting penicillamine therapy in Wilson's disease. *N Engl J Med* 1987; **317**: 209-213 [PMID: 3600712 DOI: 10.1056/NEJM198707233170405]
56 **Walshe JM**. Tetrathiomolybdate as an anticopper agent in man. Orphan disease and orphan drugs. Sheinberg. Manchester University Press, 1986: 76-85
57 **Brewer GJ**, Dick RD, Johnson V, Wang Y, Yuzbasiyan-Gurkan V, Kluin K, Fink JK, Aisen A. Treatment of Wilson's disease with ammonium tetrathiomolybdate. I. Initial therapy in 17 neurologically affected patients. *Arch Neurol* 1994; **51**: 545-554 [PMID: 8198464]
58 **Hoogenraad TU**, Van den Hamer CJ. 3 years of continuous oral zinc therapy in 4 patients with Wilson's disease. *Acta Neurol Scand* 1983; **67**: 356-364 [PMID: 6613522]
59 NIH Office of Rare Diseases Research [Internet]. 2015. Available from: URL: https://rarediseases.org/organizations/nihoffice-of-rare-disease-research/
60 Orphanet - The portal for rare diseases and orphan drugs [Internet]. 2015. Available from: URL: http://www.orpha.net/consor/cgi-bin/index.php
61 **Brewer GJ**, Yuzbasiyan-Gurkan V, Lee DY, Appelman H. Treatment of Wilson's disease with zinc. VI. Initial treatment studies. *J Lab Clin Med* 1989; **114**: 633-638 [PMID: 2592853]
62 Public summary of positive opinion for orphan designation of ammonium tetrathiomolybdate for the treatment of Wilson's disease. In: committee for orphan medicinal products, European Medicines Agency [Internet]. 2008: 1-4. Available from: URL: http://www.ema.europa.eu/docs/en_GB/document_library/Orphan_designation/2009/10/WC500005717.pdf
63 **Walshe JM**. Wilson's disease; new oral therapy. *Lancet* 1956; **270**: 25-26 [PMID: 13279157]
64 **Scheinberg IH**, Sternlieb I, Schilsky M, Stockert RJ. Penicillamine may detoxify copper in Wilson's disease. *Lancet* 1987; **2**: 95 [PMID: 2885586]
65 **Farinati F**, Cardin R, Mestriner C, Sturniolo GC, Naccarato R. Mechanisms of pennicillamine and zinc in the treatment of Wilson's disease. *Am J Gastroenterol* 1995; **90**: 2264-2265 [PMID: 8540540]
66 **Lipsky PE**, Ziff M. The effect of D-penicillamine on mitogen-induced human lymphocyte proliferation: synergistic inhibition by D-penicillamine and copper salts. *J Immunol* 1978; **120**: 1006-1013 [PMID: 632576]
67 **Nimni ME**. Mechanism of inhibition of collagen crosslinking by penicillamine. *Proc R Soc Med* 1977; **70** Suppl 3: 65-72 [PMID: 122677]
68 **Siegel RC**. Collagen cross-linking. Effect of D-penicillamine on cross-linking in vitro. *J Biol Chem* 1977; **252**: 254-259 [PMID: 13066]
69 **Cope-Yokoyama S**, Finegold MJ, Sturniolo GC, Kim K, Mescoli C, Rugge M, Medici V. Wilson disease: histopathological correlations with treatment on follow-up liver biopsies. *World J Gastroenterol* 2010; **16**: 1487-1494 [PMID: 20333789]
70 **Brewer GJ**, Terry CA, Aisen AM, Hill GM. Worsening of neurologic syndrome in patients with Wilson's disease with initial penicillamine therapy. *Arch Neurol* 1987; **44**: 490-493 [PMID: 3579660]
71 **Wiggelinkhuizen M**, Tilanus ME, Bollen CW, Houwen RH. Systematic review: clinical efficacy of chelator agents and zinc in the initial treatment of Wilson disease. *Aliment Pharmacol Ther* 2009; **29**: 947-958 [PMID: 19210288 DOI: 10.1111/j.1365-2036.2009.03959.x]
72 **Durand F**, Bernuau J, Giostra E, Mentha G, Shouval D, Degott C, Benhamou JP, Valla D. Wilson's disease with severe hepatic insufficiency: beneficial effects of early administration of D-penicillamine. *Gut* 2001; **48**: 849-852 [PMID: 11358907]
73 **Falkmer S**, Samuelson G, Sjölin S. Penicillamine-induced normalization of clinical signs, and liver morphology and histochemistry in a case of Wilson's disease. *Pediatrics* 1970; **45**: 260-268 [PMID: 5413388]
74 **Lau JY**, Lai CL, Wu PC, Pan HY, Lin HJ, Todd D. Wilson's disease: 35 years' experience. *Q J Med* 1990; **75**: 597-605 [PMID: 2217665]
75 **Merle U**, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. *Gut* 2007; **56**: 115-120 [PMID: 16709660 DOI: 10.1136/gut.2005.087262]
76 **Keen CL**, Mark-Savage P, Lönnerdal B, Hurley LS. Teratogenic effects of D-penicillamine in rats: relation to copper deficiency. *Drug Nutr Interact* 1983; **2**: 17-34 [PMID: 6678745]
77 **Medici V**, Di Leo V, Lamboglia F, Bowlus CL, Tseng SC, D' Incà R, Irato P, Burra P, Martines D, Sturniolo GC. Effect of penicillamine and zinc on iron metabolism in Wilson's disease. *Scand J Gastroenterol* 2007; **42**: 1495-1500 [PMID: 17994470 DOI: 10.1080/00365520701514495]
78 **Brewer GJ**, Turkay A, Yuzbaziyan-Gurkan V. Development of neurologic symptoms in a patient with asymptomatic Wilson's disease treated with penicillamine. *Arch Neurol* 1994; **51**: 304-305 [PMID: 8129644]
79 **Brewer GJ**, Askari F, Dick RB, Sitterly J, Fink JK, Carlson M, Kluin KJ, Lorincz MT. Treatment of Wilson's disease with tetrathiomolybdate: V. Control of free copper by tetrathiomolybdate and a comparison with trientine. *Transl Res* 2009; **154**: 70-77 [PMID: 19595438 DOI: 10.1016/j.trsl.2009.05.002]
80 **Zhang JW**, Liu JX, Hou HM, Chen DB, Feng L, Wu C, Wei LT, Li XH. Effects of tetrathiomolybdate and penicillamine on brain hydroxyl radical and free copper levels: a microdialysis study in vivo. *Biochem Biophys Res Commun* 2015; **458**: 82-85 [PMID: 25634697 DOI: 10.1016/j.bbrc.2015.01.071]
81 **Ranjan A**, Kalita J, Kumar V, Misra UK. MRI and oxidative stress markers in neurological worsening of Wilson disease following penicillamine. *Neurotoxicology* 2015; **49**: 45-49 [PMID: 26004675 DOI: 10.1016/j.neuro.2015.05.004]
82 **Kodama H**, Murata Y, Iitsuka T, Abe T. Metabolism of administered triethylene tetramine dihydrochloride in humans. *Life Sci* 1997; **61**: 899-907 [PMID: 9284083]
83 **Walshe JM**. Treatment of Wilson's disease with trientine (triethylene tetramine) dihydrochloride. *Lancet* 1982; **1**: 643-647 [PMID: 6121964]
84 **Walshe JM**. Copper chelation in patients with Wilson's disease. A comparison of penicillamine and triethylene tetramine dihydrochloride. *Q J Med* 1973; **42**: 441-452 [PMID: 4728043]
85 **Kim B**, Chung SJ, Shin HW. Trientine-induced neurological deterioration in a patient with Wilson's disease. *J Clin Neurosci* 2013; **20**: 606-608 [PMID: 23274038 DOI: 10.1016/j.jocn.2012.02.041]
86 **Brewer GJ**, Askari F, Lorincz MT, Carlson M, Schilsky M, Kluin KJ, Hedera P, Moretti P, Fink JK, Tankanow R, Dick RB, Sitterly J. Treatment of Wilson disease with ammonium tetrathiomolybdate: IV. Comparison of tetrathiomolybdate and trientine in a double-blind study of treatment of the neurologic presentation of Wilson disease. *Arch Neurol* 2006; **63**: 521-527 [PMID: 16606763 DOI: 10.1001/archneur.63.4.521]
87 **Epstein O**, Sherlock S. Triethylene tetramine dihydrochloride toxicity in primary biliary cirrhosis. *Gastroenterology* 1980; **78**: 1442-1445 [PMID: 6445305]
88 **Sarkar B**, Sass-Kortsak A, Clarke R, Laurie SH, Wei P. A comparative study of in vitro and in vivo interaction of D-penicillamine and triethylenetetramine with copper. *Proc R Soc Med* 1977; **70** Suppl 3: 13-18 [PMID: 122660]
89 **Hoogenraad TU**, Van den Hamer CJ, Koevoet R, Korver EG. Oral zinc in Wilson's disease. *Lancet* 1978; **2**: 1262 [PMID: 82772]
90 **Brewer GJ**. Zinc acetate for the treatment of Wilson's disease. *Expert Opin Pharmacother* 2001; **2**: 1473-1477 [PMID: 11585025 DOI: 10.1517/14656566.2.9.1473]
91 **Sturniolo GC**, Mestriner C, Irato P, Albergoni V, Longo G, D'

Incà R. Zinc therapy increases duodenal concentrations of metallothionein and iron in Wilson's disease patients. *Am J Gastroenterol* 1999; **94**: 334-338 [PMID: 10022625 DOI: 10.1111/j.1572-0241.1999.851_w.x]
92 **Cousins RJ**. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol Rev* 1985; **65**: 238-309 [PMID: 3885271]
93 **Farinati F**, Cardin R, D'inca R, Naccarato R, Sturniolo GC. Zinc treatment prevents lipid peroxidation and increases glutathione availability in Wilson's disease. *J Lab Clin Med* 2003; **141**: 372-377 [PMID: 12819634 DOI: 10.1016/S0022-2143(03)00027-1]
94 **Dzieżyc K**, Karliński M, Litwin T, Członkowska A. Compliant treatment with anti-copper agents prevents clinically overt Wilson's disease in pre-symptomatic patients. *Eur J Neurol* 2014; **21**: 332-337 [PMID: 24313946 DOI: 10.1111/ene.12320]
95 **Czlonkowska A**, Gajda J, Rodo M. Effects of long-term treatment in Wilson's disease with D-penicillamine and zinc sulphate. *J Neurol* 1996; **243**: 269-273 [PMID: 8936358]
96 **Członkowska A**, Litwin T, Karliński M, Dziezyc K, Chabik G, Czerska M. D-penicillamine versus zinc sulfate as first-line therapy for Wilson's disease. *Eur J Neurol* 2014; **21**: 599-606 [PMID: 24447648 DOI: 10.1111/ene.12348]
97 **Hevia FJ**. Practical management of Wilson's Disese. In: Arroyo V, editor. International Hepatology Updates, 2009
98 **Brewer GJ**, Yuzbasiyan-Gurkan V. Wilson disease. *Medicine* (Baltimore) 1992; **71**: 139-164 [PMID: 1635439]
99 **Ishida S**, Doi Y, Yamane K, Sugino M, Kimura F, Hanafusa T, Fukui H, Tamai H. Resolution of cranial MRI and SPECT abnormalities in a patient with Wilson's disease following oral zinc monotherapy. *Intern Med* 2012; **51**: 1759-1763 [PMID: 22790141]
100 **Askari FK**, Greenson J, Dick RD, Johnson VD, Brewer GJ. Treatment of Wilson's disease with zinc. XVIII. Initial treatment of the hepatic decompensation presentation with trientine and zinc. *J Lab Clin Med* 2003; **142**: 385-390 [PMID: 14713890 DOI: 10.1016/S0022-2143(03)00157-4]
101 **Brewer GJ**, Yuzbasiyan-Gurkan V, Dick R, Wang Y, Johnson V. Does a vegetarian diet control Wilson's disease? *J Am Coll Nutr* 1993; **12**: 527-530 [PMID: 8263268]
102 **Arnon R**, Calderon JF, Schilsky M, Emre S, Shneider BL. Wilson disease in children: serum aminotransferases and urinary copper on triethylene tetramine dihydrochloride (trientine) treatment. *J Pediatr Gastroenterol Nutr* 2007; **44**: 596-602 [PMID: 17460493 DOI: 10.1097/MPG.0b013e3180467715]
103 **Medici V**, Mirante VG, Fassati LR, Pompili M, Forti D, Del Gaudio M, Trevisan CP, Cillo U, Sturniolo GC, Fagiuoli S. Liver transplantation for Wilson's disease: The burden of neurological and psychiatric disorders. *Liver Transpl* 2005; **11**: 1056-1063 [PMID: 16123950 DOI: 10.1002/lt.20486]
104 **Cheng F**, Li GQ, Zhang F, Li XC, Sun BC, Kong LB, Pu LY, Wang K, Qian XF, You W, Wang XH. Outcomes of living-related liver transplantation for Wilson's disease: a single-center experience in China. *Transplantation* 2009; **87**: 751-757 [PMID: 19295322 DOI: 10.1097/TP.0b013e318198a46e]
105 **Schumacher G**, Platz KP, Mueller AR, Neuhaus R, Luck W, Langrehr JM, Settmacher U, Steinmueller T, Becker M, Neuhaus P. Liver transplantation in neurologic Wilson's disease. *Transplant Proc* 2001; **33**: 1518-1519 [PMID: 11267403]
106 **Groth CG**, Dubois RS, Corman J, Gustafsson A, Iwatsuki S, Rodgerson DO, Halgrimson CG, Starzl TE. Metabolic effects of hepatic replacement in Wilson's disease. *Transplant Proc* 1973; **5**: 829-833 [PMID: 4572145]
107 **Arnon R**, Annunziato R, Schilsky M, Miloh T, Willis A, Sturdevant M, Sakworawich A, Suchy F, Kerkar N. Liver transplantation for children with Wilson disease: comparison of outcomes between children and adults. *Clin Transplant* 2011; **25**: E52-E60 [PMID: 20946468 DOI: 10.1111/j.1399-0012.2010.01327.x]
108 **Weiss KH**, Schäfer M, Gotthardt DN, Angerer A, Mogler C, Schirmacher P, Schemmer P, Stremmel W, Sauer P. Outcome and development of symptoms after orthotopic liver transplantation for Wilson disease. *Clin Transplant* 2013; **27**: 914-922 [PMID: 24118554 DOI: 10.1111/ctr.12259]
109 **Yagci MA**, Tardu A, Karagul S, Ertugrul I, Ince V, Kirmizi S, Unal B, Isik B, Kayaalp C, Yilmaz S. Influence of Liver Transplantation on Neuropsychiatric Manifestations of Wilson Disease. *Transplant Proc* 2015; **47**: 1469-1473 [PMID: 26093745 DOI: 10.1016/j.transproceed.2015.04.017]
110 **Stracciari A**, Tempestini A, Borghi A, Guarino M. Effect of liver transplantation on neurological manifestations in Wilson disease. *Arch Neurol* 2000; **57**: 384-386 [PMID: 10714666]
111 **Medici V**, Sturniolo GC. Tetrathiomolybdate, a copper chelator for the treatment of Wilson disease, pulmonary fibrosis and other indications. *IDrugs* 2008; **11**: 592-606 [PMID: 18683094]
112 **Walshe JM**. Tetrathiomolybdate (MoS4) as an "anti-copper" agent in man. In: Scheinberg IH, Walsche J, editors. Orphan disease, orphan drugs. Manchester University Press, 1986: 76-85
113 **McQuaid A**, Mason J. A comparison of the effects of penicillamine, trientine, and trithiomolybdate on [35S]-labeled metallothionein in vitro; implications for Wilson's disease therapy. *J Inorg Biochem* 1991; **41**: 87-92 [PMID: 2033396]
114 **Brewer GJ**, Dick RD, Yuzbasiyan-Gurkin V, Tankanow R, Young AB, Kluin KJ. Initial therapy of patients with Wilson's disease with tetrathiomolybdate. *Arch Neurol* 1991; **48**: 42-47 [PMID: 1986725]
115 **Brewer GJ**, Hedera P, Kluin KJ, Carlson M, Askari F, Dick RB, Sitterly J, Fink JK. Treatment of Wilson disease with ammonium tetrathiomolybdate: III. Initial therapy in a total of 55 neurologically affected patients and follow-up with zinc therapy. *Arch Neurol* 2003; **60**: 379-385 [PMID: 12633149]
116 **Medici V**, Trevisan CP, Bigotto MA, D'Incà R, Martines D, Dal Pont E, Sturniolo GC. Adverse reaction after tetrathiomolybdate treatment for Wilson's disease: a case report. *Mov Disord* 2006; **21**: 2030-2032 [PMID: 16991142 DOI: 10.1002/mds.21109]
117 **Ogihara H**, Ogihara T, Miki M, Yasuda H, Mino M. Plasma copper and antioxidant status in Wilson's disease. *Pediatr Res* 1995; **37**: 219-226 [PMID: 7731761 DOI: 10.1203/00006450-199502000-00016]
118 **Sokol RJ**, Twedt D, McKim JM, Devereaux MW, Karrer FM, Kam I, von Steigman G, Narkewicz MR, Bacon BR, Britton RS. Oxidant injury to hepatic mitochondria in patients with Wilson's disease and Bedlington terriers with copper toxicosis. *Gastroenterology* 1994; **107**: 1788-1798 [PMID: 7958693]
119 **von Herbay A**, de Groot H, Hegi U, Stremmel W, Strohmeyer G, Sies H. Low vitamin E content in plasma of patients with alcoholic liver disease, hemochromatosis and Wilson's disease. *J Hepatol* 1994; **20**: 41-46 [PMID: 8201221]
120 **Schilsky ML**. Liver transplantation for Wilson's disease. *Ann N Y Acad Sci* 2014; **1315**: 45-49 [PMID: 24820352 DOI: 10.1111/nyas.12454]
121 **Petrasek J**, Jirsa M, Sperl J, Kozak L, Taimr P, Spicak J, Filip K, Trunecka P. Revised King's College score for liver transplantation in adult patients with Wilson's disease. *Liver Transpl* 2007; **13**: 55-61 [PMID: 17154398 DOI: 10.1002/lt.20920]
122 **Nazer H**, Ede RJ, Mowat AP, Williams R. Wilson's disease: clinical presentation and use of prognostic index. *Gut* 1986; **27**: 1377-1381 [PMID: 3792921]
123 **Dhawan A**, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. *Liver Transpl* 2005; **11**: 441-448 [PMID: 15776453 DOI: 10.1002/lt.20352]
124 **Sarles J**, Lefevre P, Picon G. Plasma exchange for fulminant Wilson disease. *Eur J Pediatr* 1992; **151**: 310 [PMID: 1499587]
125 **Akyildiz BN**, Yildirim S, Kondolot M, Arslan D. Is plasma exchange effective in prevention of hepatic transplantation in fulminant Wilson disease with hepatic failure? *J Pediatr Gastroenterol Nutr* 2011; **52**: 778-780 [PMID: 21593651 DOI: 10.1097/MPG.0b013e318208d0a3]
126 **Jhang JS**, Schilsky ML, Lefkowitch JH, Schwartz J. Therapeutic plasmapheresis as a bridge to liver transplantation in fulminant Wilson disease. *J Clin Apher* 2007; **22**: 10-14 [PMID: 17285615 DOI: 10.1002/jca.20118]
127 **Motobayashi M**, Fukuyama T, Nakayama Y, Sano K, Noda

S, Hidaka Y, Amano Y, Ikeda S, Koike K, Inaba Y. Successful treatment of fulminant Wilson's disease without liver transplantation. *Pediatr Int* 2014; **56**: 429-432 [PMID: 24894932 DOI: 10.1111/ped.12291]

128 **Nagata Y**, Uto H, Hasuike S, Ido A, Hayashi K, Eto T, Hamakawa T, Tanaka K, Tsubouchi H. Bridging use of plasma exchange and continuous hemodiafiltration before living donor liver transplantation in fulminant Wilson's disease. *Intern Med* 2003; **42**: 967-970 [PMID: 14606709]

129 **Collins KL**, Roberts EA, Adeli K, Bohn D, Harvey EA. Single pass albumin dialysis (SPAD) in fulminant Wilsonian liver failure: a case report. *Pediatr Nephrol* 2008; **23**: 1013-1016 [PMID: 18299897 DOI: 10.1007/s00467-008-0761-x]

130 **Manz T**, Ochs A, Bisse E, Strey C, Grotz W. Liver support--a task for nephrologists? Extracorporeal treatment of a patient with fulminant Wilson crisis. *Blood Purif* 2003; **21**: 232-236 [PMID: 12784049]

131 **Aagaard NK**, Thomsen KL, Holland-Fischer P, Jørgensen SP, Ott P. A 15-year-old girl with severe hemolytic Wilson's crisis recovered without transplantation after extracorporeal circulation with the Prometheus system. *Blood Purif* 2009; **28**: 102-107 [PMID: 19439930 DOI: 10.1159/000218090]

132 **Stange J**, Mitzner SR, Risler T, Erley CM, Lauchart W, Goehl H, Klammt S, Peszynski P, Freytag J, Hickstein H, Löhr M, Liebe S, Schareck W, Hopt UT, Schmidt R. Molecular adsorbent recycling system (MARS): clinical results of a new membrane-based blood purification system for bioartificial liver support. *Artif Organs* 1999; **23**: 319-330 [PMID: 10226696]

133 **Sen S**, Felldin M, Steiner C, Larsson B, Gillett GT, Olausson M, Williams R, Jalan R. Albumin dialysis and Molecular Adsorbents Recirculating System (MARS) for acute Wilson's disease. *Liver Transpl* 2002; **8**: 962-967 [PMID: 12360442 DOI: 10.1053/jlts.2002.35546]

134 **Chiu A**, Tsoi NS, Fan ST. Use of the molecular adsorbents recirculating system as a treatment for acute decompensated Wilson disease. *Liver Transpl* 2008; **14**: 1512-1516 [PMID: 18825711 DOI: 10.1002/lt.21553]

135 **Rustom N**, Bost M, Cour-Andlauer F, Lachaux A, Brunet AS, Boillot O, Bordet F, Valla F, Richard N, Javouhey E. Effect of molecular adsorbents recirculating system treatment in children with acute liver failure caused by Wilson disease. *J Pediatr Gastroenterol Nutr* 2014; **58**: 160-164 [PMID: 24458219 DOI: 10.1097/MPG.0b013e3182a853a3]

136 **Shimono N**, Ishibashi H, Ikematsu H, Kudo J, Shirahama M, Inaba S, Maeda K, Yamasaki K, Niho Y. Fulminant hepatic failure during perinatal period in a pregnant woman with Wilson's disease. *Gastroenterol Jpn* 1991; **26**: 69-73 [PMID: 2007459]

137 **Myint B**. D-penicillamine-induced cleft palate in mice. *Teratology* 1984; **30**: 333-340 [PMID: 6515561 DOI: 10.1002/tera.1420300305]

138 **Endres W**. D-penicillamine in pregnancy--to ban or not to ban? *Klin Wochenschr* 1981; **59**: 535-537 [PMID: 7265809]

139 **Tanaka H**, Inomata K, Arima M. Teratogenic effects of triethylene tetramine dihydrochloride on the mouse brain. *J Nutr Sci Vitaminol* (Tokyo) 1993; **39**: 177-188 [PMID: 8410376]

140 **Brewer GJ**, Johnson VD, Dick RD, Hedera P, Fink JK, Kluin KJ. Treatment of Wilson's disease with zinc. XVII: treatment during pregnancy. *Hepatology* 2000; **31**: 364-370 [PMID: 10655259 DOI: 10.1002/hep.510310216]

141 **Medici V**, Trevisan CP, D'Incà R, Barollo M, Zancan L, Fagiuoli S, Martines D, Irato P, Sturniolo GC. Diagnosis and management of Wilson's disease: results of a single center experience. *J Clin Gastroenterol* 2006; **40**: 936-941 [PMID: 17063115 DOI: 10.1097/01.mcg.0000225670.91722.59]

142 **Merle U**, Stremmel W, Encke J. Perspectives for gene therapy of Wilson disease. *Curr Gene Ther* 2007; **7**: 217-220 [PMID: 17584039 DOI: 10.2174/156652307780859053]

143 **Filippi C**, Dhawan A. Current status of human hepatocyte transplantation and its potential for Wilson's disease. *Ann N Y Acad Sci* 2014; **1315**: 50-55 [PMID: 24605914 DOI: 10.1111/nyas.12386]

P- Reviewer: Boucek C, Bruha R, Hoogenraad TU, Usta J
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.i29.2871

World J Hepatol 2015 December 18; 7(29): 2871-2879
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

2015 Advances in Cirrhosis

Advances in cirrhosis: Optimizing the management of hepatic encephalopathy

Andy Liu, Ryan B Perumpail, Radhika Kumari, Zobair M Younossi, Robert J Wong, Aijaz Ahmed

Andy Liu, Albert Einstein College of Medicine, Bronx, NY 10461, United States

Ryan B Perumpail, Radhika Kumari, Aijaz Ahmed, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, CA 94305, United States

Zobair M Younossi, Center for Liver Diseases, Department of Medicine, Inova Fairfax Hospital, Falls Church, VA 22042, United States

Robert J Wong, Division of Gastroenterology and Hepatology, Alameda Health System - Highland Hospital Campus, Oakland, CA 94602, United States

Author contributions: Liu A, Perumpail RB and Ahmed A contributed to the study concept and design; Liu A and Perumpail RB contributed to the systematic review, data collection and drafting of the manuscript; Liu A, Perumpail RB, Kumari R, Younossi ZM, Wong RJ and Ahmed A contributed to the critical review of the manuscript; Perumpail RB and Ahmed A contributed to the revision and finalization of the manuscript.

Conflict-of-interest statement: We declare that we have no conflicts of interest.

Correspondence to: Aijaz Ahmed, MD, Associate Professor of Medicine, Medical Director Liver Transplant Program, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, 750 Welch Road, Suite 210, Palo Alto, Stanford, CA 94305, United States. aijazahmed@stanford.edu
Telephone: +1-650-4986091

Received: April 29, 2015
Peer-review started: May 8, 2015
First decision: September 8, 2015
Revised: October 24, 2015
Accepted: November 23, 2015
Article in press: November 25, 2015
Published online: December 18, 2015

Abstract

Hepatic encephalopathy (HE) is a major complication of cirrhosis resulting in significant socioeconomic burden, morbidity, and mortality. HE can be further subdivided into covert HE (CHE) and overt HE (OHE). CHE is a subclinical, less severe manifestation of HE and requires psychometric testing for diagnosis. Due to the time consuming screening process and lack of standardized diagnostic criteria, CHE is frequently underdiagnosed despite its recognized role as a precursor to OHE. Screening for CHE with the availability of the Stroop test has provided a pragmatic method to promptly diagnose CHE. Management of acute OHE involves institution of lactulose, the preferred first-line therapy. In addition, prompt recognition and treatment of precipitating factors is critical as it may result in complete resolution of acute episodes of OHE. Treatment goals include improvement of daily functioning, evaluation for liver transplantation, and prevention of OHE recurrence. For secondary prophylaxis, intolerance to indefinite lactulose therapy may lead to non-adherence and has been identified as a precipitating factor for recurrent OHE. Rifaximin is an effective add-on therapy to lactulose for treatment and prevention of recurrent OHE. Recent studies have demonstrated comparable efficacy of probiotic therapy to lactulose use in both primary prophylaxis and secondary prophylaxis.

Key words: Overt hepatic encephalopathy; Lactulose; Rifaximin; Hepatic encephalopathy; Covert hepatic encephalopathy

Core tip: Hepatic encephalopathy (HE) is a major complication of cirrhosis resulting in significant socioeconomic burden, morbidity, and mortality. Management of acute overt HE (OHE) involves institution of lactulose, the preferred first-line therapy. In addition, prompt recognition and treatment of precipitating factors may result in complete resolution of acute episodes of OHE. Treatment goals include improvement of daily functioning and prevention of OHE recurrence. For secondary prophylaxis, intolerance to indefinite lactulose therapy may lead to non-adherence and has been identified as a precipitating factor for recurrent OHE. Rifaximin is an effective add-on therapy to lactulose for treatment and prevention of recurrent OHE.

Liu A, Perumpail RB, Kumari R, Younossi ZM, Wong RJ, Ahmed A. Advances in cirrhosis: Optimizing the management of hepatic encephalopathy. *World J Hepatol* 2015; 7(29): 2871-2879 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2871.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2871

INTRODUCTION

The prevalence of cirrhosis both worldwide and in the United States is unknown[1], although experts estimate that 5.5 million people in the United States have cirrhosis[2]. One of the primary complications of cirrhosis is hepatic encephalopathy (HE)[3]. HE represents a continuum of clinical features involving cerebral dysfunction in cirrhotic patients with hepatic decompensation[4]. The spectrum can be further divided into preclinical stage, covert HE (CHE), or a clinically symptomatic state known as overt HE (OHE).

INCIDENCE AND PREVALENCE OF HE

According to a study utilizing data from the Nationwide Inpatient Sample, annual inpatient incidence of HE ranged from 20918 in 2005 to 22931 in 2009[5]. This accounted for 0.33% of all hospitalizations in the United States. A 2010 study of 170 cirrhotic patients from United States reported 56% of patients with underlying CHE, while 30% developed an episode of OHE[6]. Among 1348 consecutive patients with cirrhosis in a multicenter European study, 34% patients were diagnosed with OHE[7]. Other studies utilizing data from British and Danish cohorts report OHE in 10% to 11% of patients in the setting of alcoholic cirrhosis[8,9]. Prevalence of CHE in patients with cirrhosis range from 27% to 70%[10,11].

MORTALITY AND MORBIDITY ASSOCIATED WITH HE

The onset of OHE is associated with high mortality rates as reported by Bustamante *et al*[12] with 42% survival at 1 year follow-up and 23% at 3 years. Based on comparison with other complications of cirrhosis, the mortality rates are comparable to bleeding from varices[13]. OHE has been associated with 54% mortality at one year in patients needing admission to intensive care unit[14], while data from the inpatient samples report a 14% to 15% mortality over the 5-year period[5]. Jepsen *et al*[9] describe 169 patients with a median survival time of 2.4 mo from disease onset with mortality rates of 45% within 1 mo, 64% within 1 year, and 85% within 5 years. In addition to morbidity and mortality associated with HE, the socioeconomic and emotional burden on patients, families and the healthcare system are significant. Patients with HE who were discharged to nursing homes or rehabilitation centers increased from 2.6% to 23.3% between 1993 and 2009[15]. A study of 104 cirrhotic patients with previous history of HE was associated with unemployment, decline in financial status, and higher caregiver burden[16].

PATHOGENESIS OF HE

Cerebral dysfunction in liver failure is a varied phenomenon that can is termed HE. Numerous, complementary mechanisms have been stipulated to underlie HE. Ammonia and other toxins, typically filtered by the liver, play a role in conjunction with altered blood-brain transport of neurotransmitter precursors, metabolism of amino acid neurotransmitters, and cerebral glucose oxidation[17-19]. These alterations result in propagation of inhibitory signals, mediated by gamma-aminobutyric acid and serotonin, and inhibition of excitatory signals, mediated by glutamate and catecholamines[20,21]. The overall effect is neural inhibition. Other contributing mechanisms include neuroinflammation and altered gut flora[22,23].

DIAGNOSIS OF HE

The diagnosis of OHE is complex, beginning with a clinical recognition of the distinctive neurologic features of HE. The most common features are confusion or coma, asterixis, loss of fine motor skills, and hyperreflexia. Also required for the diagnosis of HE are the presence of underlying cirrhosis and exclusion of all other etiologies for neurologic or metabolic abnormalities. The portal-systemic encephalopathy score (PSE score; West Haven Criteria) is used to evaluate overall severity.

The two forms of HE include CHE, which has also been called subclinical encephalopathy or minimal encephalopathy (MHE) in the past, and OHE. In contrast to clinically symptomatic OHE, the diagnosis of CHE is made difficult by a lack of agreement in which tests should be utilized[4]. CHE was originally used as the label for patients who performed poorly on psychometric tests[11]. Noted to be more sensitive than observation; psychometric tests have since served as the standard for diagnosis of CHE[24]. Recent studies have also established

Table 1 Precipitating factors for hepatic encephalopathy

Noncompliance with lactulose
Dehydration from lactulose overuse
Gastrointestinal bleeding
Infection/sepsis
Medications (narcotics, sedatives, *etc.*)
Recreational drugs (cocaine, marijuana, *etc.*)
Transjugular intrahepatic portosystemic shunt
Alcohol intoxication
Electrolyte dysfunction
Constipation
Renal failure
Constipation

Table 2 Non-hepatic encephalopathy causes of altered mental status

Acidosis
Drug intoxication
Encephalitis
Hyperglycemia
Hypoglycemia
Hypocapnia
Hypoxia
Intracranial hematoma
Severe sepsis
Thyroid dysfunction
Uremia
Wernicke encephalopathy and korsakoff syndrome (vitamin B1 deficiency)

the usefulness of the neurophysiological critical flicker frequency (CFF) test in diagnosing CHE[25,26]. A study of 132 cirrhotic patients looking at inter-index agreement and predictive validity showed that CFF did not predict CHE reliably, while abnormal electroencephalogram and psychometric HE score (PHES) were predictive of subsequent CHE episodes[27]. The availability of neuroimaging to measure brain activity adds to the variability in tests used to detect CHE. In 2013, Zheng *et al*[28] used neuroimaging to report that the gray matter cerebral blood flow of CHE patients was significantly higher than that of non-HE patients. Arterial-spin labeling magnetic resonance imaging was used to characterize patterns of cerebral blood flow. Comparing CHE, non-HE, and control patients, they identified particular brain regions such as the right putamen where cutoff values for cerebral blood flow was 93.8% sensitive for characterization of CHE. The study was limited by its small sample size but further research can elucidate the potential for non-invasive CHE screening.

The presence of CHE is associated with higher re-hospitalization and lower survival rates as shown by Patidar *et al*[6] who followed 170 cirrhotic patients to assess the rates of CHE, hospitalizations, need for liver transplantation and survival. The authors reported that patients diagnosed with CHE *via* $\geqslant$ 2 psychometric tests were at a higher risk of developing OHE (HR = 2.1, P = 0.05), hospitalization (HR = 2.5, P = 0.002), needing liver transplantation or death (HR = 3.4, P = 0.01). Despite these observations, the clinical significance of CHE is under appreciated and diagnosis is not universally pursued. The PHES includes five pencil and paper tests, but is not widely used. In a survey of American Association for the Study of Liver Diseases physicians, 38% never tested for CHE, while only 14% tested for CHE in > 80% of patients with cirrhosis[29]. The major reason cited for lack of screening for CHE was time constraints (85%), followed by tests requiring trained personnel (75%) and lack of standardization in testing protocols (69%). Additional work is needed to establish reliable and user-friendly diagnostic criteria for CHE.

The clinical classification of HE can be established according to type of underlying disease, severity of manifestations, time course, and precipitating factors[30]. Based on underlying disease HE is further subdivided into: Type A: HE resulting from acute liver failure; Type B: HE resulting from portosystemic bypass or shunting; and Type C: Resulting from cirrhosis. Clinical manifestations of Type B and Type C are similar, whereas type A includes increased intracranial pressure and risk of cerebral herniation. Grading describes severity of manifestations, with CHE covering minimal HE and grade 1, and OHE covering grades 2-4. Time course is subdivided into three types: episodic, recurrent, and persistent. Lastly, the episode of HE is described as spontaneous or precipitated by specific factors (Table 1).

MANAGEMENT OF HE

The management of HE is mainly guided by clinical impression. In cases where HE is suspected, normal or slightly elevated blood ammonia levels cannot rule out a diagnosis of HE[31]. However, when evidence of underlying chronic liver disease is lacking, elevated blood ammonia levels may provide helpful prognostic information for patients in acute liver failure, or may serve as the basis for further evaluation of uncommon metabolic disorders such as urea cycle disorders[31]. Furthermore, other causes of altered mental status should be ruled out. Laboratory tests and brain imaging can diagnose other causes such as intracranial hematomas, thyroid dysfunction, electrolyte imbalances, and sepsis (Table 2).

Acute OHE

The approach to a patient with acute OHE involves empiric treatment for HE and a focus on identifying the precipitating factor (Figure 1)[32,33]. The precipitating factors may include gastrointestinal bleeding, infection, mind-altering medications (sedatives, narcotics, recreational agents, *etc.*), electrolyte imbalances, constipation, and renal failure (Table 1)[2]. Identification and treatment of precipitating factors of HE is critical. An early study found 90% of all cases of acute OHE were successfully resolved with prompt management of the precipitating factor(s) alone[34,35]. In one sample, spontaneous bacterial peritonitis was the most common precipitant of HE (20.5%), followed by constipation (18.3%), and urinary

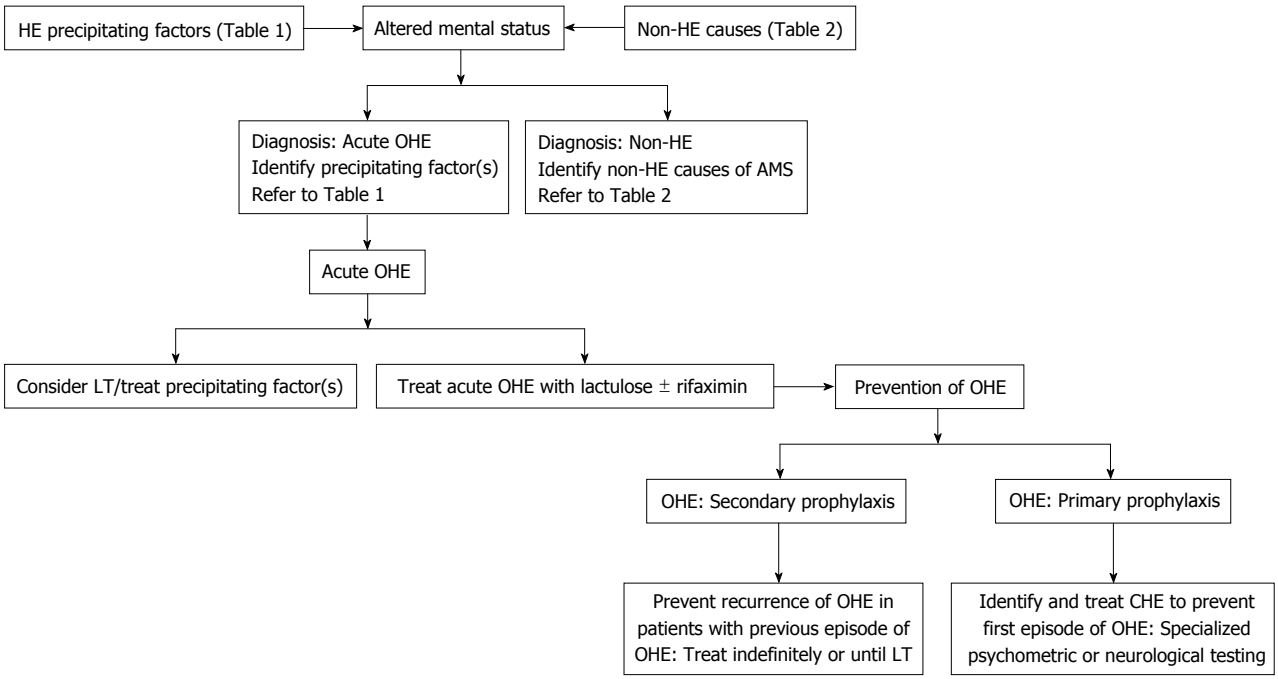

Figure 1 Algorithm to optimize the management of hepatic encephalopathy. HE: Hepatic encephalopathy; AMS: Altered mental status; OHE: Over hepatic encephalopathy; LT: Liver transplantation; CHE: Covert hepatic encephalopathy.

tract infection (15.3%)[36]. Therefore, an evaluation to search for a potential infection with a diagnostic paracentesis, blood cultures, urine culture and chest X-ray is warranted. In another study, infection (44%), gastrointestinal bleeding (38%), and constipation (38%) were the most common precipitating factors[37].

Currently, the preferred first-line treatment for an episode of acute OHE is lactulose, a poorly absorbed disaccharide that works in several ways. Lactulose decreases the blood ammonia concentration by promoting elimination of ammonia. Fermentation of cleaved lactulose by bacteria into lactic acid and hydrogen ions acidifies the colon, resulting in protonation of ammonia molecules into non-absorbable ammonium and facilitates movement of ammonia from the blood stream into the colon lumen[2]. Alteration to the gut flora also causes reduction of urease-producing bacteria. Lactitol, a second-generation unabsorbed dissacharide, is an alternative with an analogous mechanism of action to lactulose in the management of HE. Lactitol, which is formulated as a powder that can be dissolved in water for administration, is known to have superior taste properties and a defined laxative threshold. In addition, lactitol has demonstrated comparable efficacy with lactulose[38,39].

Rifaximin is an alternative first-line treatment option for OHE. It is a broad-spectrum antibiotic and it works by altering the bowel flora with net reduction in blood ammonia concentration. A 2013 study by Sharma *et al*[40] was the first prospective double-blind randomized control trial to compare rifaximin plus lactulose to lactulose alone. A total of 120 patients were randomized to the rifaximin group or placebo group, with lactulose dosed to achieve 2 to 3 semisoft stools per day. Treatment was administered through nasogastric tube and continued for a maximum of 10 d or until recovery. The rifaximin group demonstrated 76% reversal of OHE *vs* 44% reversal of OHE in the placebo group ($P = 0.004$). Mortality rates were lower in the rifaximin group when compared to the placebo group (24% *vs* 49%, $P \leqslant 0.05$). The lower mortality was due to a reduction in sepsis-related deaths. The authors hypothesize that the anti-bacterial effect of rifaximin inhibited the release of gut-related endotoxins and their transport into the bloodstream. In addition, this study demonstrated a shorter duration of hospital stay with rifaximin treatment (5.8 ± 3.4 d *vs* 8.2 ± 4.6 d, $P = 0.001$). The study was limited by lack of serial ammonia level monitoring, though this is not routinely performed in clinical practice.

Second-line agents for the treatment of acute OHE include neomycin and metronidazole. These antibiotics have fallen out of favor due to their systemic adverse effects such as ototoxicity and nephrotoxicity for neomycin, and neurotoxicity for metronidazole.

Branched-chain amino acids (BCAA) have been studied as therapeutic agents targeting HE. Randomized controlled trials have evaluated the use of BCAA-rich parenteral nutrition in patients with HE[41,42]. Based on a meta-analysis, patients receiving BCAA-rich infusions demonstrated superior mental recovery compared to controls[43]. The impact on mortality was mixed among the constituent trials, with three suggesting improved survival and two suggesting worse survival. Data regarding oral BCAA supplements is controversial. Whereas some trials have demonstrated significant benefit in mental performance with oral BCAA dietary supplementation, others have not revealed consistent benefit[44,45]. A

recent meta-analysis including 16 randomized controlled trials of both intravenous and oral BCAA supplementation determined that BCAA had a clinically beneficial effect on OHE (RR = 0.73, 95%CI: 0.61-0.88); however, there was no impact on mortality, quality of life, or nutritional parameters[46]. Increased risk of nausea and vomiting was noted. Les *et al*[45] found improvement in performance on neuropsychometric tests and an increase in mid-arm muscle circumference in patients randomized to BCAA. The design of this study was limited by its inability to discriminate between the effects of the quantity and quality of nitrogen intake. Marchesini *et al*[47] found improved benefits of BCCA in average hospital admission rate, nutritional parameters and liver function tests. The major limitation of this study was the withdrawal of a significantly greater number of patients form the BCAA arm compared to control study, primarily due to a combination of adverse effects and noncompliance.

Polyethylene glycol (PEG), a cathartic agent best known for management of constipation, results in increased fecal ammonia excretion. In a randomized controlled trial, PEG was compared head-to-head with lactulose among inpatients with HE. Among patients who were randomly assigned to four liters of PEG over four hours, there was greater improvement in HE after 24 h compared to those who were given three or more doses of lactulose, each 20 to 30 g, over 24 h. Furthermore, median time to resolution of HE was shorter in the PEG cohort[48]. The study was limited by its single-center design and lack of blinding.

Patients with hepatic decompensation in the setting of cirrhosis and HE have been noted to exhibit decreased serum zinc levels[49,50]. Based on the potential role of zinc in neurotransmission[51,52], zinc supplementation has been considered in the treatment or prevention of acute OHE. Yoshida *et al*[49] compared zinc serum levels in 10 patients with cirrhosis-related hepatic decompensation to patients with compensated cirrhosis and healthy volunteers. Zinc supplementation was found to decrease serum ammonia levels with an unknown impact on OHE clinical course. The study noted that the percentage increase in serum zinc levels was lower in cirrhotic patients. Reduced absorption and higher urinary excretion due to diuretic administration was deemed partially responsible for this observation. The relatively lower percentage increase in serum zinc levels could partially explain an earlier study of zinc-supplementation in 15 cirrhotic patients with chronic HE with no improvement in symptoms associated with chronic HE[53]. A 2013 systematic review of zinc in the treatment of HE found an improvement on the number connection test, but no reduction in recurrence of HE (RR = 0.64; 95%CI: 0.26-1.59)[54].

Secondary prophylaxis of OHE

Secondary prophylaxis is the prevention of recurrent episodes of OHE following resolution of acute OHE. Outpatient management after an episode of acute OHE includes improvement of daily function and evaluation for liver transplant candidacy. Secondary prophylaxis should continue indefinitely or until liver transplantation. In the 2009 study from New Delhi, India, Sharma *et al*[55] conducted an open-label randomized control trial to study recurrence of OHE in cirrhotic patients after recovery from a prior episode. Primary end point was development of OHE and follow-up ranged from 1 to 20 mo. The proportion of patients with recurrent episodes was found to be lower in the lactulose group compared to the placebo (19.6% *vs* 46.8%, $P = 0.001$). The most common adverse effects of lactulose were found to be diarrhea (23%), abdominal bloating (10%), and distaste (13%). In this study from New Delhi, all patients tolerated treatment and remained adherent to therapy. In the United States, adherence to lactulose therapy is a concern because many patients find the adverse effects difficult to tolerate[2]. These include severe diarrhea leading to dehydration, hypokalemia, hyponatremia, and other electrolyte disturbance. Other adverse effects related to lactulose therapy include bloating, flatulence, nausea, vomiting, and unpleasant sweet taste. Bajaj *et al*[56] studied adherence in 137 patients treated with lactulose therapy after initial episode of OHE Among the 103 patients who experienced a recurrent episode of OHE while on lactulose therapy, 38% were non-adherent, 54% were adherent, and 8% experienced lactulose-associated dehydration leading to recurrence. All non-adherent patients in the study developed recurrence, while 64% of those who were adherent to lactulose therapy developed OHE recurrence. Despite adherence to lactulose in this study, precipitating events such as sepsis and gastrointestinal bleeding resulted in recurrence of OHE. Multivariate regression demonstrated lactulose non-adherence (OR = 3.26) and MELD score (OR = 1.14) as predictive factors for recurrence. A retrospective study by Pantham *et al*[57] noted that lactulose non-compliance (39%), constipation (22%), opioids and benzodiazepines (17%), dehydration (16%), and infections (15%) as the leading precipitating factors for OHE.

Rifaximin can be used as an effective add-on therapy with lactulose to prevent OHE recurrence. After a second recurrent episode of OHE, rifaximin is recommended as an add-on therapy to lactulose for secondary prophylaxis[30]. Bass *et al*[58] performed a randomized, double-blind, placebo-controlled trial to study the effect of rifaximin plus lactulose *vs* lactulose and placebo in patients who were in remission from OHE. Patients were followed for the treatment period of 6 mo with the primary endpoint being time until a breakthrough episode of OHE. In the rifaximin group, 22.1% of patients reported a breakthrough episode *vs* 45.9% in the placebo group with a relative risk reduction of 58% for recurrence with rifaximin *vs* placebo. These data estimated the number-needed-to-treat is 4 patients for 6 mo to prevent one episode of OHE. Hospitalizations were also lower in the rifaximin group (13.6%) compared to the placebo group (22.6%). The background

use of lactulose in both groups was 91%. In another study, long term effects of rifaximin treatment were studied in 23 cirrhotic patients for up to 5 years, death, or liver transplantation[59]. Compared with controls, patients on rifaximin therapy experienced lower rates of variceal bleeding (35% *vs* 60%, P = 0.011), HE (32% *vs* 47%, P = 0.034), spontaneous bacterial peritonitis (5.5% *vs* 46%, P = 0.027), and hepatorenal syndrome (4.5% *vs* 51%, P = 0.037). The 5-year cumulative probability of survival in the rifaximin group was 61% *vs* 13.5% in the controls.

The concerns over bacterial-resistance with long-term rifaximin use is mitigated by studies demonstrating minimal long-term effects on intestinal flora[60]. *In vitro*, rifaximin is minimally absorbed and predominantly concentrated in the lumen of the gastrointestinal tract[61]. In a study that found rifaximin resistance in individuals treated with rifaximin 800 mg orally per day for 5 d, discontinuation of treatment for 1 to 2 wk resulted in reduction in resistance rates to less than 20% of intestinal flora[62]. Resistant strains were unable to maintain their presence in the intestinal flora[61]. In contrast to plasmid-mediated resistance often seen with aminoglycoside antibiotics[63], rifaximin resistance occurs through a chromosomal mutation affecting bacterial RNA synthesis.

Primary prophylaxis of OHE

Treatment of patients with CHE to prevent development of a first episode of OHE is referred to as primary prophylaxis and it has important prognostic implications[55]. Sharma *et al*[64] randomized 120 cirrhotic patients with no previous episodes of OHE to study primary prophylaxis with and without lactulose therapy. Patients were followed for 12 mo and the number of patients with CHE at baseline was similar in both groups. In the lactulose group, 11% of patients developed OHE *vs* 28% of patients in the no-lactulose group (P = 0.02). Reduction in overall mortality was found to be non-statistically significant (9% *vs* 20%, P = 0.16). Lactulose was found to improve CHE in 66% of patients. These findings demonstrate lactulose as an effective option for primary prophylaxis of OHE. Current guidelines do not recommend lactulose or rifaximin use as primary prophylactic therapy for the prevention of OHE following post-transjugular intrahepatic portosystemic shunt (TIPS). Lunia *et al*[65] performed an open-label, randomized-control trial in 2014 to study the role of probiotics in primary prophylaxis of OHE in patients with cirrhosis. At a single site in New Delhi, India, 160 patients were randomized to either probiotics or no treatment. Probiotics alter intestinal microflora by increasing non-urease producing bacteria, thus reducing the amount of ammonia production. In patients with MHE or CHE, an absolute risk reduction of 23.8% was observed, demonstrating a number-needed-to-treat of 4.2 to prevent an episode of OHE in patients with cirrhosis. In addition, a significant reduction in the number of patients with CHE was also observed in the probiotics treatment group compared to no treatment. However, the study was limited by potential treatment bias given lack of blinding the investigators to the administered treatments.

CONTROVERSIES AND FUTURE DIRECTIONS

An earlier study published in 2012 by Agrawal *et al*[66] also compared probiotic therapy to lactulose and no therapy for secondary prophylaxis. Using an open-label, randomized controlled study, patients who had recovered from an episode of OHE were followed until development of recurrent OHE or for 12 mo. Development of OHE in both the lactulose group and probiotics group were significantly lower than that of no treatment (26.2% and 34.4%, respectively, *vs* 56.9% P = 0.001, P = 0.02). Nevertheless, this study was limited by lack of blinding of the randomized intervention. The improved side effect profile of probiotics compared with lactulose offers a compelling alternative therapy for patients with difficulty tolerating lactulose therapy. In this study, all of the patients in the lactulose group were able to tolerate treatment despite the usual adverse effects; 26.4% diarrhea, 16.2% abdominal bloating, and 17.6% distaste to lactulose. In comparison, the most common adverse effect in the probiotics group was constipation (21.8%) and abdominal distention (14%). At 21.8%, the percentage of patients reporting constipation in the probiotics group paralleled that of the non-therapy group (21.5%). No patients in the probiotics group experienced fever, rash, or increased frequency of stools. While probiotic strains may differ and further studies are needed for validation, data demonstrating effective secondary prophylaxis with decreased risk of non-adherence are encouraging.

New screening test for CHE include Stroop smartphone application[67]. The Stroop application involves a test of mental speed and flexibility that asks the user to correctly identify the color of the presented stimuli. The stimuli can be presented in three forms, a neutral non-verbal cue such as "###" in red ink, a congruent stimuli such as "red" written in red ink, or an incongruent cue such as "red" in green ink. Bajaj *et al*[67] studied Stroop-app reported scores in 125 cirrhotic patients and 134 controls. Performance was significantly impaired in patients with CHE compared to patients without CHE, as measured using psychometric tests. Furthermore, scores correlated with MELD scores and were worst in those with prior OHE episodes. The study was limited by a relatively high educational status in patients. However, the findings are promising for quick, valid, and reliable screening for CHE.

CONCLUSION

Management of acute OHE involves institution of lactulose, preferred first-line therapy. In addition, prompt recognition and treatment of precipitating factors

is critical as it may result in complete resolution of acute episode of OHE. Treatment goals include improvement of daily functioning, evaluation for liver transplantation, and prevention of OHE recurrence. For secondary prophylaxis, intolerance to indefinite lactulose therapy may lead to non-adherence and has been identified as a precipitating factor for recurrent OHE. Rifaximin is an effective add-on therapy to lactulose for treatment and prevention of recurrent OHE. Recent studies have demonstrated comparable efficacy of probiotic therapy to lactulose use in both primary prophylaxis and secondary prophylaxis.

REFERENCES

1 **Schuppan D**, Afdhal NH. Liver cirrhosis. *Lancet* 2008; **371**: 838-851 [PMID: 18328931 DOI: 10.1016/S0140-6736(08)60383-9]
2 **Khungar V**, Poordad F. Management of overt hepatic encephalopathy. *Clin Liver Dis* 2012; **16**: 73-89 [PMID: 22321466 DOI: 10.1016/j.cld.2012.03.009]
3 **Lefton HB**, Rosa A, Cohen M. Diagnosis and epidemiology of cirrhosis. *Med Clin North Am* 2009; **93**: 787-799, vii [PMID: 19577114 DOI: 10.1016/j.mcna.2009.03.002]
4 **Ferenci P**, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002; **35**: 716-721 [PMID: 11870389 DOI: 10.1053/jhep.2002.31250]
5 **Stepanova M**, Mishra A, Venkatesan C, Younossi ZM. In-hospital mortality and economic burden associated with hepatic encephalopathy in the United States from 2005 to 2009. *Clin Gastroenterol Hepatol* 2012; **10**: 1034-1041.e1 [PMID: 22642955 DOI: 10.1016/j.cgh.2012.05.016]
6 **Patidar KR**, Thacker LR, Wade JB, Sterling RK, Sanyal AJ, Siddiqui MS, Matherly SC, Stravitz RT, Puri P, Luketic VA, Fuchs M, White MB, Noble NA, Unser AB, Gilles H, Heuman DM, Bajaj JS. Covert hepatic encephalopathy is independently associated with poor survival and increased risk of hospitalization. *Am J Gastroenterol* 2014; **109**: 1757-1763 [PMID: 25178701 DOI: 10.1038/ajg.2014.264]
7 **Cordoba J**, Ventura-Cots M, Simón-Talero M, Amorós À, Pavesi M, Vilstrup H, Angeli P, Domenicali M, Ginés P, Bernardi M, Arroyo V. Characteristics, risk factors, and mortality of cirrhotic patients hospitalized for hepatic encephalopathy with and without acute-on-chronic liver failure (ACLF). *J Hepatol* 2014; **60**: 275-281 [PMID: 24128414 DOI: 10.1016/j.jhep.2013.10.004]
8 **Saunders JB**, Walters JR, Davies AP, Paton A. A 20-year prospective study of cirrhosis. *Br Med J* (Clin Res Ed) 1981; **282**: 263-266 [PMID: 6779978 DOI: 10.1136/bmj.282.6260.263]
9 **Jepsen P**, Ott P, Andersen PK, Sørensen HT, Vilstrup H. Clinical course of alcoholic liver cirrhosis: a Danish population-based cohort study. *Hepatology* 2010; **51**: 1675-1682 [PMID: 20186844 DOI: 10.1002/hep.23500]
10 **Groeneweg M**, Moerland W, Quero JC, Hop WC, Krabbe PF, Schalm SW. Screening of subclinical hepatic encephalopathy. *J Hepatol* 2000; **32**: 748-753 [PMID: 10845661 DOI: 10.1016/S0168-8278(00)80243-3]
11 **Gitlin N**, Lewis DC, Hinkley L. The diagnosis and prevalence of subclinical hepatic encephalopathy in apparently healthy, ambulant, non-shunted patients with cirrhosis. *J Hepatol* 1986; **3**: 75-82 [PMID: 3745889 DOI: 10.1016/S0168-8278(86)80149-0]
12 **Bustamante J**, Rimola A, Ventura PJ, Navasa M, Cirera I, Reggiardo V, Rodés J. Prognostic significance of hepatic encephalopathy in patients with cirrhosis. *J Hepatol* 1999; **30**: 890-895 [PMID: 10365817 DOI: 10.1016/S0168-8278(99)80144-5]
13 **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]
14 **Fichet J**, Mercier E, Genée O, Garot D, Legras A, Dequin PF, Perrotin D. Prognosis and 1-year mortality of intensive care unit patients with severe hepatic encephalopathy. *J Crit Care* 2009; **24**: 364-370 [PMID: 19327960 DOI: 10.1016/j.jcrc.2009.01.008]
15 **Neff GW**, Kemmer N, Duncan C, Alsina A. Update on the management of cirrhosis - focus on cost-effective preventative strategies. *Clinicoecon Outcomes Res* 2013; **5**: 143-152 [PMID: 23626470 DOI: 10.2147/CEOR.S30675]
16 **Bajaj JS**, Wade JB, Gibson DP, Heuman DM, Thacker LR, Sterling RK, Stravitz RT, Luketic V, Fuchs M, White MB, Bell DE, Gilles H, Morton K, Noble N, Puri P, Sanyal AJ. The multi-dimensional burden of cirrhosis and hepatic encephalopathy on patients and caregivers. *Am J Gastroenterol* 2011; **106**: 1646-1653 [PMID: 21556040 DOI: 10.1038/ajg.2011.157]
17 **Aldridge DR**, Tranah EJ, Shawcross DL. Pathogenesis of hepatic encephalopathy: role of ammonia and systemic inflammation. *J Clin Exp Hepatol* 2015; **5**: S7-S20 [PMID: 26041962 DOI: 10.1016/j.jceh.2014.06.004]
18 **Grippon P**, Le Poncin Lafitte M, Boschat M, Wang S, Faure G, Dutertre D, Opolon P. Evidence for the role of ammonia in the intracerebral transfer and metabolism of tryptophan. *Hepatology* 1986; **6**: 682-686 [PMID: 2426170 DOI: 10.1002/hep.1840060424]
19 **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]
20 **Cauli O**, Rodrigo R, Llansola M, Montoliu C, Monfort P, Piedrafita B, El Mlili N, Boix J, Agustí A, Felipo V. Glutamatergic and gabaergic neurotransmission and neuronal circuits in hepatic encephalopathy. *Metab Brain Dis* 2009; **24**: 69-80 [PMID: 19085094 DOI: 10.1007/s11011-008-9115-4]
21 **Yurdaydin C**, Hörtnagl H, Steindl P, Zimmermann C, Pifl C, Singer EA, Roth E, Ferenci P. Increased serotoninergic and noradrenergic activity in hepatic encephalopathy in rats with thioacetamide-induced acute liver failure. *Hepatology* 1990; **12**: 695-700 [PMID: 1698704 DOI: 10.1002/hep.1840120413]
22 **Rodrigo R**, Cauli O, Gomez-Pinedo U, Agusti A, Hernandez-Rabaza V, Garcia-Verdugo JM, Felipo V. Hyperammonemia induces neuroinflammation that contributes to cognitive impairment in rats with hepatic encephalopathy. *Gastroenterology* 2010; **139**: 675-684 [PMID: 20303348 DOI: 10.1053/j.gastro.2010.03.040]
23 **Bajaj JS**, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014; **60**: 940-947 [PMID: 24374295 DOI: 10.1016/j.jhep.2013.12.019]
24 **Riggio O**, Ridola L, Pasquale C, Pentassuglio I, Nardelli S, Moscucci F, Merli M, Montagnese S, Amodio P, Merkel C. A simplified psychometric evaluation for the diagnosis of minimal hepatic encephalopathy. *Clin Gastroenterol Hepatol* 2011; **9**: 613-616.e1 [PMID: 21440091 DOI: 10.1016/j.cgh.2011.03.017]
25 **Kircheis G**, Wettstein M, Timmermann L, Schnitzler A, Häussinger D. Critical flicker frequency for quantification of low-grade hepatic encephalopathy. *Hepatology* 2002; **35**: 357-366 [PMID: 11826409 DOI: 10.1053/jhep.2002.30957]
26 **Romero-Gómez M**, Córdoba J, Jover R, del Olmo JA, Ramírez M, Rey R, de Madaria E, Montoliu C, Nuñez D, Flavia M, Compañy L, Rodrigo JM, Felipo V. Value of the critical flicker frequency in patients with minimal hepatic encephalopathy. *Hepatology* 2007; **45**: 879-885 [PMID: 17393525 DOI: 10.1002/hep.21586]
27 **Montagnese S**, Balistreri E, Schiff S, De Rui M, Angeli P, Zanus G, Cillo U, Bombonato G, Bolognesi M, Sacerdoti D, Gatta A, Merkel C, Amodio P. Covert hepatic encephalopathy: agreement and predictive validity of different indices. *World J Gastroenterol* 2014; **20**: 15756-15762 [PMID: 25400460 DOI: 10.3748/wjg.v20.i42.15756]

28 **Zheng G**, Zhang LJ, Zhong J, Wang Z, Qi R, Shi D, Lu GM. Cerebral blood flow measured by arterial-spin labeling MRI: a useful biomarker for characterization of minimal hepatic encephalopathy in patients with cirrhosis. *Eur J Radiol* 2013; **82**: 1981-1988 [PMID: 23849331 DOI: 10.1016/j.ejrad.2013.06.002]

29 **Bajaj JS**, Etemadian A, Hafeezullah M, Saeian K. Testing for minimal hepatic encephalopathy in the United States: An AASLD survey. *Hepatology* 2007; **45**: 833-834 [PMID: 17326210 DOI: 10.1002/hep.21515]

30 **Vilstrup H**, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, Weissenborn K, Wong P. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. *Hepatology* 2014; **60**: 715-735 [PMID: 25042402 DOI: 10.1002/hep.27210]

31 **Elgouhari HM**, O'Shea R. What is the utility of measuring the serum ammonia level in patients with altered mental status? *Cleve Clin J Med* 2009; **76**: 252-254 [PMID: 19339641 DOI: 10.3949/ccjm.76a.08072]

32 **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]

33 **Prakash R**, Mullen KD. Mechanisms, diagnosis and management of hepatic encephalopathy. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 515-525 [PMID: 20703237 DOI: 10.1038/nrgastro.2010.116]

34 **Strauss E**, Tramote R, Silva EP, Caly WR, Honain NZ, Maffei RA, de Sá MF. Double-blind randomized clinical trial comparing neomycin and placebo in the treatment of exogenous hepatic encephalopathy. *Hepatogastroenterology* 1992; **39**: 542-545 [PMID: 1483668]

35 **Khungar V**, Poordad F. Hepatic encephalopathy. *Clin Liver Dis* 2012; **16**: 301-320 [PMID: 22541700 DOI: 10.1016/j.cld.2011.12.007]

36 **Mumtaz K**, Ahmed US, Abid S, Baig N, Hamid S, Jafri W. Precipitating factors and the outcome of hepatic encephalopathy in liver cirrhosis. *J Coll Physicians Surg Pak* 2010; **20**: 514-518 [PMID: 20688015]

37 **Maqsood S**, Saleem A, Iqbal A, Butt JA. Precipitating factors of hepatic encephalopathy: experience at Pakistan Institute of Medical Sciences Islamabad. *J Ayub Med Coll Abbottabad* 2006; **18**: 58-62 [PMID: 17591012]

38 **Morgan MY**, Hawley KE. Lactitol vs. lactulose in the treatment of acute hepatic encephalopathy in cirrhotic patients: a double-blind, randomized trial. *Hepatology* 1987; **7**: 1278-1284 [PMID: 3315932 DOI: 10.1002/hep.1840070617]

39 **Blanc P**, Daures JP, Rouillon JM, Peray P, Pierrugues R, Larrey D, Gremy F, Michel H. Lactitol or lactulose in the treatment of chronic hepatic encephalopathy: results of a meta-analysis. *Hepatology* 1992; **15**: 222-228 [PMID: 1531204 DOI: 10.1002/hep.1840150209]

40 **Sharma BC**, Sharma P, Lunia MK, Srivastava S, Goyal R, Sarin SK. A randomized, double-blind, controlled trial comparing rifaximin plus lactulose with lactulose alone in treatment of overt hepatic encephalopathy. *Am J Gastroenterol* 2013; **108**: 1458-1463 [PMID: 23877348 DOI: 10.1038/ajg.2013.219]

41 **Cerra FB**, Cheung NK, Fischer JE, Kaplowitz N, Schiff ER, Dienstag JL, Bower RH, Mabry CD, Leevy CM, Kiernan T. Disease-specific amino acid infusion (F080) in hepatic encephalopathy: a prospective, randomized, double-blind, controlled trial. *JPEN J Parenter Enteral Nutr* 1985; **9**: 288-295 [PMID: 3892073 DOI: 10.1177/0148607185009003288]

42 **Michel H**, Bories P, Aubin JP, Pomier-Layrargues G, Bauret P, Bellet-Herman H. Treatment of acute hepatic encephalopathy in cirrhotics with a branched-chain amino acids enriched versus a conventional amino acids mixture. A controlled study of 70 patients. *Liver* 1985; **5**: 282-289 [PMID: 4079669 DOI: 10.1111/j.1600-0676.1985.tb00250.x]

43 **Naylor CD**, O'Rourke K, Detsky AS, Baker JP. Parenteral nutrition with branched-chain amino acids in hepatic encephalopathy. A meta-analysis. *Gastroenterology* 1989; **97**: 1033-1042 [PMID: 2506095]

44 **Marchesini G**, Dioguardi FS, Bianchi GP, Zoli M, Bellati G, Roffi L, Martines D, Abbiati R. Long-term oral branched-chain amino acid treatment in chronic hepatic encephalopathy. A randomized double-blind casein-controlled trial. The Italian Multicenter Study Group. *J Hepatol* 1990; **11**: 92-101 [PMID: 2204661 DOI: 10.1016/0168-8278(90)90278-Y]

45 **Les I**, Doval E, García-Martínez R, Planas M, Cárdenas G, Gómez P, Flavià M, Jacas C, Mínguez B, Vergara M, Soriano G, Vila C, Esteban R, Córdoba J. Effects of branched-chain amino acids supplementation in patients with cirrhosis and a previous episode of hepatic encephalopathy: a randomized study. *Am J Gastroenterol* 2011; **106**: 1081-1088 [PMID: 21326220 DOI: 10.1038/ajg.2011.9]

46 **Gluud LL**, Dam G, Les I, Córdoba J, Marchesini G, Borre M, Aagaard NK, Vilstrup H. Branched-chain amino acids for people with hepatic encephalopathy. *Cochrane Database Syst Rev* 2015; **9**: CD001939 [PMID: 26377410 DOI: 10.1002/14651858.cd001939.pub2]

47 **Marchesini G**, Bianchi G, Merli M, Amodio P, Panella C, Loguercio C, Rossi Fanelli F, Abbiati R. Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. *Gastroenterology* 2003; **124**: 1792-1801 [PMID: 12806613 DOI: 10.1016/S0016-5085(03)00323-8]

48 **Rahimi RS**, Singal AG, Cuthbert JA, Rockey DC. Lactulose vs polyethylene glycol 3350--electrolyte solution for treatment of overt hepatic encephalopathy: the HELP randomized clinical trial. *JAMA Intern Med* 2014; **174**: 1727-1733 [PMID: 25243839 DOI: 10.1001/jamainternmed.2014.4746]

49 **Yoshida Y**, Higashi T, Nouso K, Nakatsukasa H, Nakamura SI, Watanabe A, Tsuji T. Effects of zinc deficiency/zinc supplementation on ammonia metabolism in patients with decompensated liver cirrhosis. *Acta Med Okayama* 2001; **55**: 349-355 [PMID: 11779097]

50 **Loomba V**, Pawar G, Dhar KL, Setia MS. Serum zinc levels in hepatic encephalopathy. *Indian J Gastroenterol* 1995; **14**: 51-53 [PMID: 7797277]

51 **Ebadi M**, Iversen PL, Hao R, Cerutis DR, Rojas P, Happe HK, Murrin LC, Pfeiffer RF. Expression and regulation of brain metallothionein. *Neurochem Int* 1995; **27**: 1-22 [PMID: 7655341 DOI: 10.1016/0197-0186(94)00164-P]

52 **Agus ZS**, Dukes ID, Morad M. Divalent cations modulate the transient outward current in rat ventricular myocytes. *Am J Physiol* 1991; **261**: C310-C318 [PMID: 1872373]

53 **Riggio O**, Ariosto F, Merli M, Caschera M, Zullo A, Balducci G, Ziparo V, Pedretti G, Fiaccadori F, Bottari E. Short-term oral zinc supplementation does not improve chronic hepatic encephalopathy. Results of a double-blind crossover trial. *Dig Dis Sci* 1991; **36**: 1204-1208 [PMID: 1893805 DOI: 10.1007/BF01307509]

54 **Chavez-Tapia NC**, Cesar-Arce A, Barrientos-Gutiérrez T, Villegas-López FA, Méndez-Sanchez N, Uribe M. A systematic review and meta-analysis of the use of oral zinc in the treatment of hepatic encephalopathy. *Nutr J* 2013; **12**: 74 [PMID: 23742732 DOI: 10.1186/1475-2891-12-74]

55 **Sharma BC**, Sharma P, Agrawal A, Sarin SK. Secondary prophylaxis of hepatic encephalopathy: an open-label randomized controlled trial of lactulose versus placebo. *Gastroenterology* 2009; **137**: 885-891, 891.e1 [PMID: 19501587]

56 **Bajaj JS**, Sanyal AJ, Bell D, Gilles H, Heuman DM. Predictors of the recurrence of hepatic encephalopathy in lactulose-treated patients. *Aliment Pharmacol Ther* 2010; **31**: 1012-1017 [PMID: 20136802 DOI: 10.1111/j.1365-2036.2010.04257.x]

57 **Pantham G**, Waghray N, Prakash R, Biyyani RSRS, Mullen KD. Overt Hepatic Encephalopathy in Cirrhosis: Influence of Multiple Clinical Precipitants Resulting in Hospitalization. *Gastroenterology* 2012; **142**: S-950 [Abstract] [DOI: 10.1016/S0016-5085(12)63683-X]

58 **Bass NM**, Mullen KD, Sanyal A, Poordad F, Neff G, Leevy CB, Sigal S, Sheikh MY, Beavers K, Frederick T, Teperman L, Hillebrand D, Huang S, Merchant K, Shaw A, Bortey E, Forbes WP. Rifaximin treatment in hepatic encephalopathy. *N Engl J Med* 2010; **362**: 1071-1081 [PMID: 20335583 DOI: 10.1056/NEJMoa0907893]

59 **Vlachogiannakos J**, Viazis N, Vasianopoulou P, Vafiadis I,

Karamanolis DG, Ladas SD. Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis. *J Gastroenterol Hepatol* 2013; **28**: 450-455 [PMID: 23216382 DOI: 10.1111/jgh.12070]

60 **Jiang ZD**, DuPont HL. Rifaximin: in vitro and in vivo antibacterial activity--a review. *Chemotherapy* 2005; **51** Suppl 1: 67-72 [PMID: 15855749 DOI: 10.1159/000081991]

61 **Gerard L**, Garey KW, DuPont HL. Rifaximin: a nonabsorbable rifamycin antibiotic for use in nonsystemic gastrointestinal infections. *Expert Rev Anti Infect Ther* 2005; **3**: 201-211 [PMID: 15918778 DOI: 10.1586/14787210.3.2.201]

62 **De Leo C**, Eftimiadi C, Schito GC. Rapid disappearance from the intestinal tract of bacteria resistant to rifaximin. *Drugs Exp Clin Res* 1986; **12**: 979-981 [PMID: 3569008]

63 **Neu HC**. The crisis in antibiotic resistance. *Science* 1992; **257**: 1064-1073 [PMID: 1509257 DOI: 10.1126/science.257.5073.1064]

64 **Sharma P**, Sharma BC, Agrawal A, Sarin SK. Primary prophylaxis of overt hepatic encephalopathy in patients with cirrhosis: an open labeled randomized controlled trial of lactulose versus no lactulose. *J Gastroenterol Hepatol* 2012; **27**: 1329-1335 [PMID: 22606978 DOI: 10.1111/j.1440-1746.2012.07186.x]

65 **Lunia MK**, Sharma BC, Sharma P, Sachdeva S, Srivastava S. Probiotics prevent hepatic encephalopathy in patients with cirrhosis: a randomized controlled trial. *Clin Gastroenterol Hepatol* 2014; **12**: 1003-1008.e1 [PMID: 24246768 DOI: 10.1016/j.cgh.2013.11.006]

66 **Agrawal A**, Sharma BC, Sharma P, Sarin SK. Secondary prophylaxis of hepatic encephalopathy in cirrhosis: an open-label, randomized controlled trial of lactulose, probiotics, and no therapy. *Am J Gastroenterol* 2012; **107**: 1043-1050 [PMID: 22710579 DOI: 10.1038/ajg.2012.113]

67 **Bajaj JS**, Thacker LR, Heuman DM, Fuchs M, Sterling RK, Sanyal AJ, Puri P, Siddiqui MS, Stravitz RT, Bouneva I, Luketic V, Noble N, White MB, Monteith P, Unser A, Wade JB. The Stroop smartphone application is a short and valid method to screen for minimal hepatic encephalopathy. *Hepatology* 2013; **58**: 1122-1132 [PMID: 23389962 DOI: 10.1002/hep.26309]

P- Reviewer: Arai M, Karatapanis S, Lens S, Shimizu Y, Smith MA
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.i29.2880

World J Hepatol 2015 December 18; 7(29): 2880-2889
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C Virus

Oxidative stress modulation in hepatitis C virus infected cells

Sonia A Lozano-Sepulveda, Owen L Bryan-Marrugo, Carlos Cordova-Fletes, Maria C Gutierrez-Ruiz, Ana M Rivas-Estilla

Sonia A Lozano-Sepulveda, Owen L Bryan-Marrugo, Carlos Cordova-Fletes, Ana M Rivas-Estilla, Department of Biochemistry and Molecular Medicine, School of Medicine, Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon 64460, Mexico

Maria C Gutierrez-Ruiz, Department of Health Sciences, Biological Sciences and Health Division, School of Biological Sciences, Universidad Autonoma Metropolitana, Distrito Federal 09340, Mexico

Author contributions: All the authors contributed to the preparation of this manuscript.

Supported by The CONACYT, No. CB-2011-1-58781 to Ana M Rivas-Estilla (partially); and Red CA Fisiopatología de Enfermedades Hepáticas 2015.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Correspondence to: Ana M Rivas-Estilla, PhD, Department of Biochemistry and Molecular Medicine, School of Medicine, Universidad Autonoma de Nuevo Leon, Av. Francisco I. Madero and Eduardo Aguirre Pequeño, Mitras Centro, Monterrey, Nuevo Leon 64460, Mexico. amrivas1@yahoo.ca
Telephone: +52-81-83294174
Fax: +52-81-83337747

Received: April 29, 2015
Peer-review started: May 8, 2015
First decision: September 8, 2015
Revised: November 7, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: December 18, 2015

Abstract

Hepatitis C virus (HCV) replication is associated with the endoplasmic reticulum, where the virus can induce cellular stress. Oxidative cell damage plays an important role in HCV physiopathology. Oxidative stress is triggered when the concentration of oxygen species in the extracellular or intracellular environment exceeds antioxidant defenses. Cells are protected and modulate oxidative stress through the interplay of intracellular antioxidant agents, mainly glutathione system (GSH) and thioredoxin; and antioxidant enzyme systems such as superoxide dismutase, catalase, GSH peroxidase, and heme oxygenase-1. Also, the use of natural and synthetic antioxidants (vitamin C and E, N-acetylcysteine, glycyrrhizin, polyenylphosphatidyl choline, mitoquinone, quercetin, S-adenosylmethionine and silymarin) has already shown promising results as co-adjuvants in HCV therapy. Despite all the available information, it is not known how different agents with antiviral activity can interfere with the modulation of the cell redox state induced by HCV and decrease viral replication. This review describes an evidence-based consensus on molecular mechanisms involved in HCV replication and their relationship with cell damage induced by oxidative stress generated by the virus itself and cell antiviral machinery. It also describes some molecules that modify the levels of oxidative stress in HCV-infected cells.

Key words: Hepatitis C virus; Oxidative stress; Reactive oxygen species; Vitamin E; Antioxidants; Glycyrrhizin; S-adenosylmethionine; N-acetylcysteine; Silymarin

Core tip: This review focuses on the available findings regarding the relationship between viral and cellular proteins and the resulting regulation of oxidative stress. To understand the liver damage induced by hepatitis C virus and its persistence is important to know how the cell regulatory systems involved in the production and elimination of reactive oxygen species (ROS) benefit the replication of the virus, as well as their participation in the cell defense mechanisms and immune perturbation following the infection. We must also consider the involvement of ROS in signaling pathways that induce viral replication and its implication in antiviral therapies.

Lozano-Sepulveda SA, Bryan-Marrugo OL, Cordova-Fletes C, Gutierrez-Ruiz MC, Rivas-Estilla AM. Oxidative stress modulation in hepatitis C virus infected cells. *World J Hepatol* 2015; 7(29): 2880-2889 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2880.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2880

INTRODUCTION

The number of deaths as a result of liver cirrhosis and cancer rose by 50 million in the last two decades, according to the first-ever World Health Organization study on liver disease mortality. Liver cancer is largely a problem in developing countries accounting for 83% of the estimated 782000 new cases occurred in 2012. Liver cancer is the fifth most common type of cancer reported in men and the ninth in women. Liver cancer is the second leading cause of death by cancer around the world, and it is considered to be responsible for nearly 746000 deaths in 2012 (9.1% of the total). The prognosis for liver cancer patients is very poor (overall ratio of mortality to incidence of 0.95), and as such, the geographical patterns in incidence and mortality are frighteningly similar[1]. The highest hepatitis C virus (HCV) prevalence worldwide are reported in the Western Pacific, Southeast Asia, and Africa regions, with around 120 million people infected who have limited access to the new anti-HCV drugs. It is important to mention that most of the people with HCV infection do not live in North America, Europe, or Japan (approximately 3.2 million people in the United States are chronically infected with HCV), which are the primary market for current anti-HCV drugs. In addition, it is well known that low- and middle-income countries, like Egypt (22%) and China (3.2%), report the highest prevalence. Although HCV genotypes 1 and 3 are more prevalent in most countries regardless of economic status, the largest proportions of genotypes, 4 and 5 are in low-income countries[2-4]. The total population infected with HCV in Latin America in 2010 was estimated at 7.8 million, of which 4.6 million are infected with genotype 1 (approximately 70% with subtypes 1a and 1b) and genotypes 2a, 2b, 2c and 3a which constitute 25% of the remaining genotypes, whereas the other genotypes 4 to 7 are less common[5]. It is estimated that up to 3% of the global population (approximately 150-170 million persons) is infected with chronic hepatitis C. HCV has been demonstrated to be the leading cause of chronic liver disease[4], cirrhosis and hepatocellular carcinoma (HCC), and is the underlying cause of over 475000 annual deaths, worldwide[6].

There is still no anti-HCV vaccine available and until recently, the only approved treatment, based on a combination of pegylated interferon (PEG-INF) and ribavirin (RBV), was partially effective in treated patients and also had considerable side effects in most of the patients[7]. Recently, after several years of research, new therapies that specifically block the virus have been developed. In 2011, the first anti-HCV specific drugs were approved for clinical use. Since then, a new era began for HCV infected patients with the founding of new direct acting agents (DAAs)[8,9]. However, the availability and accessibility of new protease inhibitors, telaprevir, boceprevir, simeprevir; and the recently approved RNA polymerase inhibitor sofosbuvir, depends on the region where patients are located and their access through government health programs, since the costs of DAAS are high. Increasing response rates are expected in the near future due to the development of numerous new DAAs and host-targeted drugs active against HCV[10-12]. There are constant efforts to identify new cheaper and effective antiviral molecules through other therapeutic approaches. Recent antioxidant compounds reports highlight their antiviral activity against HCV and post them as anti-HCV co-adjuvants that can improve the effectiveness of treatment as well as shorten the period and reduce the overall cost of the therapy.

HCV MOLECULAR BIOLOGY

HCV is a positive-strand RNA virus, classified into the *Hepacivirus* genus (*Flaviviridae* family), with a genome around 9600 nucleotides in length. The single strand genome carries a 50 and 30 nt length in non-coding region (NCR) flanking a single open-reading frame, encoding a single polyprotein of around 3009 aminoacid residues. Interestingly, this 50 NCR forms an internal ribosome entry site that leads the translation of the viral polyprotein, which in turn is cleaved by both viral and host proteases, in order to produce the structural (core, E1 and E2) and non-structural (NS2 to NS5B) viral proteins. There are numerous reports about the interaction between viral and cellular proteins to facilitate replication of the virus, however more information is needed to understand their role in the pathophysiology of the disease[13,14].

HCV AND OXIDATIVE STRESS

It is well known that most of the viral replication cycle takes place associated with the endoplasmic reticulum

(ER), which encourages cellular stress. This effect has been associated with a specific regulation of the virus replication cycle[15]. It is reported that viral proteins such as HCV-core, E1, and NS3 can modulate and inactivate mitochondrial respiratory chain enzymes and in turn unshackle blockade of the electrons, disruption of mitochondrial transmembrane potential, and electron leakage inducing an increase of intracellular reactive oxygen species (ROS) levels[16,17]. Together, all these molecular events impair mitochondrial and cellular signaling pathways.

The generation of oxygen species in several biological processes exceeds the capacity of antioxidant systems. A greater imbalance in the pro-oxidant and antioxidant ratio in benefit of the pro-oxidant can induces cellular damage[15,18].

Several reports demonstrate that expression of HCV proteins increase ROS levels due to activation of several pathways including the activation of mitogen-activated protein kinase (MAPK), ER response, Nuclear factor κB (NF-κB) and calcium signaling[19]. In the cytoplasm, the ER is actively involved in the induction of ROS, since excessive production of viral proteins induces the unfolded proteins response which is accompanied by calcium release. Then, the mitochondria absorbed the released calcium quickly, giving as a result an elevation of ROS. The constant production of ROS is a challenge that the cell has to overcome to survive. Normally, cells have sufficient capacity to balance the demand for antioxidant compounds and enzymatic antioxidant systems with the production of ROS[20], then modulating or blocking tissue damage. A biological condition that disrupts this balance, can induce accumulation of ROS and oxidative stress. Among the mechanisms involved in cell protection against oxidative stress damage we can found several intracellular antioxidant agents (glutathione, S-adenosil-methionine and thioredoxin), and antioxidant enzymes (superoxide dismutase, catalase, GSH peroxidase and heme oxygenase-1)[21-23].

In fact, there are large amount of data demonstrating that several intracellular signaling pathways are altered by HCV proteins in order to promote replication. The HCV can do accomplish this by finely regulating the oxido-reductive state of the host cell. MAPK and phosphoinositide-3 kinase (PI3K)/Akt signaling pathways are critical controllers of HCV replication and in turn, these pathways are modulated by phosphorylation cascades and oxidative stress. The first evidences that a virus could induce oxidative stress by increasing ROS levels, were published by Peterhans *et al*[24] in 1979. They demonstrated that the infection of mouse splenocytes with Sendai virus induced an increase of chemiluminescence levels, which it meant that the luminol had been oxidized by ROS in the experimental setting. They demonstrated that virus inactivated with UV light are able to generate ROS too, whereas virus inactivated by heating could not generate ROS, suggesting that viral structure conformation are mediating this action[24].

It has been shown that oxidative damage has a major role in HCV-induced liver damage, *via* ROS accumulation, produced from HCV infected cells and infiltrating immune cells[25,26]. The high amount of either HCV-structural or non structural proteins induce oxidative stress and disrupt the antioxidant equilibrium into the cells. In addition, a direct interaction of the HCV-core protein with mitochondria is able to decrease the mitochondrial NADPH levels, reducing the activity of the electron transport complex Ⅰ and then increasing generation of ROS. It has been shown that HCV-core protein overexpression diminished GSH levels and induced GSSG levels. In addition, an expected compensatory response increases the major enzymatic antioxidant elements such as GSH reductasa, catalase, MnSOD and heme oxygenase-1 (HO-1), in infected cells[27].

It is reported that expression of viral core protein induces the expression of p21-waf1, activates the NF-κB, bind to p53 and induces oxidative stress markers (ROS and peroxidated lipids)[28-30]. There is a differential effect on the enzymatic antioxidant systems in response to the presence of different viral proteins. Abdalla *et al*[31] reported that HCV infection reduced *in vivo* hepatic expression of HO-1 and *in vitro* HCV-core protein expression causes a similar effect, but this effect is not detected in superoxide dismutase (SOD) and catalase enzymes. This group also studied the effect of HCV-core expression in regulation of GSH levels. They findings demonstrated that GSH levels were diminished upon HCV-core expression, but at the same time oxidized GSH (GSSG) levels were undetectable. This finding led them to evaluate if thioredoxin (Trx) was also regulated by HCV-core protein, and they found that in fact Trx was also oxidated. As expected there is a compensatory induction of anti-oxidant defenses in cells expressing viral proteins[31]. On the other hand, expression of non-structural protein 5A (NS5A) upregulates mitochondrial ROS levels because it induces the release of calcium from ER, binds to PI3K and triggers NF-κB signaling[19,32-34]. This event is followed by a translocation of NF-κB to the nucleus, where it binds to DNA and activates several gene promoters. In addition, activation of NF-κB signaling can be blocked by several antioxidants[35]. Figure 1 Possible interactions/mechanisms of antioxidant agents with reported anti-HCV effect.

On the other hand, twenty years ago, Suematsu *et al*[36] showed that patients with hepatitis C had increased serum lipid peroxides, and further this increase was also confirmed by Higueras *et al*[37], but interestingly they reported that lipid peroxides levels were decreased in patients treated with alpha-interferon. Still, it has not been defined whether this effect was due to the decrease of viral replication and inflammation or a combination of induction of antioxidant enzymes by the alpha-interferon treatment[37]. Another important component in this viral redox imbalance is the transcription factor Nrf2, reported by Waris *et al*[33] in 2010. An independent study by Ivanov *et al*[38], on Huh7 cells reported that induction of

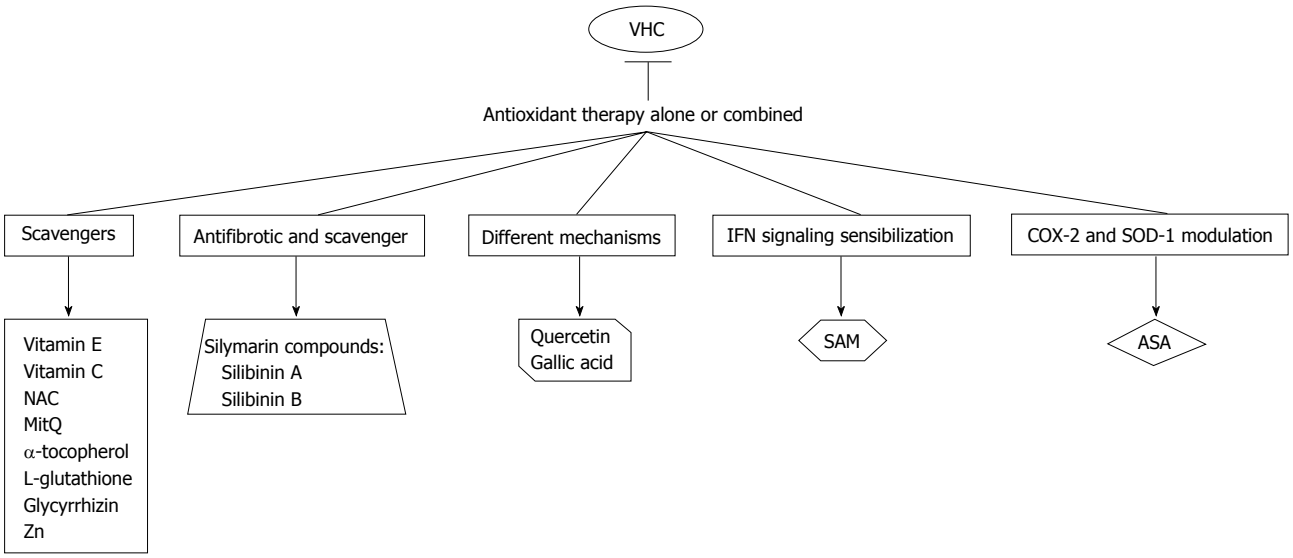

Figure 1 Possible interactions/mechanisms of antioxidant agents with reported anti-hepatitis C virus effect. SOD: Superoxide dismutase; SAM: S-adenosylmethionine; ASA: Acetylsalicylic acid; Zn: Zinc; IFN: Interferon.

Table 1 Most common antioxidant used in several clinical trials and *in vitro* experiments

Antioxidant	Dose range
Scavengers	
Vitamin E[47,48]	400-544 IU/d or 600 mg[1]
Vitamin C[48]	500 mg-10 g
N-acetylcysteine[78]	600 mg or 1800 mg/d[1]
Mitoquine Q[65,66]	40 or 80 mg/d
α-tocopherol[79]	600 mg, 500 mg/d, 800 IU/d
Glycyrrhizin[40,42,53,54]	500 mg, 120 mg
Different mechanisms	
Silymarin (Silibinin A, Silibinin B, *etc.*)[40,64,80-82]	250 mg or 5-20 mg/kg[1]
S-adenosylmethionine[69]	1600 mg/d[1]
Acetylsalicylic acid[73]	4 mmol/L (*in vitro*)
Gallic acid	300 mg/mL (*in vitro*)

[1]Combined treatment with interferon.

the Nrf2/ARE signaling pathway is triggered at least by five of the HCV viral proteins (core, E1, E2, NS4B, and NS5A). It is reported that virus can affect the cell redox equilibrium by inducing cellular pro-oxidants such as $Fe^{2+/3+}$ ions, nitric oxide and by decreasing the synthesis of antioxidant enzyme systems. In addition, ROS could regulate virus replication modulating oxidative stress present in the infected cell in order to choose survivors viral mutants, by inducing mutations and also by activating transcription factors such as NF-κB that could participate in viral protein expression[39]. Based on the multiple oxidant and antioxidant activities performed *in vivo* and *in vitro* systems during viral infections, and the variable ability of antioxidants to cross cell membranes, the systematic use of antioxidants as antiviral therapies had been limited[40].

On the other hand, HCV is potentially lymphotropic, because it can invade and propagate in cells of the immune system. It is known that processing of HCV proteins are performed in the ER but HCV proteins accumulate at the space between the mitochondrial outer membrane and ER. It has been reported that HCV proteins can move from the ER synthesis site to the mitochondria, therefore, there is an interaction between HCV proteins with the mitochondrial machinery in hepatic and extra-hepatic sites. Impairment of mitochondria-nuclear cross talk through involvement of PI3 kinases has been published by Bhargava *et al*[17], 2011.

ANTIOXIDANT THERAPY

It has been shown that HCV modifies antioxidant defense mechanisms yielding a cellular oxidative imbalance[29,31,41]. Several authors, including our research group, have demonstrated that HCV effectively modifies gene expression regulatory proteins of oxidative stress as SOD and catalase[26,31,33]. The limitations of interfering with such mechanisms in viral diseases are similar to those when interfering with oxidant generation. This is a result of the association between these pathways with the normal host physiology as well as with host pathology. It is clear that antioxidant therapy could be criticized because it involves a wide variety of drugs, rather than the magic one or two putatively specific ones used in modern pharmacotherapy. The most common antioxidant used in several clinical trials and *in vitro* experiments are described in Table 1. However, because the symptoms and pathology of viral diseases are ultimately the result of complex host reactions in addition to direct viral effects, there is a scientific basis for this strategy of viral disease therapy. Clearly, this does not obviate the need for further research to identify a drugs that may specifically interfere with viral replication[40,42,43]. The modulation of major signaling

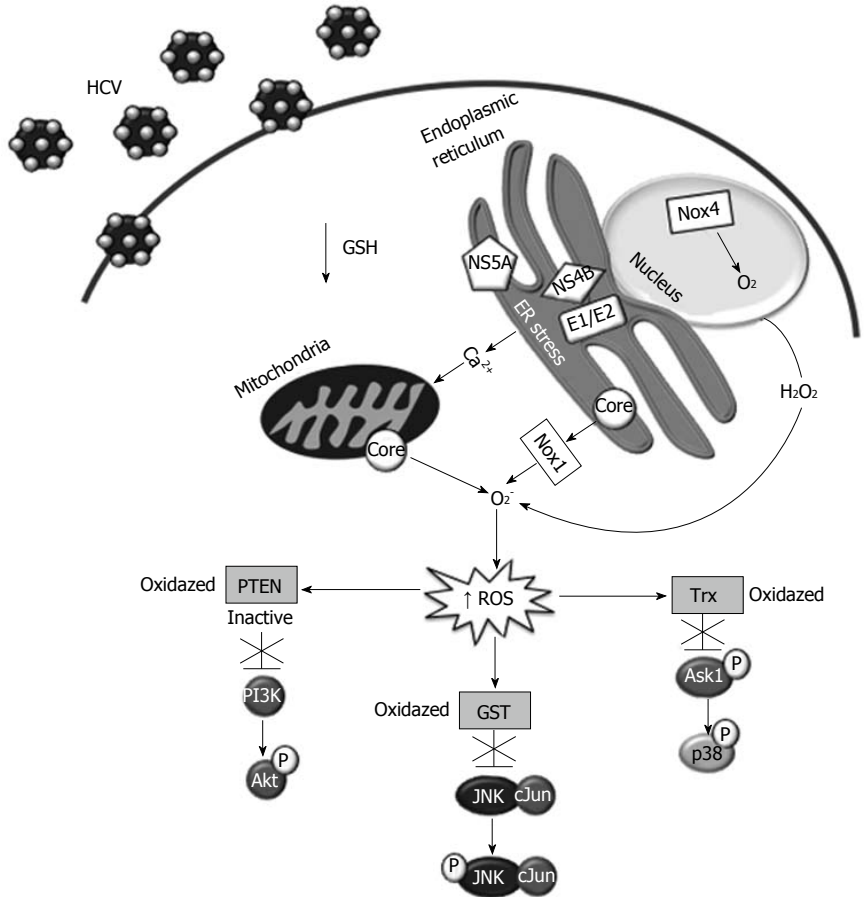

Figure 2 Cell signaling pathways modulated by increased reactive oxygen species levels in hepatitis C virus infected cells. ROS: Reactive oxygen species; HCV: Hepatitis C virus; GSH: Glutathione system; Trx: Thioredoxin; PI3K: Phosphoinositide-3 kinase.

pathways by high cell ROS levels are shown in Figure 2.

VITAMINS C AND E

Vitamin C (ascorbic acid) is as an electron donor in enzymatic reactions and thus can block free radical chain reactions. Vitamins C, A, and E are the most important natural antioxidants associated with cell-mediated immunity and toxic hepatitis[44]. Vitamin C defends cells from free radical damage by reducing radicals, scavenging lipid-peroxidation-derived radicals, or reducing tocopherol radicals to tocopherol[45]. In cells, vitamin E is the major lipid soluble chain-breaking antioxidant in mitochondria, microsomes, and lipo-proteins[46]. von Herbay *et al*[47] reported that vitamin E decreases the hepatic aminotransferase levels in patients whit chronic HCV infection. Murakami *et al*[48] observed that antioxidant vitamins (E and C) supplemented during interferon alpha treatment inhibited the decrease in eicosapentaenoic acid of mononuclear cell. They also stated that it might be possible to enhance the efficacy of combination therapy of interferon alfa-2b and RBV[48]. There are other reports, with conflicting or non-reproducible results, so there is still controversy on their usefulness.

ZINC

It has been reported that zinc (Zn) supplementation down-regulated oxidative stress products such as malondialdehyde (MDA), 4-hydroxyalkenals, and 8-hydroxydeoxyguanine in plasma; inhibited induction of tumor necrosis factor-α and interleukin-1β mRNA in mononuclear cells; and protected against NF-κB activation in mononuclear cells[49]. Polaprezinc (zinc plus l-carnosine), has been studied in many studies as an antioxidant adjuvant to IFN as treatment for chronic HCV infection, showing promising results[50]. There are several potential mechanisms supporting the antioxidant effects of zinc, such as the decrease of hepatic fibrosis, antioxidant activity, down-regulation of ferritin, and improvement in hepatic encephalopathy. Zn also is able to decrease HCV replication, then it has been used as an adjuvant for treatment of HCV infection, but further studies are needed to understand the involved mechanisms[51].

N-ACETYLCYSTEINE

On the other hand, Look *et al*[52] demonstrated that adjuvant antioxidant therapy with N-acetylcysteine/

Selenium co-supplementation has not beneficial effect to improve antiviral activity in chronic hepatitis C patients upon a six-month interferon alpha monotherapy. These data suggest that antioxidant compounds could have a beneficial effect on necro-inflammatory variables, but have no effect on the viral cycle reduction.

GLYCYRRHIZIN

Another antioxidant evaluated in several clinical trials to combat HCV is glycyrrhizin, a free radical scavenger. Intravenous administration of glycyrrhizin, diminished transaminase enzyme levels, and decrease cellular damage in patients with chronic HCV infections[53,54]. In the Melhem *et al*[55] study, 50 HCV-infected patients were treated for 20 wk with a mix of seven oral antioxidants (glycyrrhizin, schisandra, silymarin, lipoic acid, ascorbic acid, L-glutathione, and alpha-tocopherol), along with four different intravenous preparations (glycyrrhizin, ascorbic acid, L-glutathione, B-complex) twice weekly for the first 10 wk. In patients who underwent this treatment, liver enzymes normalization occurred in 44% who had elevated ALT levels previous to treatment. Twenty five percent of the patients showed a decrease in viral load by one log or more. Histological improvement was also noted in 36.1% of patients. There were no major adverse reactions noticed[55]. These data portrays promising results for the use of glycyrrhizin in different liver diseases.

SILYMARIN COMPLEX

Silibinin is a major component of milk thistle (silymarin), which has been demonstrated in several *in vitro* and clinical trials that is a strong antioxidant and antifibrotic agent. The mechanisms of action of silymarin include different events, such as the stimulation of ribosomal RNA transcription and levels, protecting the cell membrane from oxidative stress damage and blockage of the uptake of toxins. A combination of antioxidant compounds that have been shown to improve cellular and biochemical markers in chronic HCV patients is formed by alpha-lipoic acid, silymarin, and selenium[56,57]. There are conflicting results on the efficacy of silymarin. As an example a phase Ⅰ trial assessing the efficacy of oral doses of silymarin in non-responders HCV infected patients demonstrated no adverse effects but also showed no antiviral efficacy against HCV[41]. However, in other report the administration of intravenous (*iv*) silibilin in non-responders HCV infected patients decreased HCV-RNA levels in a dose-dependent manner[58]. It is reported that silibinin monotherapies of 5, 10, 15 or 20 mg/kg for one week decrease viral load (from 0.55 to 3.02 logs). The addition of combination of PEG-IFN/RBV at day 8 resulted in a higher reduction of viremia, but some patients had slight rebound viremia upon *iv* silibinin treatment was discontinued despite continued standard of care.

In another study, antiviral activity of silymarin complex was evaluated *in vitro* by using the HCV-RNA polymerase and NS3/4A protease enzyme assays. Results demonstrated that silibinin A, silibinin B, their water-soluble dihydrogen succinate forms and Legalon SIL (a commercially available *iv* preparation of silibinin), were able to inhibit HCV-RNA polymerase activity (IC_{50} of the order of 75-100 μmol/L). In addition, Silibinin A and silibinin B also decreased HCV genotype 1b replication and HCV genotype 2a strain JFH1 replication in cell culture. Interestingly, none of these agents affected the HCV protease function[59]. The antioxidant effects of silibinin have been showed in several cell studies. Silibinin is classified as an antioxidant compound because it blocks radical formation, binds many radical species (scavenger), interferes with lipid peroxidation mechanisms of membranes, and then increases the intracellular content of scavengers[60].

Gomez *et al*[61] in 2010 investigated the role of Viusid as an antioxidant and an immunomodulator in nonresponder HCV infected patients. Authors reported that MDA and 4-hydroxyalkenal levels were significantly decreased in serum of the treated patients when compared with placebo.

GALLIC ACID

Gallic acid (GA) is a phenolic compound present in several natural sources and it has been reported to have various biological effects such as antioxidant, anti-inflammatory, antibiotic, anticancer, antiviral and cardiovascular protection. Recently, our group started to investigate whether GA is able to have an effect on HCV replication. Recently, we examined the effects of GA on HCV expression using a subgenomic HCV replicon cell culture system that expresses HCV-nonstructural proteins (unpublished data). We observed that GA down-regulated NS5A-HCV protein expression (around 55%) and HCV-RNA levels (nearly 50%) in a time-dependent fashion compared with untreated cells. Interestingly, we observed that GA treatment decreased ROS levels at early times of exposure in cells expressing HCV proteins (manuscript in press). Similar results were found upon PDTC exposure. These findings suggest the possibility that antioxidant capacity of GA could contribute to the mechanism(s) involved in the down-regulation of HCV replication in hepatoma cells, however further experiments are needed to confirm these findings.

HEME OXYGENASE REGULATION

There are some reports regarding to modulation of antioxidant enzymes as a HCV therapy, Zhu *et al*[62] worked with HO which catalyzes the rate-limiting reaction in the catabolism of heme which produces equimolar amounts of biliverdin, carbon monoxide and free iron. They reported that increased expression of HO-1 is associated with diminished HCV replication and also with an increase of the resistance of hepatocytes to oxidative damage. Based on this findings, heme

oxygenase regulation could be useful as an adjunctive antiviral therapy[62-64].

MITOQUINONE

Mitoquinone or MitQ (Antipodean Pharmaceuticals, Auckland, New Zeland) is a potent antioxidant that bonds the antioxidant moiety of coenzyme Q10 (known as ubiquinone) to a triphenylphosphonium cation. The cation causes the attached antioxidant to accumulate several-hundred fold within mitochondria *in vivo* upon oral administration, protecting them from oxidative injury and cell death. A phase-2 study of 28 d of MitQ revealed a decrease in serum aminotransferase in HCV treated patients[65,66].

QUERCETIN

Quercetin is a flavonoid antioxidant. *In vitro* treatment of HCV infected cells with quercetin diminished viral replication and infectious particle production. It has been shown that quercetin blocks viral protein production independently of viral genome replication; may help to elucidate the replication cycle and has potential use as antiviral agent helping to reduce virus production with low toxicity[67]. Additionally, dihydroquercetin has been shown to be beneficial as a hepatoprotective substance in the treatment of toxic hepatitis and liver fibrosis by enhancing antioxidant enzyme activity and decreasing the pro-oxidant effect[67,68].

S-ADENOSYLMETHIONINE

Further information into the hepatoprotective mechanisms of antioxidant agents might be discovered by analyzing the relationship between glutathione precursor S-adenosylmethionine (SAM) and involved signaling pathways. Administration of SAM to non-responders patients infected with HCV, showed beneficial effects in combination with PEG-IFN and RBV. Feld *et al*[69] also suggest that this effect is through the methylation of STAT-1, a transcription factor responsible of interferon stimulated gene expression, which enhances its translocation to the nucleus. Our recent results indicate that there is a combination of actions regarding to the molecular mechanism of SAM on HCV replication. Administration of SAM to HCV replicon cells, leads to an increase of glutathione synthesis (unpublished data). There are also reports of SAM benefits in patients with alcoholic liver cirrhosis. The possible mechanisms of action of SAM include: (1) function as a methyl donor compound and restoring of mitochondrial glutathione levels, which is necessary to counterbalance the oxidant environment in cirrhotic liver; and (2) decreasing the hepatic production of nitric oxide (NO), through the modulation of iNOS enzyme. SAM perform these effects in part by accelerating synthesis of inhibitor of κB-alpha and regulating the activation of NF-κB, thereby decreasing the transactivation of iNOS promoter[70]. Nevertheless, further experiments are needed to explore the participation of NO synthase-2 promoter and the effect of SAM in HCV replication.

We are currently investigating the molecular mechanisms of SAM against HCV in a subgenomic replicon cell model. We found that SAM is capable of modulate the antioxidant defense systems at transcriptional and translational level (SOD1, SOD2 and thioredoxin 1); we also found that biosynthesis of GSH in presence of SAM is increased in short periods of time (2-6 h). In addition, MAT1/MAT2 turnover is switched in presence of SAM (unpublished data). MAT1 is the enzyme responsible of the conversion of methionine to s-adenosylmethionine, and it is down-regulated in hepatocarcinoma and liver diseases. Presently, we need more experimental data to understand the role of SAM in the modulation of HCV.

ACETYLSALICYLIC ACID

Several studies including our reported findings demonstrated that sodium salicylate and acetylsalicylic acid (ASA) block the replication of flaviviruses, such as Japanese encephalitis virus, HCV and dengue virus[71,72]. Liao *et al*[71] demonstrated that salicylates inhibit flavivirus infection through a mechanism including p38-MAPK activity, but not NF-κB activation. In other hand, Mazur *et al*[72] reported inhibition of influenza virus replication *in vitro* and *in vivo* by ASA. This antiviral activity was mediated by a mechanism including expression of proapoptotic factors, decrease of caspase activation, and blocking of the nuclear export of viral ribonucleoproteins[72]. In 2008, our research group demonstrated that ASA presented anti-HCV properties in HCV-replicon cells through inhibition of COX-2 activity and expression, which is mediated in part by the activation of (MEK1/2)/p38 MAPKs[73,74]. Our results suggest that ASA in combination with standard therapy could be an excellent adjuvant in the treatment of chronic HCV infection.

As antiviral agents, antioxidants could be used in four different ways: (1) to deteriorate cellular mechanisms involved in HCV replication; (2) to improve liver enzyme activity and levels; (3) to counteract and protect against liver cell injury; and (4) to improve interferon anti-viral response. Triple antioxidants therapies have been tested in clinical trials, which include alpha-lipoic acid, silymarin and selenium in order to suppress HCV-induced liver damage, when used together with Vitamins C and E, and in a healthy diet and exercise regime[43,75,76].

Several studies are in favor of a positive control of HCV replication by oxidative stress and to search counteracting this effect. Taking all these data together, there is plenty of evidence suggesting that antioxidants can effectively improve the response of HCV infected patients, even if they are non-responders. There are also proofs that antioxidants ameliorate the oxidative and nitrosative stress in liver disease, ultimately decreasing inflammation and fibrosis progression. Some investigators think that although it is important testing

these antioxidant compounds in clinical trials, they make emphasis on the need of researching the side effects of different antioxidants on HCV replication before its use as therapy. However, there are conflicting data published by Nakamura *et al*[77], where they reported an enhanced HCV replication with resveratrol treatment, so further research is needed.

CONCLUSION

It is well established that HCV infection leads to strong cellular oxidative stress, triggering several HCV-associated metabolic disorders including HCC, steatosis, liver fibrosis, cirrhosis and iron overload. Today, several molecular interplays and signaling pathways involving viral proteins, host cell factors, ROS-generating enzymes and cellular antioxidant systems have been elucidated. In fact, several intracellular signaling pathways are altered by the expression of HCV proteins in favor of virus replication and they are finely regulated by the cellular redox state. Additional mechanisms by which HCV induces and modulate oxidative stress still remain to be discovered and require further studies. With the current findings regarding the dual function of the oxidative stress induced by the virus and the host cell, it may be possible to establish new and more effective therapeutic targets for HCV treatment.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin D, Forman D, Bray F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. [Internet]. Lyon, Fr. Int. Agency Res Cancer 2013: 11. Available from: URL: http://globocan.iarc.fr

2 **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]

3 **Messina JP**, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]

4 **WHO**. Hepatitis C Fact sheet N°164 [Internet]. [Updated 2015 Jul; accessed 2015 Aug 20]. Available from: URL: http://www.who.int/mediacentre/factsheets/fs164/en/

5 **Kershenobich D**, Razavi HA, Sánchez-Avila JF, Bessone F, Coelho HS, Dagher L, Gonçales FL, Quiroz JF, Rodriguez-Perez F, Rosado B, Wallace C, Negro F, Silva M. Trends and projections of hepatitis C virus epidemiology in Latin America. *Liver Int* 2011; **31** Suppl 2: 18-29 [PMID: 21651701 DOI: 10.1111/j.1478-3231.2011.02538.x]

6 **Torresi J**, Johnson D, Wedemeyer H. Progress in the development of preventive and therapeutic vaccines for hepatitis C virus. *J Hepatol* 2011; **54**: 1273-1285 [PMID: 21236312 DOI: 10.1016/j.jhep.2010.09.040]

7 **El-Shamy A**, Hotta H. Impact of hepatitis C virus heterogeneity on interferon sensitivity: an overview. *World J Gastroenterol* 2014; **20**: 7555-7569 [PMID: 24976696 DOI: 10.3748/wjg.v20.i24.7555]

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 **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]

10 **FDA**. FDA approves Sovaldi for chronic hepatitis C [Internet]. US Food Drug Adm, 2013. Available from: URL: http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm377888.htm

11 **Hill A**, Khoo S, Fortunak J, Simmons B, Ford N. Minimum costs for producing hepatitis C direct-acting antivirals for use in large-scale treatment access programs in developing countries. *Clin Infect Dis* 2014; **58**: 928-936 [PMID: 24399087 DOI: 10.1093/cid/ciu012]

12 **Tencate V**, Sainz B Jr, Cotler SJ, Uprichard SL. Potential treatment options and future research to increase hepatitis C virus treatment response rate. *Hepat Med* 2010; **2010**: 125-145 [PMID: 21331152 DOI: 10.2147/HMER.S7193]

13 **Xu Z**, Choi J, Lu W, Ou JH. Hepatitis C virus f protein is a short-lived protein associated with the endoplasmic reticulum. *J Virol* 2003; **77**: 1578-1583 [PMID: 12502871 DOI: 10.1128/JVI.77.2.1578-1583.2003]

14 **Ploss A**, Evans MJ. Hepatitis C virus host cell entry. *Curr Opin Virol* 2012; **2**: 14-19 [PMID: 22440961 DOI: 10.1016/j.coviro.2011.12.007]

15 **Oberley LW**, Buettner GR. Role of superoxide dismutase in cancer: a review. *Cancer Res* 1979; **39**: 1141-1149 [PMID: 217531]

16 **Machida K**, McNamara G, Cheng KT, Huang J, Wang CH, Comai L, Ou JH, Lai MM. Hepatitis C virus inhibits DNA damage repair through reactive oxygen and nitrogen species and by interfering with the ATM-NBS1/Mre11/Rad50 DNA repair pathway in monocytes and hepatocytes. *J Immunol* 2010; **185**: 6985-6998 [PMID: 20974981 DOI: 10.4049/jimmunol.1000618]

17 **Bhargava A**, Raghuram GV, Pathak N, Varshney S, Jatawa SK, Jain D, Mishra PK. Occult hepatitis C virus elicits mitochondrial oxidative stress in lymphocytes and triggers PI3-kinase-mediated DNA damage response. *Free Radic Biol Med* 2011; **51**: 1806-1814 [PMID: 21893189 DOI: 10.1016/j.freeradbiomed.2011.08.009]

18 **Halliwell B**. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 1994; **344**: 721-724 [PMID: 7915779 DOI: 10.1016/S0140-6736(94)92211-X]

19 **Gong G**, Waris G, Tanveer R, Siddiqui A. Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-kappa B. *Proc Natl Acad Sci USA* 2001; **98**: 9599-9604 [PMID: 11481452 DOI: 10.1073/pnas.171311298]

20 **Halliwell B**. Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: measurement, mechanism and the effects of nutrition. *Mutat Res* 1999; **443**: 37-52 [PMID: 10415430 DOI: 10.1016/S1383-5742(99)00009-5]

21 **Tsan MF**. Superoxide dismutase and pulmonary oxygen toxicity: lessons from transgenic and knockout mice (Review). *Int J Mol Med* 2001; **7**: 13-19 [PMID: 11115602]

22 **Immenschuh S**, Ramadori G. Gene regulation of heme oxygenase-1 as a therapeutic target. *Biochem Pharmacol* 2000; **60**: 1121-1128 [PMID: 11007950 DOI: 10.1016/S0006-2952(00)00443-3]

23 **Guo X**, Shin VY, Cho CH. Modulation of heme oxygenase in tissue injury and its implication in protection against gastrointestinal diseases. *Life Sci* 2001; **69**: 3113-3119 [PMID: 11758836 DOI: 10.1016/S0024-3205(01)01417-5]

24 **Peterhans E**. Sendai virus stimulates chemiluminescence in mouse spleen cells. *Biochem Biophys Res Commun* 1979; **91**: 383-392 [PMID: 229848 DOI: 10.1016/0006-291X(79)90630-2]

25 **Lieber CS**. Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. *Adv Pharmacol* 1997; **38**: 601-628 [PMID: 8895826 DOI: 10.1016/S1054-3589(08)61001-7]

26 **Okuda M**, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core

protein. *Gastroenterology* 2002; **122**: 366-375 [PMID: 11832451 DOI: 10.1053/gast.2002.30983]
27 **Abdalla MY**, Ahmad IM, Spitz DR, Schmidt WN, Britigan BE. Hepatitis C virus-core and non structural proteins lead to different effects on cellular antioxidant defenses. *J Med Virol* 2005; **76**: 489-497 [PMID: 15977232 DOI: 10.1002/jmv.20388]
28 **Otsuka M**, Kato N, Lan K, Yoshida H, Kato J, Goto T, Shiratori Y, Omata M. Hepatitis C virus core protein enhances p53 function through augmentation of DNA binding affinity and transcriptional ability. *J Biol Chem* 2000; **275**: 34122-34130 [PMID: 10924497 DOI: 10.1074/jbc.M000578200]
29 **Yoshida H**, Kato N, Shiratori Y, Otsuka M, Maeda S, Kato J, Omata M. Hepatitis C virus core protein activates nuclear factor kappa B-dependent signaling through tumor necrosis factor receptor-associated factor. *J Biol Chem* 2001; **276**: 16399-16405 [PMID: 11278312 DOI: 10.1074/jbc.M006671200]
30 **Wen F**, Abdalla MY, Aloman C, Xiang J, Ahmad IM, Walewski J, McCormick ML, Brown KE, Branch AD, Spitz DR, Britigan BE, Schmidt WN. Increased prooxidant production and enhanced susceptibility to glutathione depletion in HepG2 cells co-expressing HCV core protein and CYP2E1. *J Med Virol* 2004; **72**: 230-240 [PMID: 14695664 DOI: 10.1002/jmv.10567]
31 **Abdalla MY**, Britigan BE, Wen F, Icardi M, McCormick ML, LaBrecque DR, Voigt M, Brown KE, Schmidt WN. Down-regulation of heme oxygenase-1 by hepatitis C virus infection in vivo and by the in vitro expression of hepatitis C core protein. *J Infect Dis* 2004; **190**: 1109-1118 [PMID: 15319861 DOI: 10.1086/423488]
32 **He Y**, Nakao H, Tan SL, Polyak SJ, Neddermann P, Vijaysri S, Jacobs BL, Katze MG. Subversion of cell signaling pathways by hepatitis C virus nonstructural 5A protein via interaction with Grb2 and P85 phosphatidylinositol 3-kinase. *J Virol* 2002; **76**: 9207-9217 [PMID: 12186904 DOI: 10.1128/JVI.76.18.9207-9217.2002]
33 **Waris G**, Livolsi A, Imbert V, Peyron JF, Siddiqui A. Hepatitis C virus NS5A and subgenomic replicon activate NF-kappaB via tyrosine phosphorylation of IkappaBalpha and its degradation by calpain protease. *J Biol Chem* 2003; **278**: 40778-40787 [PMID: 12909638 DOI: 10.1074/jbc.M303248200]
34 **Qadri I**, Iwahashi M, Capasso JM, Hopken MW, Flores S, Schaack J, Simon FR. Induced oxidative stress and activated expression of manganese superoxide dismutase during hepatitis C virus replication: role of JNK, p38 MAPK and AP-1. *Biochem J* 2004; **378**: 919-928 [PMID: 14670077 DOI: 10.1042/BJ20031587]
35 **Schreck R**, Meier B, Männel DN, Dröge W, Baeuerle PA. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *J Exp Med* 1992; **175**: 1181-1194 [PMID: 1314883]
36 **Suematsu T**, Kamada T, Abe H, Kikuchi S, Yagi K. Serum lipoperoxide level in patients suffering from liver diseases. *Clin Chim Acta* 1977; **79**: 267-270 [PMID: 890958 DOI: 10.1016/0009-8981(77)90486-7]
37 **Higueras V**, Raya A, Rodrigo JM, Serra MA, Romá J, Romero FJ. Interferon decreases serum lipid peroxidation products of hepatitis C patients. *Free Radic Biol Med* 1994; **16**: 131-133 [PMID: 7507878]
38 **Ivanov AV**, Bartosch B, Smirnova OA, Isaguliants MG, Kochetkov SN. HCV and oxidative stress in the liver. *Viruses* 2013; **5**: 439-469 [PMID: 23358390 DOI: 10.3390/v5020439]
39 **Ruggieri A**, Anticoli S, Nencioni L, Sgarbanti R, Garaci E, Palamara AT. Interplay between Hepatitis C Virus and Redox Cell Signaling. *Int J Mol Sci* 2013; **14**: 4705-4721 [PMID: 23443167 DOI: 10.3390/ijms14034705]
40 **Hsiang CY**, Lin LJ, Kao ST, Lo HY, Chou ST, Ho TY. Glycyrrhizin, silymarin, and ursodeoxycholic acid regulate a common hepatoprotective pathway in HepG2 cells. *Phytomedicine* 2015; **22**: 768-777 [PMID: 26141764]
41 **Hawke RL**, Schrieber SJ, Soule TA, Wen Z, Smith PC, Reddy KR, Wahed AS, Belle SH, Afdhal NH, Navarro VJ, Berman J, Liu QY, Doo E, Fried MW. Silymarin ascending multiple oral dosing phase I study in noncirrhotic patients with chronic hepatitis C. *J Clin Pharmacol* 2010; **50**: 434-449 [PMID: 19841158 DOI: 10.1177/0091270009347475]
42 **Gabbay E**, Zigmond E, Pappo O, Hemed N, Rowe M, Zabrecky G, Cohen R, Ilan Y. Antioxidant therapy for chronic hepatitis C after failure of interferon: results of phase II randomized, double-blind placebo controlled clinical trial. *World J Gastroenterol* 2007; **13**: 5317-5323 [PMID: 17879400]
43 **Esrefoglu M**. Oxidative stress and benefits of antioxidant agents in acute and chronic hepatitis. *Hepat Mon* 2012; **12**: 160-167 [PMID: 22550523 DOI: 10.5812/hepatmon.837]
44 **Stehbens WE**. Oxidative stress, toxic hepatitis, and antioxidants with particular emphasis on zinc. *Exp Mol Pathol* 2003; **75**: 265-276 [PMID: 14611818 DOI: 10.1016/S0014-4800(03)00097-2]
45 **Villalba JM**, Navarro F, Gómez-Díaz C, Arroyo A, Bello RI, Navas P. Role of cytochrome b5 reductase on the antioxidant function of coenzyme Q in the plasma membrane. *Mol Aspects Med* 1997; **18** Suppl: S7-13 [PMID: 9266501 DOI: 10.1016/S0098-2997(97)00015-0]
46 **Tappel AL**. Lipid peroxidation damage to cell components. *Fed Proc* 1973; **32**: 1870-1874 [PMID: 4352451]
47 **von Herbay A**, Stahl W, Niederau C, Sies H. Vitamin E improves the aminotransferase status of patients suffering from viral hepatitis C: a randomized, double-blind, placebo-controlled study. *Free Radic Res* 1997; **27**: 599-605 [PMID: 9455695]
48 **Murakami Y**, Nagai A, Kawakami T, Hino K, Kitase A, Hara Y, Okuda M, Okita K, Okita M. Vitamin E and C supplementation prevents decrease of eicosapentaenoic acid in mononuclear cells in chronic hepatitis C patients during combination therapy of interferon alpha-2b and ribavirin. *Nutrition* 2006; **22**: 114-122 [PMID: 16459223 DOI: 10.1016/j.nut.2005.05.014]
49 **Prasad AS**, Bao B, Beck FW, Kucuk O, Sarkar FH. Antioxidant effect of zinc in humans. *Free Radic Biol Med* 2004; **37**: 1182-1190 [PMID: 15451058 DOI: 10.1016/j.freeradbiomed.2004.07.007]
50 **Nagamine T**, Takagi H, Takayama H, Kojima A, Kakizaki S, Mori M, Nakajima K. Preliminary study of combination therapy with interferon-alpha and zinc in chronic hepatitis C patients with genotype 1b. *Biol Trace Elem Res* 2000; **75**: 53-63 [PMID: 11051596]
51 **Yuasa K**, Naganuma A, Sato K, Ikeda M, Kato N, Takagi H, Mori M. Zinc is a negative regulator of hepatitis C virus RNA replication. *Liver Int* 2006; **26**: 1111-1118 [PMID: 17032412 DOI: 10.1111/j.1478-3231.2006.01352.x]
52 **Look MP**, Gerard A, Rao GS, Sudhop T, Fischer HP, Sauerbruch T, Spengler U. Interferon/antioxidant combination therapy for chronic hepatitis C--a controlled pilot trial. *Antiviral Res* 1999; **43**: 113-122 [PMID: 10517313]
53 **van Rossum TG**, Vulto AG, Hop WC, Brouwer JT, Niesters HG, Schalm SW. Intravenous glycyrrhizin for the treatment of chronic hepatitis C: a double-blind, randomized, placebo-controlled phase I/II trial. *J Gastroenterol Hepatol* 1999; **14**: 1093-1099 [PMID: 10574137 DOI: 10.1046/j.1440-1746.1999.02008.x]
54 **Abe Y**, Ueda T, Kato T, Kohli Y. [Effectiveness of interferon, glycyrrhizin combination therapy in patients with chronic hepatitis C]. *Nihon Rinsho* 1994; **52**: 1817-1822 [PMID: 7521424]
55 **Melhem A**, Stern M, Shibolet O, Israeli E, Ackerman Z, Pappo O, Hemed N, Rowe M, Ohana H, Zabrecky G, Cohen R, Ilan Y. Treatment of chronic hepatitis C virus infection via antioxidants: results of a phase I clinical trial. *J Clin Gastroenterol* 2005; **39**: 737-742 [PMID: 16082287 DOI: 10.1097/01.mcg.0000174023.73472.29]
56 **Salmi HA**, Sarna S. Effect of silymarin on chemical, functional, and morphological alterations of the liver. A double-blind controlled study. *Scand J Gastroenterol* 1982; **17**: 517-521 [PMID: 6753109 DOI: 10.3109/00365528209182242]
57 **Buzzelli G**, Moscarella S, Giusti A, Duchini A, Marena C, Lampertico M. A pilot study on the liver protective effect of silybin-phosphatidylcholine complex (IdB1016) in chronic active hepatitis. *Int J Clin Pharmacol Ther Toxicol* 1993; **31**: 456-460 [PMID: 8225695]
58 **Ferenci P**, Scherzer TM, Kerschner H, Rutter K, Beinhardt S,

Hofer H, Schöniger-Hekele M, Holzmann H, Steindl-Munda P. Silibinin is a potent antiviral agent in patients with chronic hepatitis C not responding to pegylated interferon/ribavirin therapy. *Gastroenterology* 2008; **135**: 1561-1567 [PMID: 18771667 DOI: 10.1053/j.gastro.2008.07.072]

59 **Ahmed-Belkacem A**, Ahnou N, Barbotte L, Wychowski C, Pallier C, Brillet R, Pohl RT, Pawlotsky JM. Silibinin and related compounds are direct inhibitors of hepatitis C virus RNA-dependent RNA polymerase. *Gastroenterology* 2010; **138**: 1112-1122 [PMID: 19962982 DOI: 10.1053/j.gastro.2009.11.053]

60 **Trouillas P**, Marsal P, Svobodová A, Vostálová J, Gazák R, Hrbác J, Sedmera P, Kren V, Lazzaroni R, Duroux JL, Walterová D. Mechanism of the antioxidant action of silybin and 2,3-dehydrosilybin flavonolignans: a joint experimental and theoretical study. *J Phys Chem A* 2008; **112**: 1054-1063 [PMID: 18193843 DOI: 10.1021/jp075814h]

61 **Gomez EV**, Perez YM, Sanchez HV, Forment GR, Soler EA, Bertot LC, Garcia AY, del Rosario Abreu Vazquez M, Fabian LG. Antioxidant and immunomodulatory effects of Viusid in patients with chronic hepatitis C. *World J Gastroenterol* 2010; **16**: 2638-2647 [PMID: 20518086 DOI: 10.3748/wjg.v16.i21.2638]

62 **Zhu Z**, Wilson AT, Mathahs MM, Wen F, Brown KE, Luxon BA, Schmidt WN. Heme oxygenase-1 suppresses hepatitis C virus replication and increases resistance of hepatocytes to oxidant injury. *Hepatology* 2008; **48**: 1430-1439 [PMID: 18972446 DOI: 10.1002/hep.22491]

63 **Chen WC**, Wang SY, Chiu CC, Tseng CK, Lin CK, Wang HC, Lee JC. Lucidone suppresses hepatitis C virus replication by Nrf2-mediated heme oxygenase-1 induction. *Antimicrob Agents Chemother* 2013; **57**: 1180-1191 [PMID: 23254429 DOI: 10.1128/AAC.02053-12]

64 **Mehrab-Mohseni M**, Sendi H, Steuerwald N, Ghosh S, Schrum LW, Bonkovsky HL. Legalon-SIL downregulates HCV core and NS5A in human hepatocytes expressing full-length HCV. *World J Gastroenterol* 2011; **17**: 1694-1700 [PMID: 21483629 DOI: 10.3748/wjg.v17.i13.1694]

65 **Gane EJ**, Weilert F, Orr DW, Keogh GF, Gibson M, Lockhart MM, Frampton CM, Taylor KM, Smith RA, Murphy MP. The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients. *Liver Int* 2010; **30**: 1019-1026 [PMID: 20492507 DOI: 10.1111/j.1478-3231.2010.02250.x]

66 **Gane E**, Orr D, Weilert F, Keogh G, Gibson M, Murphy M, Smith R, Lockhart M, Frampton C, Taylor K. Phase II study of the mitochondrial antioxidant mitoquinone for hepatitis C. *J Hepatol* 2008: **48** Supplement 2: S318 [DOI: 10.1016/S0168-8278(08)60849-1]

67 **Gonzalez O**, Fontanes V, Raychaudhuri S, Loo R, Loo J, Arumugaswami V, Sun R, Dasgupta A, French SW. The heat shock protein inhibitor Quercetin attenuates hepatitis C virus production. *Hepatology* 2009; **50**: 1756-1764 [PMID: 19839005 DOI: 10.1002/hep.23232]

68 **Dionisio N**, Garcia-Mediavilla MV, Sanchez-Campos S, Majano PL, Benedicto I, Rosado JA, Salido GM, Gonzalez-Gallego J. Hepatitis C virus NS5A and core proteins induce oxidative stress-mediated calcium signalling alterations in hepatocytes. *J Hepatol* 2009; **50**: 872-882 [PMID: 19303156 DOI: 10.1016/j.jhep.2008.12.026]

69 **Feld JJ**, Modi AA, El-Diwany R, Rotman Y, Thomas E, Ahlenstiel G, Titerence R, Koh C, Cherepanov V, Heller T, Ghany MG, Park Y, Hoofnagle JH, Liang TJ. S-adenosyl methionine improves early viral responses and interferon-stimulated gene induction in hepatitis C nonresponders. *Gastroenterology* 2011; **140**: 830-839 [PMID: 20854821 DOI: 10.1053/j.gastro.2010.09.010]

70 **Majano PL**, García-Monzón C, García-Trevijano ER, Corrales FJ, Cámara J, Ortiz P, Mato JM, Avila MA, Moreno-Otero R. S-Adenosylmethionine modulates inducible nitric oxide synthase gene expression in rat liver and isolated hepatocytes. *J Hepatol* 2001; **35**: 692-699 [PMID: 11738094]

71 **Liao CL**, Lin YL, Wu BC, Tsao CH, Wang MC, Liu CI, Huang YL, Chen JH, Wang JP, Chen LK. Salicylates inhibit flavivirus replication independently of blocking nuclear factor kappa B activation. *J Virol* 2001; **75**: 7828-7839 [PMID: 11483726 DOI: 10.1128/JVI.75.17.7828-7839.2001]

72 **Mazur I**, Wurzer WJ, Ehrhardt C, Pleschka S, Puthavathana P, Silberzahn T, Wolff T, Planz O, Ludwig S. Acetylsalicylic acid (ASA) blocks influenza virus propagation via its NF-kappaB-inhibiting activity. *Cell Microbiol* 2007; **9**: 1683-1694 [PMID: 17324159 DOI: 10.1111/j.1462-5822.2007.00902.x]

73 **Trujillo-Murillo K**, Rincón-Sánchez AR, Martínez-Rodríguez H, Bosques-Padilla F, Ramos-Jiménez J, Barrera-Saldaña HA, Rojkind M, Rivas-Estilla AM. Acetylsalicylic acid inhibits hepatitis C virus RNA and protein expression through cyclooxygenase 2 signaling pathways. *Hepatology* 2008; **47**: 1462-1472 [PMID: 18393288 DOI: 10.1002/hep.22215]

74 **Rivas-Estilla AM**, Bryan-Marrugo OL, Trujillo-Murillo K, Pérez-Ibave D, Charles-Niño C, Pedroza-Roldan C, Ríos-Ibarra C, Ramírez-Valles E, Ortiz-López R, Islas-Carbajal MC, Nieto N, Rincón-Sánchez AR. Cu/Zn superoxide dismutase (SOD1) induction is implicated in the antioxidative and antiviral activity of acetylsalicylic acid in HCV-expressing cells. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G1264-G1273 [PMID: 22442156 DOI: 10.1152/ajpgi.00237.2011]

75 **Moreno-Otero R**, Trapero-Marugán M. Hepatoprotective effects of antioxidants in chronic hepatitis C. *World J Gastroenterol* 2010; **16**: 1937-1938 [PMID: 20397276 DOI: 10.3748/wjg.v16.i15.1937]

76 **Quarato G**, Scrima R, Ripoli M, Agriesti F, Moradpour D, Capitanio N, Piccoli C. Protective role of amantadine in mitochondrial dysfunction and oxidative stress mediated by hepatitis C virus protein expression. *Biochem Pharmacol* 2014; **89**: 545-556 [PMID: 24726442 DOI: 10.1016/j.bcp.2014.03.018]

77 **Nakamura M**, Saito H, Ikeda M, Hokari R, Kato N, Hibi T, Miura S. An antioxidant resveratrol significantly enhanced replication of hepatitis C virus. *World J Gastroenterol* 2010; **16**: 184-192 [PMID: 20066737 DOI: 10.3748/wjg.v16.i2.184]

78 **Beloqui O**, Prieto J, Suárez M, Gil B, Qian CH, García N, Civeira MP. N-acetyl cysteine enhances the response to interferon-alpha in chronic hepatitis C: a pilot study. *J Interferon Res* 1993; **13**: 279-282 [PMID: 8228388]

79 **Takagi H**, Kakizaki S, Sohara N, Sato K, Tsukioka G, Tago Y, Konaka K, Kabeya K, Kaneko M, Takayama H, Hashimoto Y, Yamada T, Takahashi H, Shimojo H, Nagamine T, Mori M. Pilot clinical trial of the use of alpha-tocopherol for the prevention of hepatocellular carcinoma in patients with liver cirrhosis. *Int J Vitam Nutr Res* 2003; **73**: 411-415 [PMID: 14743544]

80 **El-Kamary SS**, Shardell MD, Abdel-Hamid M, Ismail S, El-Ateek M, Metwally M, Mikhail N, Hashem M, Mousa A, Aboul-Fotouh A, El-Kassas M, Esmat G, Strickland GT. A randomized controlled trial to assess the safety and efficacy of silymarin on symptoms, signs and biomarkers of acute hepatitis. *Phytomedicine* 2009; **16**: 391-400 [PMID: 19303273 DOI: 10.1016/j.phymed.2009.02.002]

81 **Loguercio C**, Festi D. Silybin and the liver: from basic research to clinical practice. *World J Gastroenterol* 2011; **17**: 2288-2301 [PMID: 21633595 DOI: 10.3748/wjg.v17.i18.2288]

82 **Fried MW**, Navarro VJ, Afdhal N, Belle SH, Wahed AS, Hawke RL, Doo E, Meyers CM, Reddy KR. Effect of silymarin (milk thistle) on liver disease in patients with chronic hepatitis C unsuccessfully treated with interferon therapy: a randomized controlled trial. *JAMA* 2012; **308**: 274-282 [PMID: 22797645 DOI: 10.1001/jama.2012.8265]

P- Reviewer: Kittner JM, Mishra PK, Tsukiyama-Kohara K
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.i29.2890

World J Hepatol 2015 December 18; 7(29): 2890-2895
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

2015 Advances in Liver Transplantation

Management of biliary complications after liver transplantation

Riccardo Memeo, Tullio Piardi, Federico Sangiuolo, Daniele Sommacale, Patrick Pessaux

Riccardo Memeo, Federico Sangiuolo, Patrick Pessaux, Hepato-Biliary and Pancreatic Surgical Unit, General, Digestive and Endocrine Surgery, IRCAD, IHU Mix-Surg, Institute for Minimally Invasive Image-Guided Surgery, University of Strasbourg, 1 place de l'Hôpital, 67091 Strasbourg, France

Tullio Piardi, Daniele Sommacale, Department of General, Digestive and Endocrine Surgery, Hôpital Robert Debré, Centre Hospitalier Universitaire de Reims, Université de Reims Champagne-Ardenne, 51100 Reims, France

Author contributions: Memeo R and Sangiuolo F contributed to study conception and design; Piardi T contributed to acquisition of the data; Memeo R, Pessaux P and Sommacale D contributed to drafting of manuscript; Memeo R and Pessaux P contributed to critical review.

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

Correspondence to: Patrick Pessaux, MD, PhD, Professor, Hepato-Biliary and Pancreatic Surgical Unit, General, Digestive and Endocrine Surgery, IRCAD, IHU Mix-Surg, Institute for Minimally Invasive Image-Guided Surgery, University of Strasbourg, 1 place de l'Hopital, 67091 Strasbourg, France. patrick.pessaux@chru-strasbourg.fr
Telephone: +33-3-69550552
Fax: +33-3-69551745

Received: April 28, 2015
Peer-review started: May 5, 2015
First decision: June 25, 2015
Revised: November 9, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015

Published online: December 18, 2015

Abstract

Biliary complications (BC) currently represent a major source of morbidity after liver transplantation. Although refinements in surgical technique and medical therapy have had a positive influence on the reduction of post-operative morbidity, BC affect 5% to 25% of trans-planted patients. Bile leak and anastomotic strictures represent the most common complications. Nowadays, a multidisciplinary approach is required to manage such complications in order to prevent liver failure and retransplantation.

Key words: Biliary complication; Bile leak; Anastomotic stricture; Endoscopic treatment; Liver transplantation

Core tip: Biliary complications (BC) represent the downside of liver transplantation, impacting postoperative morbidity as well as patient and graft survival. In this paper, we will analyze the most common BC, along with diagnosis, management and treatment modalities.

Memeo R, Piardi T, Sangiuolo F, Sommacale D, Pessaux P. Management of biliary complications after liver transplantation. *World J Hepatol* 2015; 7(29): 2890-2895 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2890.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2890

INTRODUCTION

Liver transplantation (LT) is the standard of care for

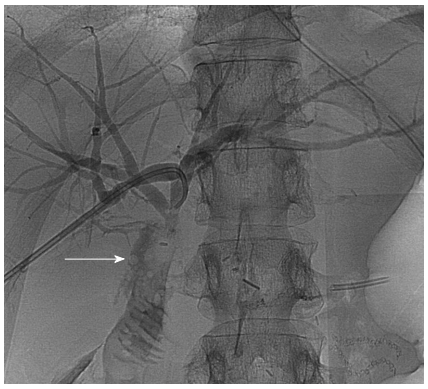

Figure 1 Biliary fistula post hepatojejunostomy.

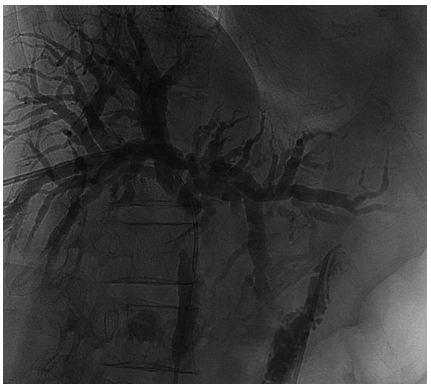

Figure 2 Ischemic anastomotic stricture after duct-to-duct anastomosis.

end-stage liver disease. Although LT can currently be considered as a consolidate procedure and various refinements in surgical techniques are required, organ preservation and immunosuppressive management have reduced complications and contribute to better outcomes[1], biliary complications (BC) remain the main downside of this procedure, affecting 5% to 25% of transplanted patients[2]. The most common causes of BC are bile leakage, anastomotic and non-anastomotic strictures, and bile duct obstruction. According to the literature[3-8], the main risk factors are technical complications, ischemia/reperfusion injury, ABO mismatch, hepatic artery complications (thrombosis and stenosis), donor age and cytomegalovirus infection. The aim of this article is to review and focus on the treatment of BC after LT.

Surgical technique

Two different types of biliary anastomoses can be performed in LT: Duct-to-duct (DD) and hepatojejunostomy (HJ). Different factors determine the choice of biliary reconstruction. In most cases, a DD anastomosis is preferred (90% of deceased donor LT[9,10] and 60% in living donor LT[11] due to its simpler technical feasibility, preserved function of Oddi's sphincter, and endoscopic access preservation. In the literature, two kinds of DD anastomoses have been described: End-to-end and side-to-side. Davidson *et al*[12] have described an equal effectiveness of both reconstructions. HJ is preferred in case of size disparity between donor and recipient bile duct, recipient diseased duct or previous transplant or biliary surgery. In addition to the type of reconstruction, no evidence has been demonstrated regarding the suturing method (interrupted or continuous[13]) or materials. However, an inadequate surgical technique can be responsible for BC[14]. A T tube was routinely used after DD anastomosis to reduce the incidence of BC. Most retrospective studies cannot conclude in favor of the use of a T tube[15], and some of them point towards a potential negative effect[16-18] on anastomosis. Recently, a meta-analysis[19] including six prospective randomized trials demonstrated no benefit in terms of T tube, and consequently it could not be recommended.

Diagnosis

The onset modality of BCs varied. In the presence of pain, abnormal liver function tests, increased levels of inflammatory markers, fever, and bilious secretion in the abdominal drain, further radiological examinations were necessary. In case of ongoing clinical suspicion, cholangiography remains the gold standard for the exploration of BCs. In the presence of a T tube, a simple cholangiography could reveal the origin of BCs. If a T tube is not used, endoscopic retrograde cholangiopancreatography (ERCP) is the method of choice in case of DD anastomosis. In case of HJ anastomosis, percutaneous transhepatic cholangiography is the first-line diagnostic exam. Recently, magnetic resonance cholangiopancreatography has guaranteed a good sensitivity and specificity in order to prevent invasive exploration. Ultrasonography may be helpful to detect the presence of biloma, biliary tree dilatation, and to explore all vascular anastomoses by means of a Doppler examination. Further procedures such as liver biopsy could be necessary in case of suspected graft dysfunction.

Types of BC

BC can be divided into several categories which are anastomotic leak (Figure 1), biliary strictures (Figure 2), non-anastomotic strictures (Figure 3), and biliary obstruction (stones, sludge and cast). Each complication usually occurs in different postoperative periods and requires different management options.

Anastomotic leaks: Bile leaks occur in 5% to 10% of deceased donor LT[20] and in 10% to 15% of living donor LT[21]. They can occur during the anastomosis, at T tube insertion, on the cystic duct stump and on the cut surface in case of partial graft. Early postoperative leak can be identified. They usually occur within one month after LT. Late bile leaks[22] rarely occur. In the early postoperative period, bile leak can originate directly from the anastomosis, and it is most likely due to technical problems or insufficient arterial bile duct vascularization. Other rare causes of bile leak are due to an incorrect suture of the cystic duct stump

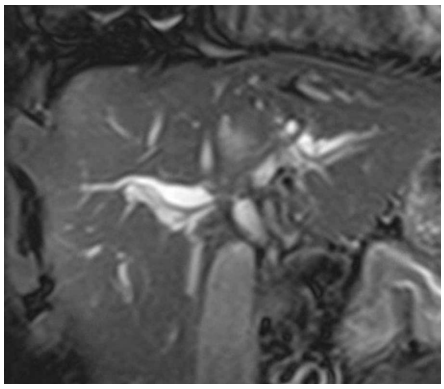

Figure 3 Non anastomotic stricture six months post liver transplantation.

and may originate from the resection surface in case of living donor or split liver. In particular, caudate lobe biliary branches, which usually drain in the left liver are sometimes drained in the right liver, increasing the risk of leak either in donors or recipients[20,23]. Late bile leaks are related to T tube[24] removal in nearly 1% of cases, with a fistula arising directly from the insertion site. In some cases, especially in case of early accidental T tube removal, a biliary peritonitis can occur, due to the incomplete T tube tract scarring.

Biliary strictures: As described in the literature, biliary strictures represent a complication which can occur in 13% in deceased donor LT and in 19% in in living donor LT[9]. Biliary strictures can be divided into two categories, *i.e.*, anastomotic strictures and non-anastomotic strictures. The main cause responsible for anastomotic strictures can be inadequate anastomoses, usually occurring in the early postoperative period and inflammatory strictures due to ischemic events or biliary fistulas. The mechanism of non-anastomotic strictures remains unclear, but it is often related to ischemic events. Non-anastomotic strictures are present in the entire biliary tree, especially in the hilum. Non-anastomotic strictures, also called ischemic cholangiopathies, can be caused by long-lasting cold ischemia times[25]. Generally, biliary strictures are considered as late complications, occurring within 6 mo[26,27] after LT, even though some cases may occur later, especially when associated with ischemic cholangitis for arterial thrombosis[28] or immunologic disorders[29].

Other complications: Bile leaks and anastomotic strictures represent the main BC. Liver test perturbation may be due to other causes. Outflow bile duct obstruction can be caused by Oddi's sphincter dysfunction, as described in about 2% to 7% of patients[30]. It is often associated with a stump denervation of the recipient bile duct or with a chronic sphincter inflammation. Other causes of bile duct dilatation can be stones, sludge or cast. The formation of stones is often due to an underlying stenosis which induces an increased bile viscosity, occurring in 3% to 12% of cases[31]. Sludge is like a mixture of particles precipitated from the bile, often composed of cholesterol and calcium salt which generates a progressive bile duct lumen obstruction in 2% of cases[32]. Damage (ischemia, rejection, infection, obstruction) of the biliary tree mucosa can provoke a cast syndrome, defined as the presence of cast (desquamated epithelial cells mixed with bile products) within the intrahepatic and extrahepatic biliary system. Its incidence varies from 3% to 18% in the literature[33]. Mucoceles, an uncommon complication after LT occurring in 2% of patients[34], are defined as a collection of mucus present in the remnant cystic duct. This abnormal dilatation can cause extrinsic compression on the bile duct.

MANAGEMENT

Bile leaks

Even if its role is still debated[35], the T tube can be a useful tool for a rapid diagnosis of bile leak in case of early postoperative bile leak. A T tube cholangiogram could be used diagnostically and a simple drainage through the tube opening could be therapeutic, preventing any invasive treatment. Without the use of a T tube, endoscopic treatment is the standard of care[22,36,37]. A simple sphincterotomy could be therapeutic in case of small bile leak[38]. An endoscopic stent could be necessary for major bile leaks, with a short-term removal[27] of the prosthesis in case of successful treatment. In case of HJ anastomosis, in case of endoscopic treatment failure (even if expert teams substantiate the use of double-balloon enteroscopy[39]), transhepatic percutaneous[40] treatment becomes an alternative. Although percutaneous transanastomotic internal-external drainage guarantees good postoperative results, it is technically difficult due to the absence of leak-induced bile duct dilatation. Re-operation and re-transplantation[9] for bile leak, although described in the literature, become increasingly anecdotal due to improvements in radiology and endoscopy.

Biliary strictures

Biliary strictures are usually classified into anastomotic strictures and non-anastomotic strictures. Anastomotic strictures usually involve the anastomotic site whereas non-anastomotic strictures could be multiple and present either in the hilum and in the intrahepatic portion of the bile duct, with a guarded prognosis as compared to anastomotic strictures[41]. Concerning anastomotic strictures, the treatment aims to dilate the stenotic segment. Considering the access route, endoscopy is preferred to the percutaneous approach[42] due to a reduced morbidity, better efficacy, and increased comfort for the patient[43]. Endoscopic treatment, performed *via* ERCP, consists of sphincterotomy and several sessions of dilatation followed by placement of a plastic stent. Some studies compared simple balloon dilatation to balloon dilatation and simultaneous plastic stent placement, showing a lower recurrence rate and requiring fewer sessions in combined treatment[44,45]. The success rate of treatment is approximately 75%[45,46]. Multiple

sessions are usually performed bimonthly and could be necessary to obtain satisfying results[47,48]. The necessity of a repetitive approach, entailed the increased use of metallic stents, which can achieve a higher diameter and develop less obstruction as compared to plastic stents. This concept is theoretically correct but has not found practical evidence neither with the use of partially nor fully covered stents as compared to plastic stents[41]. The percutaneous approach, mostly used in case of HJ anastomosis, guarantees goods results in terms of success and recurrence rates[29]. In case of endoscopic/percutaneous treatment failure, surgery represents a valid treatment alternative, considering the effect of prolonged biliary obstruction on liver function. Surgical revision was necessary in 10% to 20% of cases with anastomotic stricture[29].

Early arterial thrombosis represents the main risk factor for non-anastomotic strictures. Thrombosis associated with the absence of arterial collateral perfusion (otherwise present in late arterial thrombosis) is strongly associated with non-anastomotic strictures in 50% of cases[14]. Early detection could well reduce the rate of retransplantation[28], requiring a rapid revascularization in order to prevent graft loss. Ischemic-type lesions are difficult to manage, especially in case of multiple intrahepatic strictures. Medical treatment can be attempted, even if not evidence-based, with the use of ursodesoxicolic acid to increase bile flow and reduce lithogenicity. Considering the frequent association with cholangitis, a large use of antibiotic therapy and prophylaxis is often necessary[49]. Endoscopic treatment with multiple stent placement for non-anastomotic strictures usually requires long-term stenting, and despite encouraging results described in the literature[48,49], 30% to 50% of patients undergo re-transplantation[22,27,49] due to the progressive onset of liver dysfunction caused by chronic biliary cirrhosis. An immunomediate mechanism is responsible for late (> 1 year) non-anastomotic strictures[50,51], requiring the same management of ischemia-mediated strictures.

Other complications

Most other pathological conditions are related to a difficult bile duct emptying. On radiological findings, they can be identified as common bile duct filling defects. Different causes contribute to this dysfunction, arising from the presence of stones, sludge, cast syndrome or cystic duct mucoceles[52]. Most of these complications are treated using an endoscopic approach, consisting of sphincterotomy and ERCP with balloon dilatation and basket extraction. In case of endoscopic treatment failure, surgery represents a valid alternative.

REFERENCES

1 **Adam R**, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, Castaing D, Neuhaus P, Jamieson N, Salizzoni M, Pollard S, Lerut J, Paul A, Garcia-Valdecasas JC, Rodríguez FS, Burroughs A. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol* 2012; **57**: 675-688 [PMID: 22609307 DOI: 10.1016/j.jhep.2012.04.015]

2 **Gastaca M**. Biliary complications after orthotopic liver transplantation: a review of incidence and risk factors. *Transplant Proc* 2012; **44**: 1545-1549 [PMID: 22841209 DOI: 10.1016/j.transproceed.2012.05.008]

3 **Memeo R**, de'Angelis N, Salloum C, Compagnon P, Laurent A, Feray C, Duvoux C, Azoulay D. Clinical outcomes of right-lobe split-liver versus orthotopic liver transplants from donors more than 70 years old. *Prog Transplant* 2015; **25**: 243-250 [PMID: 26308784 DOI: 10.7182/pit2015303]

4 **Seehofer D**, Eurich D, Veltzke-Schlieker W, Neuhaus P. Biliary complications after liver transplantation: old problems and new challenges. *Am J Transplant* 2013; **13**: 253-265 [PMID: 23331505 DOI: 10.1111/ajt.12034]

5 **Yuan D**, Wei YG, Lin HM, Li FQ, Yang M, Liu XL, Li B, Yan LN, Zeng Y, Wen TF, Zhao JC, Yang JY. Risk factors of biliary complications following liver transplantation: retrospective analysis of a single centre. *Postgrad Med J* 2009; **85**: 119-123 [PMID: 19351636 DOI: 10.1136/pgmj.2008.075176]

6 **Qian YB**, Liu CL, Lo CM, Fan ST. Risk factors for biliary complications after liver transplantation. *Arch Surg* 2004; **139**: 1101-1105 [PMID: 15492152 DOI: 10.1001/archsurg.139.10.1101]

7 **Ayoub WS**, Esquivel CO, Martin P. Biliary complications following liver transplantation. *Dig Dis Sci* 2010; **55**: 1540-1546 [PMID: 20411422 DOI: 10.1007/s10620-010-1217-2]

8 **Duailibi DF**, Ribeiro MA. Biliary complications following deceased and living donor liver transplantation: a review. *Transplant Proc* 2010; **42**: 517-520 [PMID: 20304182 DOI: 10.1016/j.transproceed.2010.01.017]

9 **Akamatsu N**, Sugawara Y, Hashimoto D. Biliary reconstruction, its complications and management of biliary complications after adult liver transplantation: a systematic review of the incidence, risk factors and outcome. *Transpl Int* 2011; **24**: 379-392 [PMID: 21143651 DOI: 10.1111/j.1432-2277.2010.01202.x]

10 **Sharma S**, Gurakar A, Jabbour N. Biliary strictures following liver transplantation: past, present and preventive strategies. *Liver Transpl* 2008; **14**: 759-769 [PMID: 18508368 DOI: 10.1002/lt.21509]

11 **Zhang S**, Zhang M, Xia Q, Zhang JJ. Biliary reconstruction and complications in adult living donor liver transplantation: systematic review and meta-analysis. *Transplant Proc* 2014; **46**: 208-215 [PMID: 24507053 DOI: 10.1016/j.transproceed.2013.05.014]

12 **Davidson BR**, Rai R, Kurzawinski TR, Selves L, Farouk M, Dooley JS, Burroughs AK, Rolles K. Prospective randomized trial of end-to-end versus side-to-side biliary reconstruction after orthotopic liver transplantation. *Br J Surg* 1999; **86**: 447-452 [PMID: 10215812 DOI: 10.1046/j.1365-2168.1999.01073.x]

13 **Castaldo ET**, Pinson CW, Feurer ID, Wright JK, Gorden DL, Kelly BS, Chari RS. Continuous versus interrupted suture for end-to-end biliary anastomosis during liver transplantation gives equal results. *Liver Transpl* 2007; **13**: 234-238 [PMID: 17256781 DOI: 10.1002/lt.20986]

14 **Koneru B**, Sterling MJ, Bahramipour PF. Bile duct strictures after liver transplantation: a changing landscape of the Achilles' heel. *Liver Transpl* 2006; **12**: 702-704 [PMID: 16628684 DOI: 10.1002/lt.20753]

15 **Riediger C**, Müller MW, Michalski CW, Hüser N, Schuster T, Kleeff J, Friess H. T-Tube or no T-tube in the reconstruction of the biliary tract during orthotopic liver transplantation: systematic review and meta-analysis. *Liver Transpl* 2010; **16**: 705-717 [PMID: 20517904 DOI: 10.1002/lt.22070]

16 **Randall HB**, Wachs ME, Somberg KA, Lake JR, Emond JC, Ascher NL, Roberts JP. The use of the T tube after orthotopic liver transplantation. *Transplantation* 1996; **61**: 258-261 [PMID: 8600634]

17 **Ferraz-Neto BH**, Mirza DF, Gunson BK, Ismail T, Mayer AD, Buckels JA, McMaster P. Bile duct splintage in liver transplantation: is it necessary? *Transpl Int* 1996; **9** Suppl 1: S185-S187 [PMID: 8959822]

18 **Pratschke J**, Weiss S, Neuhaus P, Pascher A. Review of nonim-

munological causes for deteriorated graft function and graft loss after transplantation. *Transpl Int* 2008; **21**: 512-522 [PMID: 18266771 DOI: 10.1111/j.1432-2277.2008.00643.x]
19 **Sun N**, Zhang J, Li X, Zhang C, Zhou X, Zhang C. Biliary tract reconstruction with or without T-tube in orthotopic liver transplantation: a systematic review and meta-analysis. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 529-538 [PMID: 25583036 DOI: 10.1586/17474124.2015.1002084]
20 **Kapoor VK**. Bile duct injury repair - earlier is not better. *Front Med* 2015; Epub ahead of print [PMID: 26482065]
21 **Iida T**, Ogura Y, Oike F, Hatano E, Kaido T, Egawa H, Takada Y, Uemoto S. Surgery-related morbidity in living donors for liver transplantation. *Transplantation* 2010; **89**: 1276-1282 [PMID: 20216482 DOI: 10.1097/TP.0b013e3181d66c55]
22 **Londoño MC**, Balderramo D, Cárdenas A. Management of biliary complications after orthotopic liver transplantation: the role of endoscopy. *World J Gastroenterol* 2008; **14**: 493-497 [PMID: 18203278]
23 **Jassem W**, Heaton ND, Rela M. Reducing bile leak following segmental liver transplantation: understanding biliary anatomy of the caudate lobe. *Am J Transplant* 2008; **8**: 271-274 [PMID: 18162089 DOI: 10.1111/j.1600-6143.2007.02069.x]
24 **Gastaca M**, Matarranz A, Muñoz F, Valdivieso A, Aguinaga A, Testillano M, Bustamante J, Terreros I, Suarez MJ, Montejo M, Ortiz de Urbina J. Biliary complications in orthotopic liver transplantation using choledochocholedochostomy with a T-tube. *Transplant Proc* 2012; **44**: 1554-1556 [PMID: 22841211 DOI: 10.1016/j.transproceed.2012.05.025]
25 **Sanchez-Urdazpal L**, Gores GJ, Ward EM, Maus TP, Wahlstrom HE, Moore SB, Wiesner RH, Krom RA. Ischemic-type biliary complications after orthotopic liver transplantation. *Hepatology* 1992; **16**: 49-53 [PMID: 1618482]
26 **Mahajani RV**, Cotler SJ, Uzer MF. Efficacy of endoscopic management of anastomotic biliary strictures after hepatic transplantation. *Endoscopy* 2000; **32**: 943-949 [PMID: 11147942 DOI: 10.1055/s-2000-9619]
27 **Thuluvath PJ**, Pfau PR, Kimmey MB, Ginsberg GG. Biliary complications after liver transplantation: the role of endoscopy. *Endoscopy* 2005; **37**: 857-863 [PMID: 16116539 DOI: 10.1055/s-2005-870192]
28 **Memeo R**, Ciacio O, Pittau G, Cherqui D, Castaing D, Adam R, Vibert E. Systematic computer tomographic scans 7 days after liver transplantation surgery can lower rates of repeat-transplantation due to arterial complications. *Transplant Proc* 2014; **46**: 3536-3542 [PMID: 25498085 DOI: 10.1016/j.transproceed.2014.04.017]
29 **Verdonk RC**, Buis CI, Porte RJ, van der Jagt EJ, Limburg AJ, van den Berg AP, Slooff MJ, Peeters PM, de Jong KP, Kleibeuker JH, Haagsma EB. Anastomotic biliary strictures after liver transplantation: causes and consequences. *Liver Transpl* 2006; **12**: 726-735 [PMID: 16628689 DOI: 10.1002/lt.20714]
30 **Gholson CF**, Zibari G, McDonald JC. Endoscopic diagnosis and management of biliary complications following orthotopic liver transplantation. *Dig Dis Sci* 1996; **41**: 1045-1053 [PMID: 8654132]
31 **Thuluvath PJ**, Atassi T, Lee J. An endoscopic approach to biliary complications following orthotopic liver transplantation. *Liver Int* 2003; **23**: 156-162 [PMID: 12955878]
32 **Gor NV**, Levy RM, Ahn J, Kogan D, Dodson SF, Cohen SM. Biliary cast syndrome following liver transplantation: Predictive factors and clinical outcomes. *Liver Transpl* 2008; **14**: 1466-1472 [PMID: 18825683 DOI: 10.1002/lt.21492]
33 **Shah JN**, Haigh WG, Lee SP, Lucey MR, Brensinger CM, Kochman ML, Long WB, Olthoff K, Shaked A, Ginsberg GG. Biliary casts after orthotopic liver transplantation: clinical factors, treatment, biochemical analysis. *Am J Gastroenterol* 2003; **98**: 1861-1867 [PMID: 12907345 DOI: 10.1111/j.1572-0241.2003.07617.x]
34 **Caputo M**, Piolanti M, Riccioli LA, Pazienza L, Fabbro E, Gruppioni F, Grazi G, Gavelli G. [Nonobstructive residual mucocele of the cystic duct. Reassessment of complications in our 13 years' experience with liver transplantation]. *Radiol Med* 2000; **100**: 354-356 [PMID: 11213414]
35 **Sankarankutty AK**, Mente ED, Cardoso NM, Castro-E-Silva O. T-tube or no T-tube for bile duct anastomosis in orthotopic liver transplantation. *Hepatobiliary Surg Nutr* 2013; **2**: 171-173 [PMID: 24570938 DOI: 10.3978/j.issn.2304-3881.2013.05.04]
36 **Rossi AF**, Grosso C, Zanasi G, Gambitta P, Bini M, De Carlis L, Rondinara G, Arcidiacono R. Long-term efficacy of endoscopic stenting in patients with stricture of the biliary anastomosis after orthotopic liver transplantation. *Endoscopy* 1998; **30**: 360-366 [PMID: 9689509 DOI: 10.1055/s-2007-1001283]
37 **Sanna C**, Saracco GM, Reggio D, Moro F, Ricchiuti A, Strignano P, Mirabella S, Ciccone G, Salizzoni M. Endoscopic retrograde cholangiopancreatography in patients with biliary complications after orthotopic liver transplantation: outcomes and complications. *Transplant Proc* 2009; **41**: 1319-1321 [PMID: 19460551 DOI: 10.1016/j.transproceed.2009.03.086]
38 **Llach J**, Bordas JM, Elizalde JI, Enrico C, Ginès A, Pellisé M, Mondelo F, Piqué JM. Sphincterotomy in the treatment of biliary leakage. *Hepatogastroenterology* 2012; **49**: 1496-1498 [PMID: 12397716]
39 **Gutierrez JP**, Smith IB, Wilcox CM, Mönkemüller K. Bile leak from the duct of Luschka treated with double-balloon enteroscopy ERCP in a patient with Roux-en-Y gastric bypass. *Endoscopy* 2013; **45** Suppl 2 UCTN: E404 [PMID: 24285072 DOI: 10.1055/s-0033-1344864]
40 **de Jong EA**, Moelker A, Leertouwer T, Spronk S, Van Dijk M, van Eijck CH. Percutaneous transhepatic biliary drainage in patients with postsurgical bile leakage and nondilated intrahepatic bile ducts. *Dig Surg* 2013; **30**: 444-450 [PMID: 24434644 DOI: 10.1159/000356711]
41 **Nacif LS**, Bernardo WM, Bernardo L, Andraus W, Torres L, Chaib E, D'Albuquerque LC, Maluf-Filho F. Endoscopic treatment of post-liver transplantation anastomotic biliary stricture: systematic review and meta-analysis. *Arq Gastroenterol* 2014; **51**: 240-249 [PMID: 25296086]
42 **Piardi T**, Greget M, Audet M, Calandra G, Gheza F, Ellero B, Woehl-Jaegle ML, Cantu M, Portolani N, Wolf P, Pessaux P. Biliary strictures after liver transplantation: is percutaneous treatment indicated? *Ann Transplant* 2015; **16**: 5-13 [PMID: 21716179]
43 **Lee SH**, Ryu JK, Woo SM, Park JK, Yoo JW, Kim YT, Yoon YB, Suh KS, Yi NJ, Lee JM, Han JK. Optimal interventional treatment and long-term outcomes for biliary stricture after liver transplantation. *Clin Transplant* 2015; **22**: 484-493 [PMID: 18318735 DOI: 10.1111/j.1399-0012.2008.00813.x]
44 **Zoepf T**, Maldonado-Lopez EJ, Hilgard P, Malago M, Broelsch CE, Treichel U, Gerken G. Balloon dilatation vs. balloon dilatation plus bile duct endoprostheses for treatment of anastomotic biliary strictures after liver transplantation. *Liver Transpl* 2006; **12**: 88-94 [PMID: 16382450 DOI: 10.1002/lt.20548]
45 **Kulaksiz H**, Weiss KH, Gotthardt D, Adler G, Stremmel W, Schaible A, Dogan A, Stiehl A, Sauer P. Is stenting necessary after balloon dilation of post-transplantation biliary strictures? Results of a prospective comparative study. *Endoscopy* 2008; **40**: 746-751 [PMID: 18702031 DOI: 10.1055/s-2008-1077489]
46 **Park JS**, Kim MH, Lee SK, Seo DW, Lee SS, Han J, Min YI, Hwang S, Park KM, Lee YJ, Lee SG, Sung KB. Efficacy of endoscopic and percutaneous treatments for biliary complications after cadaveric and living donor liver transplantation. *Gastrointest Endosc* 2003; **57**: 78-85 [PMID: 12518136 DOI: 10.1067/mge.2003.11]
47 **Tashiro H**, Itamoto T, Sasaki T, Ohdan H, Fudaba Y, Amano H, Fukuda S, Nakahara H, Ishiyama K, Ohshita A, Kohashi T, Mitsuta H, Chayama K, Asahara T. Biliary complications after duct-to-duct biliary reconstruction in living-donor liver transplantation: causes and treatment. *World J Surg* 2007; **31**: 2222-2229 [PMID: 17885788 DOI: 10.1007/s00268-007-9217-x]
48 **Kochar K**, Vallance K, Mathew G, Jadhav V. Intrahepatic perforation of the gall bladder presenting as liver abscess: case report, review of literature and Niemeier's classification. *Eur J Gastroenterol Hepatol* 2008; **20**: 240-244 [PMID: 18301308 DOI:

10.1097/MEG.0b013e3282eeb520]

49 **Verdonk RC**, Buis CI, van der Jagt EJ, Gouw AS, Limburg AJ, Slooff MJ, Kleibeuker JH, Porte RJ, Haagsma EB. Nonanastomotic biliary strictures after liver transplantation, part 2: Management, outcome, and risk factors for disease progression. *Liver Transpl* 2007; **13**: 725-732 [PMID: 17457935]

50 **Guichelaar MM**, Benson JT, Malinchoc M, Krom RA, Wiesner RH, Charlton MR. Risk factors for and clinical course of non-anastomotic biliary strictures after liver transplantation. *Am J Transplant* 2003; **3**: 885-890 [PMID: 12814481]

51 **Buis CI**, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, Porte RJ. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. *Liver Transpl* 2007; **13**: 708-718 [PMID: 17457932]

52 **Piardi T**, Panaro F, Gheza F, Wolf P. 'Cystic' lesion in the porta hepatis after liver transplantation. *Liver Int* 2011; **31**: 859 [PMID: 21645217 DOI: 10.1111/j.1478-3231.2011.02460.x]

P- Reviewer: Kobayashi A **S- Editor**: Song XX
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.i29.2896

World J Hepatol 2015 December 18; 7(29): 2896-2905
ISSN 1948-5182 (online)

REVIEW

Recurrence of autoimmune liver diseases after liver transplantation

Nabiha Faisal, Eberhard L Renner

Nabiha Faisal, Eberhard L Renner, Liver Transplant Program/Multiorgan Transplant Program, University Health Network/Toronto General Hospital, University of Toronto, Toronto, ON M5G 2N2, Canada

Author contributions: Both authors contributed equally regarding conception and design of this review; Faisal N searched and reviewed the literature, and drafted the manuscript; Renner EL reviewed the literature, and revised the manuscript.

Conflict-of-interest statement: None of the authors has a conflict of interest with regards to this review.

Correspondence to: Eberhard L Renner, MD, FRCP(C), Professor of Medicine, Director GI Transplantation, Liver Transplant Program/Multiorgan Transplant Program, University Health Network/Toronto General Hospital, University of Toronto, 585 University Avenue, 11 PMB 176, Toronto, ON M5G 2N2, Canada. eberhard.renner@uhn.ca
Telephone: +1-416-3405221
Fax: +1-416-3403126

Received: July 14, 2015
Peer-review started: July 22, 2015
First decision: August 25, 2015
Revised: November 7, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: December 18, 2015

Abstract

Liver transplantation (LT) is the most effective treatment modality for end stage liver disease caused by many etiologies including autoimmune processes. That said, the need for transplantation for autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), but not for primary sclerosing cholangitis (PSC), has decreased over the years due to the availability of effective medical treatment. Autoimmune liver diseases have superior transplant outcomes than those of other etiologies. While AIH and PBC can recur after LT, recurrence is of limited clinical significance in most, but not all cases. Recurrent PSC, however, often progresses over years to a stage requiring re-transplantation. The exact incidence and the predisposing factors of disease recurrence remain debated. Better understanding of the pathogenesis and the risk factors of recurrent autoimmune liver diseases is required to develop preventive measures. In this review, we discuss the current knowledge of incidence, diagnosis, risk factors, clinical course, and treatment of recurrent autoimmune liver disease (AIH, PBC, PSC) following LT.

Key words: Recurrent autoimmune hepatitis; Recurrent primary biliary cirrhosis; Recurrent primary sclerosing cholangitis; Liver transplantation; Immunosuppression; Risk factors; Outcomes; Autoimmune liver diseases

Core tip: There is compelling evidence that autoimmune liver disease recur after liver transplantation, with incidence rates ranging from 10% to 50%. Recurrent autoimmune hepatitis and primary biliary cirrhosis do rarely impair patient and graft survival, but may require changing the immunosuppressive regimen, using corticosteroids or adding ursodeoxycholic acid, respectively. In a proportion of patients, recurrent primary sclerosing cholangitis progresses over years to a stage requiring re-transplantation.

Faisal N, Renner EL. Recurrence of autoimmune liver diseases

after liver transplantation. *World J Hepatol* 2015; 7(29): 2896-2905 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2896.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2896

INTRODUCTION

Liver transplantation (LT) remains the most effective treatment for patients with end stages of autoimmune liver diseases [autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC)]. Overall, autoimmune liver diseases account for approximately one quarter of LT performed in Europe and the United States[1], with a 5-year post-LT survival rate of around 85%[2]. Despite these excellent outcomes, autoimmune liver diseases recur not infrequently in the allograft. The exact rates of recurrence are somewhat obscured by inconsistency in diagnostic approach and criteria employed. Since recurrent autoimmune liver disease may be asymptomatic and, at least early on, occur in the absence of biochemical or clinical abnormalities, centers that use protocol biopsies will report greater rates of recurrence. In addition, it has been reported that AIH in the graft can occur *de novo*, *i.e.*, after LT for non-autoimmune liver disorders[2,3].

In this review, we discuss the incidence, risk factors and newer developments in the understanding of the diagnosis, clinical course, and treatment of recurrent autoimmune liver diseases after LT. *De novo* autoimmune liver disease in the graft after LT is beyond the scope of this review.

SEARCH STRATEGY

This review is based on a systematic literature search in PubMed, supplemented by the authors' own clinical experience. Specifically, the following search terms were used: "liver transplantation", "recurrence", "autoimmune liver diseases", "AIH", "primary sclerosing cholangitis", "primary biliary cirrhosis" and "incidence". We also searched manually for articles of interest referenced in publications identified in the PubMed search.

PSC

PSC is a chronic progressive inflammatory disease affecting the extra- and/or intra-hepatic bile ducts that often progresses with biliary stricturing and fibrosis within a decade from diagnosis to cirrhosis, recurrent cholangitis and/or cholangiocarcinoma (CCA). PSC is the fourth leading diagnosis for LT in the United States[4]. Approximately 4% to 5% of adult LT in the western world are performed for PSC[5].

PSC is believed to be an autoimmune disease associated with certain immunologic factors including certain subtypes of human leukocyte antigens (HLA)[6] and the presence of antineutrophil cytoplasmic antibodies[7]. There is a strong association of PSC with inflammatory bowel disease of the colon (IBD; see below). In colonic IBD, gut bacteria and endotoxins may translocate across a chronically inflamed and more permeable colonic mucosa into the portal circulation. This may subsequently lead to activation of Kupffer cells and the release of pro-inflammatory cytokines mediating biliary tree inflammation[8-10]. Activated intestinal lymphocytes, which are released into the enterohepatic circulation and may persist as memory cells, may also be involved in generating hepatic inflammation[11]. Increased hepatic expression of adhesion molecules (vascular adhesion protein-1 and mucosal addressin cell adhesion molecule) in PSC contribute to recruitment of immune cells into the tissue[12,13]. It has been hypothesized that persistence of such aberrant homing of lymphocytes from the intestine into the liver may contribute to disease recurrence in the graft[13].

PSC develops in approximately 5% of patients with IBD. Conversely, up to 85% of patients with PSC ultimately develop IBD[14]. Prospective studies in children with PSC and IBD suggest that the progression of the liver disease is independent of the severity of IBD[15,16]. Studies in adult populations have yielded conflicting results regarding potential interaction between disease severity of IBD and PSC both, pre- and post-LT. Marelli *et al*[17] reported that PSC patients needing LT have often a relative benign clinical course of ulcerative colitis (UC). Similarly, Navaneethan *et al*[18] reported that UC tends to remain in remission or to improve after LT. On the contrary, Moncrief *et al*[19] found that the activity of IBD, both clinically and histologically, increases after LT.

Patents with PSC are at increased risk of developing hepatobiliary (CCA/gallbladder carcinoma) and colorectal neoplasias[20,21]. Since there is no effective medical therapy available for PSC, LT is the sole potentially curative therapeutic option for advanced disease. LT for PSC is associated with an excellent long term survival of more than 80% and 70% at 5 and 10 years, respectively[22,23]. Post LT, the disease recurs in about 20% (5%-50%) with a median time from LT to diagnosis of recurrence usually ranging from 3 to 5 years, depending on the type and timing of diagnostic procedures employed during follow-up[24-27].

DIAGNOSIS OF RECURRENT PSC

Diagnosing recurrent PSC is often challenging due to difficulty in distinguishing it from other conditions potentially leading to non-anastomotic biliary strictures. This includes ischemia related biliary insults associated with ischemia/reperfusion injury, hepatic artery thrombosis and/or chronic ductopenic rejection, bacterial or fungal cholangitis, and ABO incompatibility between the donor and the recipient[1,28,29]. The diagnosis largely relies on radiological demonstration of diffuse, non-anastomotic biliary strictures in a patient transplanted for PSC, provided any other of the aforementioned etiologies for diffuse, non-anastomotic biliary stricturing has been excluded. The diagnosis may be further supported by

Table 1 Mayo clinic criteria for recurrent primary sclerosing cholangitis after liver transplantation[12]

Inclusion criteria
Confirmed diagnosis of PSC before LT
Cholangiography showing non-anastomotic intra- and/or extra-hepatic biliary strictures with beading and irregularities of bile ducts at least 90 d after LT and/or
Histopathological findings of fibrous cholangitis and/or fibro-obliterative lesions
Exclusion criteria
Hepatic artery thrombosis or stenosis
Chronic ductopenic rejection
Anastomotic and non-anastomotic strictures before day 90 after LT
ABO incompatible LT

LT: Liver transplantation; PSC: Primary sclerosing cholangitis.

liver biopsy, but established fibro-obliterative bile duct lesion or periductal concentric fibrosis are seen in only a minority of patients with PSC recurrence[30]. Most commonly used criteria for the diagnosis of recurrent PSC after LT are the so called Mayo clinic criteria originally proposed by Graziadei *et al*[23] (Table 1). These criteria are conservative and, as mentioned above, require to rule out all aforementioned other causes for non-anastomotic biliary strictures in the graft.

RISK FACTORS FOR PSC RECURRENCE

Numerous risk factors for the PSC recurrence have been proposed including the following: Certain HLA associations with recipient or donor (HLA-DRB1*08, HLA DR52)[31,32]; male recipient[33], as with PSC in general, and a recipient-donor gender mismatch[29]; recipient age - albeit inconsistently (older or younger)[32,34]; an intact colon in the recipient prior to transplantation[33], and the presence of UC after LT[35]; use of extended donor criteria grafts[26]; acute cellular rejection (ACR)[32], steroid-resistant ACR[32,36] or use of OKT3[4]; maintenance of steroid therapy for UC for more than 3 mo[35]; the presence of CCA prior to LT[25]; and cytomegalovirus infection in the recipient[32,37]. A high rate of recurrence has been reported in recipients of grafts from first-degree living related donors, with PSC recurrence being observed in two relatively small single center series from Japan in 55% and 59% of recipients, respectively[38,39]. Standard immunosuppressive agents either cyclosporine or tacrolimus did not seem to affect PSC recurrence, neither did post-transplant (prophylactic) use of ursodeoxycholic acid (UDCA)[4,26].

Alabraba *et al*[27] observed in a large cohort (n = 230) that PSC recurred less frequently (P = 0.028) in patients who had undergone colectomy before or at the time of LT compared to patients with an intact colon post-LT. This seems to indicate that a residual (inflamed) colon is linked to PSC recurrence in the graft in a proportion of patients. Regardless of whether this association is related to an immune mechanism or a toxic effect[4,40], it underscores the importance of adequate control of colonic IBD post LT.

Several of the aforementioned associations, however, seem spurious such as the contradictory observations on recipient age. Others seem questionable, in particular, those linking PSC recurrence to risk factors for graft ischemia and to rejection (ACR, steroid resistant ACR, OKT3 use). These events predispose to ischemic type and/or alloimmune type non-anastomotic biliary stricturing, respectively, which casts doubt on the correct diagnosis of recurrent PSC in these cases. In addition, all reported associations are derived from retrospective analyses of relatively small single center series with all their inherent limitations. Before accepting them and drawing firm conclusions, they would need to be confirmed, ideally in a prospective manner, in independent patient cohorts.

MANAGEMENT OF RECURRENT PSC

There is no treatment of proven efficacy for recurrent PSC after LT. Prophylaxis and/or treatment with UDCA is practiced in many centers because it improves the liver biochemical profile, but whether its use affects outcomes remains uncertain[41,42]. In fact, the latter seems rather questionable, given the fact that UDCA does not benefit outcomes of PSC in the non-transplant setting[41]. That said, UDCA lowers the risk of developing colon dysplasia leading to colon adenomas and carcinomas in patients with UC and PSC; its use may be justified for that reason in this patient population also post LT[43]. In the absence of any effective medical prophylaxis/therapy of PSC recurrence post LT, symptomatic treatment of biliary strictures and their complications, such as cholangitis or choledocholithiasis, remains the only option. As in the non-transplant setting, dominant strictures may be managed temporarily by percutaneous or endoscopic means, but many patients with PSC recurrence post LT will eventually have to be considered for re-transplantation.

CLINICAL IMPACT OF RECURRENT PSC

Short and mid-term patient and graft survival do not appear to be impaired by PSC recurrence. However, it is now well recognized that PSC recurrence can affect graft outcome and may increase the need for re-transplantation and perhaps also impairs patient survival with longer patient follow-up[27,41,44]. Thus, the need for re-transplantation for graft failure secondary to recurrent disease is relevantly higher in PSC (12.4%) than in PBC (1%-5%)[45].

PBC

PBC is an immune mediated chronic cholestatic liver disease predominantly affecting middle aged women. It is characterized by circulating anti-mitochondrial antibodies (AMA) and progressive immune-mediated destruction of mid-sized intrahepatic bile ducts (intra-

Table 2 Diagnostic criteria for recurrent primary biliary cirrhosis after liver transplantation[49]

Confirmed diagnosis of PBC in the explant histology
Characteristic histologic features[1]
Lymphoplasmacytic portal infiltrate
Lymphoid aggregates
Epithelioid granulomas
Evidence of bile duct injury
Persistence of AMA or AMA-M2
Exclusion of other causes of graft dysfunction
Acute and chronic rejection
Graft *vs* host disease
Bile flow impairment or cholangitis
Vascular complications
Viral hepatitis
Drug induced hepatitis

[1]Definite recurrent PBC: 3 of 4 portal tract lesions are observed; Probable recurrent PBC: 2 of 4 portal tract lesions are observed. PBC: Primary biliary cirrhosis; AMA: Anti-mitochondrial antibodies.

lobular bile ductules). Over a decade or more, the persistent immune attack leads to bile duct paucity, fibrosis, and, ultimately, cirrhosis with its associated morbidity and mortality[46]. LT is the sole effective treatment option for end-stage PBC.

PBC is the third most common indication for LT (9%) in the European Liver Transplant Registry, after virus (hepatitis C and B) and alcohol related cirrhosis[47], and one of the top six indications for LT in the United States[2,48]. Of note, however, the number of patients transplanted and even that wait-listed for PBC has markedly decreased during more recent periods[49,50]. Moreover, PBC related deaths have decreased in both men and women over the last few decades. This decreased mortality/need for transplantation seems to be attributable to increased use of UDCA, which has been shown to slow down histological progression to cirrhosis and to improve overall and transplant-free survival[51].

Fatigue (85%) and pruritus (70%) are leading symptoms of PBC patients[52,53]. Environmental factors, genetic predispositions, and molecular mimicry triggering autoimmunity have all been implicated in the pathogenesis PBC, but their relative significance and the exact patho-mechanism(s) involved remain controversial[54]. PBC is considered one of "the best" indications for LT with 1, 5, and 10-year survival rates of 86%, 80% and 72%, respectively, according to the European Liver Transplant Registry[47]. The first report of PBC recurrence after LT was published in 1982[55]. Reported disease recurrence rates range from 10% to 50%[24,56-58], during a median time of 3-5.5 years of follow-up post LT[59]. However, the exact frequency of recurrent PBC, its time course, and its effect on patient and graft survival remain ill-defined, as routine follow-up with protocol liver biopsy is not standard for PBC patients in most LT programs.

DIAGNOSIS OF RECURRENT PBC

The diagnosis of recurrent PBC can be difficult, as the diagnostic criteria used in the pre-transplant setting are obscured post LT by the following: Only a minority (12%) of patients with recurrent PBC report potentially disease-related, but typically non-specific symptoms[60]. AMA, the serologic hallmark of PBC, and elevated serum immunoglobulins M remain present beyond LT and therefore lose their diagnostic value for disease recurrence. This also implies that the (auto)immune mechanism(s) driving PBC are not affected by replacing the liver, nor by post-LT immunosuppression[2]. A cholestatic liver enzyme pattern with elevated alkaline phosphatase and gamma glutamyl transpeptidase is entirely non-specific after LT and found in many other common conditions including acute or chronic rejection, viral infections, and bile duct or hepatic vein/artery pathology, to name just a few. Even the hallmark histologic feature of PBC in the non-transplant setting, immune mediated injury of small bile ducts and bile duct paucity maybe mimicked post LT by acute and chronic rejection. Only if these typical histologic findings co-occur in the presence of biliary epitheloid granulomas the diagnosis can be regarded as histologically proven. The following criteria first described by Hubscher *et al*[61], have been widely adopted to diagnose recurrent PBC after LT (Table 2).

RISK FACTORS FOR RECURRENT PBC

A potential impact on the development of recurrent PBC of donor and recipient age, duration of cold and warm ischemia, number of HLA mismatches, and immunosuppressive regimen post LT remain controversial. Morioka *et al*[62] reported that little donor/recipient HLA mismatch was an independent risk factor for disease recurrence following living related donor LT. Two other large studies have shown an association between HLA-mismatches, especially in the DR-locus, and recurrent PBC also in deceased donor LT[63,64]. In contrast, Jacob *et al*[65] found that patients without a HLA-B mismatch were at higher risk of disease recurrence.

Several, but not all analyses (mostly retrospective, single center series) reported that, when compared with cyclosporine, tacrolimus-based immunosuppression is associated with a higher frequency and shorter time to PBC recurrence post LT[50,66,67]. However, the large meta-analysis by Gautam *et al*[24] evaluating 16 studies summarizing a total of 1241 patients, failed to confirm that tacrolimus and cyclosporine based immunosuppressive regimens are differentially associated with PBC recurrence. An appropriately sized, prospective, randomized control trial would be required to definitely resolve this issue. Such a study, however, seems unlikely to be ever conducted for various reasons not the least of which being the rarity of PBC as an indication for LT

these days.

MANAGEMENT OF RECURRENT PBC

The Mayo group was the first to report that treatment with UDCA normalizes liver enzymes in about half of the patients with PBC recurrence, but in only one fifth of untreated patients[68]. Despite that, UDCA treatment was not shown to be associated with significant changes in liver histology, patient and graft survival[63]. Whether UDCA affects the course of recurrent PBC post LT remains, therefore, somewhat unclear, although a beneficial effect might be anticipated given its well documented effects on disease progression and transplant-free survival in the non-transplant setting[69,70].

Moreover, the preliminary results of a French multicenter study presented in 2015 at the International Liver Meeting of the European Association for the Study of the Liver suggested a beneficial effect of UDCA in preventing PBC recurrence post LT[71]. The authors reported on 90 PBC LT recipients followed for an average of 12 years, 19 of which were on UDCA since LT. Recurrence was diagnosed in 48 (53%) patients. In both, univariate and multivariate Cox models, use of UDCA was the only factor that significantly affected the risk of PBC recurrence (HR = 0.31, 95%CI: 0.11-0.85). While this may suggest a role for UDCA treatment as prophylaxis for PBC recurrence after LT, neither disease recurrence itself, nor UDCA prophylaxis did affect post-LT patent/graft survival.

That said, and given the ever increasing survival rate and life-expectancy after LT, PBC recurrence might become clinically relevant in the future. Thus, a proportion of patients may live long enough to develop clinically relevant disease in the graft. While the French study supports the use of UDCA as prophylaxis for PBC recurrence after LT, its final publication, and confirmation in independent patient cohorts, ideally with hard end-points such as patient and graft survival, are required before accepting UDCA prophylaxis as standard of care in clinical practice.

In the non-transplant setting, several novel drugs have recently been suggested to be benefit PBC patients who do not completely respond to UDCA[72]. The preliminary report of the phase Ⅲ POISE multinational trial revealed promising results in treating PBC patients with obeticholic acid, a farnesoid X receptor agonist[73]. Several small studies suggested Bezafibrate, targeting the pregnane X receptor and peroxisome proliferator-activated receptor alfa, to hold promise for PBC treatment[72]. Whether these newer therapies will in the future play a role in the management of recurrent PBC after LT remains to be seen.

CLINICAL IMPACT OF RECURRENT PBC

Even if recurring, PBC in the graft seems to hardly ever affect outcomes. Thus, overall long-term graft and patient survival was not affected in any of the published reports[45,59,65]. In the two largest reported experiences with LT for PBC, only 3 out of 485 and 2 out of 154 cases, respectively, required re-transplantation[69,74]. While recurrent PBC has also been described after a second and third LT, the proportion of graft failure due to disease recurrence seems again low after re-LT (7%-14%)[75].

AUTOIMMUNE HEPATITIS

AIH is associated with human leukocytes antigens DR3 or DR4. AIH is a relatively rare, progressive, inflammatory chronic liver disease that mainly affects women and is typically associated with circulating autoantibodies (in particular, high titers of antinuclear and anti-smooth muscle antibodies) and increased levels of serum immunoglobulin G (IgG). Its etio-pathogenesis remains ill defined, but both, genetic predisposition and triggering environmental factors are thought to play a role[76,77]. LT may be indicated in AIH for both, end-stage cirrhosis with liver failure and/or complications of portal hypertension, and severe acute flairs. The latter can present as a picture mimicking fulminant hepatic failure, but in most cases is an acute exacerbation of the pre-existing, underlying chronic disease. That said, AIH is globally one of the rarer indications for LT, likely due to a combination of low incidence rates and highly effective medical therapy (corticosteroids and other immunosuppressive regimens). Medical therapy is able to prevent disease progression and to avoid LT in almost 90% of patients. In the non-transplant setting, treatment of AIH with immunosuppressive regimens has been reported to be associated with excellent 10-year survival rates ranging from 80% to 93%[78-81]. That said, around 10% of patients will eventually require LT, in particular when medical treatment does not lead to remission within 4 years[82].

Thus, AIH accounts for only 4%-6% of LT in the United States[83] and 3% in Europe[84]. LT for end-stage AIH achieves excellent outcomes, patient survival rates at 1 and 5 years amounting to approximately 90 and 80%, respectively[85-87]. AIH recurrence in the allograft was first reported by the King's College group in 1984[88] and, subsequently, confirmed by several other reports[89,90]. Recurrence rates between 16% and 43% have been reported for patients transplanted for AIH-related cirrhosis[85,91-93]. That said, similarly to PSC and PBC, the exact frequency of recurrent AIH, its time course, and its impact on patient and graft survival remains ill defined, as routine follow-up with protocol liver biopsy is not standard for AIH patients in most LT programs.

DIAGNOSIS OF RECURRENT AIH

There is no single specific biomarker to diagnose recurrent AIH after LT. The diagnosis rests on the presence of the diagnostic criteria summarized in Table 3. It is essential to rule out other etiologies causing a hepatitic

Table 3 Diagnostic criteria of recurrent autoimmune hepatitis[60,64,69]

Liver transplantation for confirmed diagnosis of autoimmune hepatitis
Elevated transaminases
Hyper-gammaglobulinemia (elevation of IgG)
Presence of autoantibodies (ANA, SMA and/or anti-LKM1)
Compatible histopathology (interface hepatitis with portal inflammation and/or lymphoplasmacytic inflammatory infiltrates)
Response to corticosteroid
Exclusion of differential diagnostic considerations (including late/atypcial, acute cellular rejection)

IgG: Immunoglobulin G; ANA: Antinuclear antibodies; SMA: Smooth muscle antibodies; LKM1: Liver/kidney microsomal antibodies type 1.

pattern of liver damage including ACR, viral infections/hepatitis, and drug induced liver injury[77,85,94]. In our experience, it can be particularly difficult to discriminate between late, often histologically somewhat atypical ACR and recurrent AIH. However, treatment of both requires increased immunosuppression and distinction between these latter two differentials may therefore be of limited consequence for immediate clinical management.

RISK FACTORS FOR RECURRENT AIH

Several risk factors including certain HLA antigen patterns have been reported to predispose to recurrent AIH post LT, but the results are conflicting and risk factors for recurrent AIH remain ill defined. A strong association of HLA DR3 or HLA DR4 was observed by some, but others failed to find an association with HLA phenotype[85,88,95-97]. Recurrent disease seems not associated with incidence rates of ACR nor with the degree of donor/recipient HLA mismatching[85]. In addition, the incidence of AIH recurrence was not different with cyclosporine *vs* tacrolimus based immunosuppression, nor associated with pre-transplant or post-transplant overall dose and duration of corticosteroid treatment[24]. Rapid weaning of steroids post LT has been suggested to be associated with higher recurrence rates and may therefore need to be exercised with caution in patients transplanted for AIH[86,87,95]. AIH that is poorly controlled prior to LT has been reported to be associated with a higher risk of recurrence post LT. Thus, Ayata *et al*[98] found that severe necro-inflammatory activity in the explant predicts AIH recurrence. In addition, coexistent autoimmune diseases, and high transaminases and IgG prior to transplant have been reported to be associated with an increased risk of AIH recurrence[97]. Collectively, however, the risk factors and their relative contribution to AIH recurrence post LT remain ill defined.

CLINICAL IMPACT AND MANAGEMENT OF RECURRENCE AIH

The majority of patients with recurrent AIH responds to intensified immunosuppressive therapy either in the form of re-introduction/addition or increasing the dose of corticosteroids and/or other immunosuppressive agents[98,99]. In treatment failure, augmenting the standard immunosuppressive regimen with a mycophenolate preparation[75], switching from one to the other calcineurin inhibitor (CNI)[98], or replacing CNI with sirolimus have all been successfully tried[100]. With this in mind, early diagnosis is key for successfully managing AIH recurrence[101], and long-term outcomes do not appear to be impaired in the vast majority of patients, fewer than 5% requiring re-LT for disease recurrence[40,102-104].

CONCLUSION

LT for end-stages of autoimmune liver diseases is associated with excellent long term patient and graft survival. While the underlying autoimmune liver disease recurs after LT in a proportion of patients, disease recurrence rarely affects graft and patients survival, with the exception of recurrent PSC. The latter not infrequently progresses over years to a stage requiring re-transplantation.

That said, the exact incidence rates, outcomes, and risk factors for the post LT recurrence of the underlying autoimmune liver disease remain somewhat ill defined due to diagnostic difficulties. Moreover, the risk/benefit ratio of protocol biopsies during long-term follow-up in this patient population remains unclear. While beyond the scope of this review, this may explain, at least in part, the absence of systematic screening (including protocol biopsies) in most programs.

In order to address these uncertainties and to elucidate potential risk factors for recurrent disease allowing to develop preventative strategies, in particular for recurrent PSC, prospective, adequately powered multicenter studies with longer follow-up is required.

REFERENCES

1 **Ilyas JA**, O'Mahony CA, Vierling JM. Liver transplantation in autoimmune liver diseases. *Best Pract Res Clin Gastroenterol* 2011; **25**: 765-782 [PMID: 22117641 DOI: 10.1016/j.bpg.09.008]

2 **Vergani D**, Mieli-Vergani G. Autoimmunity after liver transplantation. *Hepatology* 2002; **36**: 271-276 [PMID: 12143033 DOI: 10.1053/jhep.02.35339]

3 **Czaja AJ**. Autoimmune hepatitis after liver transplantation and other lessons of self-intolerance. *Liver Transpl* 2002; **8**: 505-513 [PMID: 12037780 DOI: 10.1053/jlts.2002.33485]

4 **Kugelmas M**, Spiegelman P, Osgood MJ, Young DA, Trotter JF, Steinberg T, Wachs ME, Bak T, Kam I, Everson GT. Different immunosuppressive regimens and recurrence of primary sclerosing cholangitis after liver transplantation. *Liver Transpl* 2003; **9**: 727-732 [PMID: 12827560 DOI: 10.1053/jlts.2003.50143]

5 **Duclos-Vallee JC**, Sebagh M. Recurrence of autoimmune disease, primary sclerosing cholangitis, primary biliary cirrhosis, and autoimmune hepatitis after liver transplantation. *Liver Transpl* 2009; **15** Suppl 2: S25-S34 [PMID: 19876939]

6 **Quigley EM**, LaRusso NF, Ludwig J, MacSween RN, Birnie GG, Watkinson G. Familial occurrence of primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology* 1983; **85**: 1160-1165 [PMID: 6618106]

7 **Bansi D**, Chapman R, Fleming K. Antineutrophil cytoplasmic antibodies in chronic liver diseases: prevalence, titre, specificity and IgG subclass. *J Hepatol* 1996; **24**: 581-586 [PMID: 8773914 DOI: 10.1016/S0168-8278(96)80144-9]

8 **Lee YM**, Kaplan MM. Primary sclerosing cholangitis. *N Engl J Med* 1995; **332**: 924-933 [PMID: 7877651 DOI: 10.1056/NEJM199504063321406]

9 **Fausa O**, Schrumpf E, Elgjo K. Relationship of inflammatory bowel disease and primary sclerosing cholangitis. *Semin Liver Dis* 1991; **11**: 31-39 [PMID: 2047887 DOI: 10.1055/s-2008-1040420]

10 **Aoki CA**, Bowlus CL, Gershwin ME. The immunobiology of primary sclerosing cholangitis. *Autoimmun Rev* 2005; **4**: 137-143 [PMID: 15823499 DOI: 10.1016/j.autrev.2004.09.003]

11 **Adams DH**, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. *Nat Rev Immunol* 2006; **6**: 244-251 [PMID: 16498453 DOI: 10.1038/nri1784]

12 **Salmi M**, Jalkanen S. Endothelial ligands and homing of mucosal leukocytes in extraintestinal manifestations of IBD. *Inflamm Bowel Dis* 1998; **4**: 149-156 [PMID: 9589300]

13 **Grant AJ**, Lalor PF, Hübscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). *Hepatology* 2001; **33**: 1065-1072 [PMID: 11343233 DOI: 10.1053/jhep.2001.24231]

14 **Loftus EV**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [PMID: 15591511 DOI: 10.1136/gut.2004.046615]

15 **Mieli-Vergani G**, Vergani D. Paediatric autoimmune liver disease. *Arch Dis Child* 2013; **98**: 1012-1017 [PMID: 24001955 DOI: 10.1136/archdischild-2013-303848]

16 **Miloh T**, Anand R, Yin W, Vos M, Kerkar N, Alonso E. Pediatric liver transplantation for primary sclerosing cholangitis. *Liver Transpl* 2011; **17**: 925-933 [PMID: 21506253 DOI: 10.1002/lt.22320]

17 **Marelli L**, Xirouchakis E, Kalambokis G, Cholongitas E, Hamilton MI, Burroughs AK. Does the severity of primary sclerosing cholangitis influence the clinical course of associated ulcerative colitis? *Gut* 2011; **60**: 1224-1228 [PMID: 21402617 DOI: 10.1136/gut.2010]

18 **Navaneethan U**, Venkatesh PG, Mukewar S, Lashner BA, Remzi FH, McCullough AJ, Kiran RP, Shen B, Fung JJ. Progressive primary sclerosing cholangitis requiring liver transplantation is associated with reduced need for colectomy in patients with ulcerative colitis. *Clin Gastroenterol Hepatol* 2012; **10**: 540-546 [PMID: 22245961 DOI: 10.1016/j.cgh.2012.01.006]

19 **Moncrief KJ**, Savu A, Ma MM, Bain VG, Wong WW, Tandon P. The natural history of inflammatory bowel disease and primary sclerosing cholangitis after liver transplantation--a single-centre experience. *Can J Gastroenterol* 2010; **24**: 40-46 [PMID: 20186355]

20 **Boberg KM**, Lind GE. Primary sclerosing cholangitis and malignancy. *Best Pract Res Clin Gastroenterol* 2011; **25**: 753-764 [PMID: 22117640 DOI: 10.1016/j.bpg.2011.10.002]

21 **Ngu JH**, Gearry RB, Wright AJ, Stedman CA. Inflammatory bowel disease is associated with poor outcomes of patients with primary sclerosing cholangitis. *Clin Gastroenterol Hepatol* 2011; **9**: 1092-1097; quiz e135 [PMID: 21893134 DOI: 10.1016/j.cgh.2011.08.027]

22 **Tischendorf JJ**, Hecker H, Krüger M, Manns MP, Meier PN. Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study. *Am J Gastroenterol* 2007; **102**: 107-114 [PMID: 17037993 DOI: 10.1111/j.1572-0241.2006.00872.x]

23 **Graziadei IW**, Wiesner RH, Marotta PJ, Porayko MK, Hay JE, Charlton MR, Poterucha JJ, Rosen CB, Gores GJ, LaRusso NF, Krom RA. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. *Hepatology* 1999; **30**: 1121-1127 [PMID: 10534330 DOI: 10.1002/hep.510300501]

24 **Gautam M**, Cheruvattath R, Balan V. Recurrence of autoimmune liver disease after liver transplantation: a systematic review. *Liver Transpl* 2006; **12**: 1813-1824 [PMID: 17031826 DOI: 10.1002/lt.20910]

25 **Charatcharoenwitthaya P**, Lindor KD. Recurrence of primary sclerosing cholangitis: what do we learn from several transplant centers? *Liver Transpl* 2008; **14**: 130-132 [PMID: 18236444 DOI: 10.1002/lt.21403]

26 **Campsen J**, Zimmerman MA, Trotter JF, Wachs M, Bak T, Steinberg T, Kam I. Clinically recurrent primary sclerosing cholangitis following liver transplantation: a time course. *Liver Transpl* 2008; **14**: 181-185 [PMID: 18236392 DOI: 10.1002/lt.21313]

27 **Alabraba E**, Nightingale P, Gunson B, Hubscher S, Olliff S, Mirza D, Neuberger J. A re-evaluation of the risk factors for the recurrence of primary sclerosing cholangitis in liver allografts. *Liver Transpl* 2009; **15**: 330-340 [PMID: 19243003 DOI: 10.1002/lt.21679]

28 **Fosby B**, Karlsen TH, Melum E. Recurrence and rejection in liver transplantation for primary sclerosing cholangitis. *World J Gastroenterol* 2012; **18**: 1-15 [PMID: 22228965 DOI: 10.3748/wjg.v18.i1.1]

29 **Khettry U**, Keaveny A, Goldar-Najafi A, Lewis WD, Pomfret EA, Pomposelli JJ, Jenkins RL, Gordon FD. Liver transplantation for primary sclerosing cholangitis: a long-term clinicopathologic study. *Hum Pathol* 2003; **34**: 1127-1136 [PMID: 14652814 DOI: 10.1053/j.humpath.2003.07.015]

30 **O'Grady JG**. Phenotypic expression of recurrent disease after liver transplantation. *Am J Transplant* 2010; **10**: 1149-1154 [PMID: 20353464 DOI: 10.1111/j.1600-6143.2010.03080]

31 **Alexander J**, Lord JD, Yeh MM, Cuevas C, Bakthavatsalam R, Kowdley KV. Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation. *Liver Transpl* 2008; **14**: 245-251 [PMID: 18236405 DOI: 10.1002/lt.21394]

32 **Jeyarajah DR**, Netto GJ, Lee SP, Testa G, Abbasoglu O, Husberg BS, Levy MF, Goldstein RM, Gonwa TA, Tillery GW, Crippin JS, Klintmalm GB. Recurrent primary sclerosing cholangitis after orthotopic liver transplantation: is chronic rejection part of the disease process? *Transplantation* 1998; **66**: 1300-1306 [PMID: 9846512]

33 **Vera A**, Moledina S, Gunson B, Hubscher S, Mirza D, Olliff S, Neuberger J. Risk factors for recurrence of primary sclerosing cholangitis of liver allograft. *Lancet* 2002; **360**: 1943-1944 [PMID: 12493264]

34 **Abu-Elmagd K**, Demetris J, Rakela J, Kang Y, Martin D, Dvorchik I, McMicheal J, Balan V, Starzl T, Fung J. Recurrence of primary sclerosing cholangitis (PSC) after hepatic transplantation (HTx): single center experience with 380 grafts. Abstract of the 18th Annual Meeting of the American Society of Transplantation (AST), May 15-19, 1999. *Transplantation* 1999; **67**: S236 [DOI: 10.1097/00007890-199904150-00941]

35 **Cholongitas E**, Shusang V, Papatheodoridis GV, Marelli L, Manousou P, Rolando N, Patch D, Rolles K, Davidson B, Burroughs AK. Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation. *Liver Transpl* 2008; **14**: 138-143 [PMID: 18236447 DOI: 10.1002/lt.21260]

36 **Brandsaeter B**, Schrumpf E, Bentdal O, Brabrand K, Smith HJ, Abildgaard A, Clausen OP, Bjoro K. Recurrent primary sclerosing cholangitis after liver transplantation: a magnetic resonance cholangiography study with analyses of predictive factors. *Liver Transpl* 2005; **11**: 1361-1369 [PMID: 16184522 DOI: 10.1002/lt.20444]

37 **Egawa H**, Taira K, Teramukai S, Haga H, Ueda Y, Yonezawa A, Masuda S, Tsuji H, Ashihara E, Takada Y, Uemoto S. Risk factors for recurrence of primary sclerosing cholangitis after living donor liver transplantation: a single center experience. *Dig Dis Sci* 2009; **54**: 1347-1354 [PMID: 19267192 DOI: 10.1007/s10620-009-0773-9]

38 **Tamura S**, Sugawara Y, Kaneko J, Matsui Y, Togashi J, Makuuchi

M. Recurrence of primary sclerosing cholangitis after living donor liver transplantation. *Liver Int* 2007; **27**: 86-94 [PMID: 17241386 DOI: 10.1111/j.1478-3231.2006.01395.x]

39 **Haga H**, Miyagawa-Hayashino A, Taira K, Morioka D, Egawa H, Takada Y, Manabe T, Uemoto S. Histological recurrence of autoimmune liver diseases after living-donor liver transplantation. *Hepatol Res* 2007; **37** Suppl 3: S463-S469 [PMID: 17931204 DOI: 10.1111/j.1872-034X.2007.00245.x]

40 **Schreuder TC**, Hübscher SG, Neuberger J. Autoimmune liver diseases and recurrence after orthotopic liver transplantation: what have we learned so far? *Transpl Int* 2009; **22**: 144-152 [PMID: 18662365 DOI: 10.1111/j.1432-2277.2008.00729]

41 **Carbone M**, Neuberger J. Liver transplantation in PBC and PSC: indications and disease recurrence. *Clin Res Hepatol Gastroenterol* 2011; **35**: 446-454 [PMID: 21459072 DOI: 10.1016/j.clinre.2011.02.007]

42 **Graziadei IW**. Recurrence of nonviral liver diseases after liver transplantation. *Clin Liver Dis* 2014; **18**: 675-685 [PMID: 25017083 DOI: 10.1016/j.cld.2014.05.009]

43 **Pardi DS**, Loftus EV, Kremers WK, Keach J, Lindor KD. Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. *Gastroenterology* 2003; **124**: 889-893 [PMID: 12671884 DOI: 10.1053/gast.2003.50156]

44 **Rowe IA**, Webb K, Gunson BK, Mehta N, Haque S, Neuberger J. The impact of disease recurrence on graft survival following liver transplantation: a single centre experience. *Transpl Int* 2008; **21**: 459-465 [PMID: 18225996 DOI: 10.1111/j.1432-2277.2007.00628]

45 **Maheshwari A**, Yoo HY, Thuluvath PJ. Long-term outcome of liver transplantation in patients with PSC: a comparative analysis with PBC. *Am J Gastroenterol* 2004; **99**: 538-542 [PMID: 15056099 DOI: 10.1111/j.1572-0241.2004.04050.x]

46 **Vergani D**, Bogdanos DP, Baum H. Unusual suspects in primary biliary cirrhosis. *Hepatology* 2004; **39**: 38-41 [PMID: 14752820 DOI: 10.1002/hep.2002]

47 **European Liver Transplant Registry**. Available from: URL: http://www.eltr.org/

48 **Neuberger J**. Liver transplantation for primary biliary cirrhosis. *Autoimmun Rev* 2003; **2**: 1-7 [PMID: 12848968 DOI: 10.1016/S1568-9972(02)00103-9]

49 **Lee J**, Belanger A, Doucette JT, Stanca C, Friedman S, Bach N. Transplantation trends in primary biliary cirrhosis. *Clin Gastroenterol Hepatol* 2007; **5**: 1313-1315 [PMID: 17900996]

50 **Liermann Garcia RF**, Evangelista Garcia C, McMaster P, Neuberger J. Transplantation for primary biliary cirrhosis: retrospective analysis of 400 patients in a single center. *Hepatology* 2001; **33**: 22-27 [PMID: 11124816]

51 **Mendes FD**, Kim WR, Pedersen R, Therneau T, Lindor KD. Mortality attributable to cholestatic liver disease in the United States. *Hepatology* 2008; **47**: 1241-1247 [PMID: 18318437 DOI: 10.1002/hep.22178]

52 **Huet PM**, Deslauriers J, Tran A, Faucher C, Charbonneau J. Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. *Am J Gastroenterol* 2000; **95**: 760-767 [PMID: 10710071 DOI: 10.1111/j.1572-0241.2000.01857.x]

53 **Bergasa NV**. Pruritus in primary biliary cirrhosis: pathogenesis and therapy. *Clin Liver Dis* 2008; **12**: 385-406; x [PMID: 18456187 DOI: 10.1016/j.cld.2008.02.013]

54 **Gershwin ME**, Mackay IR. The causes of primary biliary cirrhosis: Convenient and inconvenient truths. *Hepatology* 2008; **47**: 737-745 [PMID: 18098322 DOI: 10.1002/hep.22042]

55 **Neuberger J**, Portmann B, Macdougall BR, Calne RY, Williams R. Recurrence of primary biliary cirrhosis after liver transplantation. *N Engl J Med* 1982; **306**: 1-4 [PMID: 7031471 DOI: 10.1056/NEJM198201073060101]

56 **Hashimoto E**, Shimada M, Noguchi S, Taniai M, Tokushige K, Hayashi N, Takasaki K, Fuchinoue S, Ludwig J. Disease recurrence after living liver transplantation for primary biliary cirrhosis: a clinical and histological follow-up study. *Liver Transpl* 2001; **7**: 588-595 [PMID: 11460225]

57 **Sylvestre PB**, Batts KP, Burgart LJ, Poterucha JJ, Wiesner RH. Recurrence of primary biliary cirrhosis after liver transplantation: Histologic estimate of incidence and natural history. *Liver Transpl* 2003; **9**: 1086-1093 [PMID: 14526404 DOI: 10.1053/jlts.2003.50213]

58 **Sanchez EQ**, Levy MF, Goldstein RM, Fasola CG, Tillery GW, Netto GJ, Watkins DL, Weinstein JS, Murray NG, Byers D, Christensen LL, Klintmalm GB. The changing clinical presentation of recurrent primary biliary cirrhosis after liver transplantation. *Transplantation* 2003; **76**: 1583-1588 [PMID: 14702528 DOI: 10.1097/01.TP.0000090867.83666.F7]

59 **Khettry U**, Anand N, Faul PN, Lewis WD, Pomfret EA, Pomposelli J, Jenkins RL, Gordon FD. Liver transplantation for primary biliary cirrhosis: a long-term pathologic study. *Liver Transpl* 2003; **9**: 87-96 [PMID: 12514778 DOI: 10.1053/jlts.2003.36392]

60 **Silveira MG**, Talwalkar JA, Lindor KD, Wiesner RH. Recurrent primary biliary cirrhosis after liver transplantation. *Am J Transplant* 2010; **10**: 720-726 [PMID: 20199502 DOI: 10.1111/j.1600-6143.2010.03038]

61 **Hubscher SG**, Elias E, Buckels JA, Mayer AD, McMaster P, Neuberger JM. Primary biliary cirrhosis. Histological evidence of disease recurrence after liver transplantation. *J Hepatol* 1993; **18**: 173-184 [PMID: 8409333 DOI: 10.1016/S0168-8278(05)80244-2]

62 **Morioka D**, Egawa H, Kasahara M, Jo T, Sakamoto S, Ogura Y, Haga H, Takada Y, Shimada H, Tanaka K. Impact of human leukocyte antigen mismatching on outcomes of living donor liver transplantation for primary biliary cirrhosis. *Liver Transpl* 2007; **13**: 80-90 [PMID: 16964594 DOI: 10.1002/lt.20856]

63 **Guy JE**, Qian P, Lowell JA, Peters MG. Recurrent primary biliary cirrhosis: peritransplant factors and ursodeoxycholic acid treatment post-liver transplant. *Liver Transpl* 2005; **11**: 1252-1257 [PMID: 16184542 DOI: 10.1002/lt.20511]

64 **Balan V**, Ruppert K, Demetris AJ, Ledneva T, Duquesnoy RJ, Detre KM, Wei YL, Rakela J, Schafer DF, Roberts JP, Everhart JE, Wiesner RH. Long-term outcome of human leukocyte antigen mismatching in liver transplantation: results of the National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Hepatology* 2008; **48**: 878-888 [PMID: 18752327 DOI: 10.1002/hep.22435]

65 **Jacob DA**, Neumann UP, Bahra M, Klupp J, Puhl G, Neuhaus R, Langrehr JM. Long-term follow-up after recurrence of primary biliary cirrhosis after liver transplantation in 100 patients. *Clin Transplant* 2006; **20**: 211-220 [PMID: 16640529 DOI: 10.1111/j.1399-0012.2005.00471]

66 **Wong PY**, Portmann B, O'Grady JG, Devlin JJ, Hegarty JE, Tan KC, Williams R. Recurrence of primary biliary cirrhosis after liver transplantation following FK506-based immunosuppression. *J Hepatol* 1993; **17**: 284-287 [PMID: 7686192]

67 **Dmitrewski J**, Hubscher SG, Mayer AD, Neuberger JM. Recurrence of primary biliary cirrhosis in the liver allograft: the effect of immunosuppression. *J Hepatol* 1996; **24**: 253-257 [PMID: 8778189 DOI: 10.1016/S0168-8278(96)80001-8]

68 **Neuberger J**, Gunson B, Hubscher S, Nightingale P. Immunosuppression affects the rate of recurrent primary biliary cirrhosis after liver transplantation. *Liver Transpl* 2004; **10**: 488-491 [PMID: 15048790 DOI: 10.1002/lt.20123]

69 **Charatcharoenwitthaya P**, Pimentel S, Talwalkar JA, Enders FT, Lindor KD, Krom RA, Wiesner RH. Long-term survival and impact of ursodeoxycholic acid treatment for recurrent primary biliary cirrhosis after liver transplantation. *Liver Transpl* 2007; **13**: 1236-1245 [PMID: 17763401 DOI: 10.1002/lt.21124]

70 **Poupon RE**, Lindor KD, Cauch-Dudek K, Dickson ER, Poupon R, Heathcote EJ. Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. *Gastroenterology* 1997; **113**: 884-890 [PMID: 9287980 DOI: 10.1016/S0016-5085(97)70183-5]

71 **Bosch A**, Dumortier J, Boulch DM, Conti F, Morard I, Rubbia-Brandt L, Scoazec JY, Wendum D, Terris B, Boillot O, Radenne

S, Chazouillères O, Calmus Y, Giostra E, Corpechot C. Long term administration of UDCA prevents recurrence of primary biliary cirrhosis after liver transplantation. Poster P1148 EASL ILCA 2015. *J Hepatol* 2015; **62** Supplement 2: Page S783 [DOI: 10.1016/S0168-8278(15)31345-3]

72 **Karlsen TH**, Vesterhus M, Boberg KM. Review article: controversies in the management of primary biliary cirrhosis and primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2014; **39**: 282-301 [PMID: 24372568 DOI: 10.1111/apt.12581]

73 **Nevens F**, Andreone P, Mazzella G, Strasser s, Bowlus C, Invernizzi P. The first primary biliary cirrhosis (PBC) phase 3 trial in two decades – an international study of the FXR agonist obeticholic acid in PBC patients. *J Hepatol* 2014; **60** (S1): 525 [DOI: 10.1016/S0168-8278(14)61463-X]

74 **Jacob DA**, Neumann UP, Bahra M, Langrehr JM, Neuhaus P. Liver transplantation for primary biliary cirrhosis: influence of primary immunosuppression on survival. *Transplant Proc* 2005; **37**: 1691-1692 [PMID: 15919432 DOI: 10.1016/j.transproceed.2005.03.130]

75 **Jacob DA**, Bahra M, Schmidt SC, Schumacher G, Weimann A, Neuhaus P, Neumann UP. Mayo risk score for primary biliary cirrhosis: a useful tool for the prediction of course after liver transplantation? *Ann Transplant* 2008; **13**: 35-42 [PMID: 18806733]

76 **Krawitt EL**. Autoimmune hepatitis. *N Engl J Med* 2006; **354**: 54-66 [PMID: 16394302]

77 **Manns MP**, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, Vierling JM. Diagnosis and management of autoimmune hepatitis. *Hepatology* 2010; **51**: 2193-2213 [PMID: 20513004 DOI: 10.1002/hep.23584]

78 **Kogan J**, Safadi R, Ashur Y, Shouval D, Ilan Y. Prognosis of symptomatic versus asymptomatic autoimmune hepatitis: a study of 68 patients. *J Clin Gastroenterol* 2002; **35**: 75-81 [PMID: 12080231]

79 **Roberts SK**, Therneau TM, Czaja AJ. Prognosis of histological cirrhosis in type 1 autoimmune hepatitis. *Gastroenterology* 1996; **110**: 848-857 [PMID: 8608895 DOI: 10.1053/gast.1996.v110.pm8608895]

80 **Floreani A**, Niro G, Rosa Rizzotto E, Antoniazzi S, Ferrara F, Carderi I, Baldo V, Premoli A, Olivero F, Morello E, Durazzo M. Type I autoimmune hepatitis: clinical course and outcome in an Italian multicentre study. *Aliment Pharmacol Ther* 2006; **24**: 1051-1057 [PMID: 16984499 DOI: 10.1111/j.1365-2036.2006.03104.x]

81 **Seo S**, Toutounjian R, Conrad A, Blatt L, Tong MJ. Favorable outcomes of autoimmune hepatitis in a community clinic setting. *J Gastroenterol Hepatol* 2008; **23**: 1410-1414 [PMID: 18373564 DOI: 10.1111/j.1440-1746.2008.05365.x]

82 **Hoeroldt B**, McFarlane E, Dube A, Basumani P, Karajeh M, Campbell MJ, Gleeson D. Long-term outcomes of patients with autoimmune hepatitis managed at a nontransplant center. *Gastroenterology* 2011; **140**: 1980-1989 [PMID: 21396370 DOI: 10.1053/j.gastro.2011.02.065]

83 **Seaberg EC**, Belle SH, Beringer KC, Schivins JL, Detre KM. Long-term patient and retransplantation-free survival by selected recipient and donor characteristics: an update from the Pitt-UNOS Liver Transplant Registry. *Clin Transpl* 1997: 15-28 [PMID: 9919388]

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

85 **Molmenti EP**, Netto GJ, Murray NG, Smith DM, Molmenti H, Crippin JS, Hoover TC, Jung G, Marubashi S, Sanchez EQ, Gogel B, Levy MF, Goldstein RM, Fasola CG, Gonwa TA, Klintmalm GB. Incidence and recurrence of autoimmune/alloimmune hepatitis in liver transplant recipients. *Liver Transpl* 2002; **8**: 519-526 [PMID: 12037782 DOI: 10.1053/jlts.2002.32981]

86 **Mottershead M**, Neuberger J. Transplantation in autoimmune liver diseases. *World J Gastroenterol* 2008; **14**: 3388-3395 [PMID: 18528936 DOI: 10.3748/wjg.14.3388]

87 **Futagawa Y**, Terasaki PI, Waki K, Cai J, Gjertson DW. No improvement in long-term liver transplant graft survival in the last decade: an analysis of the UNOS data. *Am J Transplant* 2006; **6**: 1398-1406 [PMID: 16686763]

88 **Neuberger J**, Portmann B, Calne R, Williams R. Recurrence of autoimmune chronic active hepatitis following orthotopic liver grafting. *Transplantation* 1984; **37**: 363-365 [PMID: 6369666]

89 **Ratziu V**, Samuel D, Sebagh M, Farges O, Saliba F, Ichai P, Farahmand H, Gigou M, Féray C, Reynès M, Bismuth H. Long-term follow-up after liver transplantation for autoimmune hepatitis: evidence of recurrence of primary disease. *J Hepatol* 1999; **30**: 131-141 [PMID: 9927160 DOI: 10.1016/S0168-8278(99)80017-8]

90 **Vogel A**, Heinrich E, Bahr MJ, Rifai K, Flemming P, Melter M, Klempnauer J, Nashan B, Manns MP, Strassburg CP. Long-term outcome of liver transplantation for autoimmune hepatitis. *Clin Transplant* 2004; **18**: 62-69 [PMID: 15108772 DOI: 10.1111/j.1399-0012.2004.00117.x]

91 **El-Masry M**, Puig CA, Saab S. Recurrence of non-viral liver disease after orthotopic liver transplantation. *Liver Int* 2011; **31**: 291-302 [PMID: 21281429 DOI: 10.1111/j.1478-3231.2010.02434]

92 **Campsen J**, Zimmerman MA, Trotter JF, Wachs M, Bak T, Steinberg T, Kaplan M, Wright F, Kam I. Liver transplantation for autoimmune hepatitis and the success of aggressive corticosteroid withdrawal. *Liver Transpl* 2008; **14**: 1281-1286 [PMID: 18756454 DOI: 10.1002/lt.21525]

93 **Devlin J**, Donaldson P, Portmann B, Heaton N, Tan KC, Williams R. Recurrence of autoimmune hepatitis following liver transplantation. *Liver Transpl Surg* 1995; **1**: 162-165 [PMID: 9346559]

94 **Demetris AJ**, Adeyi O, Bellamy CO, Clouston A, Charlotte F, Czaja A, Daskal I, El-Monayeri MS, Fontes P, Fung J, Gridelli B, Guido M, Haga H, Hart J, Honsova E, Hubscher S, Itoh T, Jhala N, Jungmann P, Khettry U, Lassman C, Ligato S, Lunz JG, Marcos A, Minervini MI, Mölne J, Nalesnik M, Nasser I, Neil D, Ochoa E, Pappo O, Randhawa P, Reinholt FP, Ruiz P, Sebagh M, Spada M, Sonzogni A, Tsamandas AC, Wernerson A, Wu T, Yilmaz F. Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology* 2006; **44**: 489-501 [PMID: 16871565 DOI: 10.1002/hep.21280]

95 **González-Koch A**, Czaja AJ, Carpenter HA, Roberts SK, Charlton MR, Porayko MK, Rosen CB, Wiesner RH. Recurrent autoimmune hepatitis after orthotopic liver transplantation. *Liver Transpl* 2001; **7**: 302-310 [PMID: 11303289 DOI: 10.1053/jlts.2001.21449]

96 **Duclos-Vallée JC**, Sebagh M, Rifai K, Johanet C, Ballot E, Guettier C, Karam V, Hurtova M, Feray C, Reynes M, Bismuth H, Samuel D. A 10 year follow up study of patients transplanted for autoimmune hepatitis: histological recurrence precedes clinical and biochemical recurrence. *Gut* 2003; **52**: 893-897 [PMID: 12740348 DOI: 10.1136/gut.52.6.893]

97 **Wright HL**, Bou-Abboud CF, Hassanein T, Block GD, Demetris AJ, Starzl TE, Van Thiel DH. Disease recurrence and rejection following liver transplantation for autoimmune chronic active liver disease. *Transplantation* 1992; **53**: 136-139 [PMID: 1733061]

98 **Ayata G**, Gordon FD, Lewis WD, Pomfret E, Pomposelli JJ, Jenkins RL, Khettry U. Liver transplantation for autoimmune hepatitis: a long-term pathologic study. *Hepatology* 2000; **32**: 185-192 [PMID: 10915722 DOI: 10.1053/jhep.2000.9077]

99 **Czaja AJ**. The immunoreactive propensity of autoimmune hepatitis: is It corticosteroid-dependent after liver transplantation? *Liver Transpl Surg* 1999; **5**: 460-463 [PMID: 10477851 DOI: 10.1002/lt.500050512]

100 **Montano-Loza AJ**, Mason AL, Ma M, Bastiampillai RJ, Bain VG, Tandon P. Risk factors for recurrence of autoimmune hepatitis after liver transplantation. *Liver Transpl* 2009; **15**: 1254-1261 [PMID:

19790153 DOI: 10.1002/lt.21796]
101 **Neuberger J**. Transplantation for autoimmune hepatitis. *Semin Liver Dis* 2002; **22**: 379-386 [PMID: 12447709 DOI: 10.1055/s-2002-35707]
102 **Kerkar N**, Dugan C, Rumbo C, Morotti RA, Gondolesi G, Shneider BL, Emre S. Rapamycin successfully treats post-transplant autoimmune hepatitis. *Am J Transplant* 2005; **5**: 1085-1089 [PMID: 15816890 DOI: 10.1111/j.1600-6143.2005.00801.x]
103 **Liberal R**, Longhi MS, Grant CR, Mieli-Vergani G, Vergani D. Autoimmune hepatitis after liver transplantation. *Clin Gastroenterol Hepatol* 2012; **10**: 346-353 [PMID: 22056300 DOI: 10.1016/j.cgh.2011.10.028]
104 **Prados E**, Cuervas-Mons V, de la Mata M, Fraga E, Rimola A, Prieto M, Clemente G, Vicente E, Casanovas T, Fabrega E. Outcome of autoimmune hepatitis after liver transplantation. *Transplantation* 1998; **66**: 1645-1650 [PMID: 9884253]

P- Reviewer: Andreone P, Gong JP, Invernizzi P, Ozden I, Sugawara Y
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.i29.2906

World J Hepatol 2015 December 18; 7(29): 2906-2912
ISSN 1948-5182 (online)

MINIREVIEWS

Therapeutic and clinical aspects of portal vein thrombosis in patients with cirrhosis

Massimo Primignani, Giulia Tosetti, Vincenzo La Mura

Massimo Primignani, Giulia Tosetti, U.O. Gastroenterologia ed Epatologia, IRCCS-Ca' Granda, Ospedale Maggiore Policlinico, 20122 Milano, Italy

Vincenzo La Mura, U.O. Medicina Interna, IRCCS-San Donato, Dipartimento di Scienze Biomediche per la Salute, Università degli studi di Milano, 20097 Milan, Italy

Author contributions: Primignani M contributed to review concept and design; all authors contributed to drafting of the manuscript.

Conflict-of-interest statement: No potential conflict of interest.

Correspondence to: Massimo Primignani, MD, PhD, U.O. Gastroenterologia ed Epatologia, IRCCS-Ca' Granda, Ospedale Maggiore Policlinico, via F. Sforza 35, 20122 Milano, Italy. massimo.primignani@policlinico.mi.it
Telephone: +39-02-55035432
Fax: +39-02-50320410

Received: July 3, 2015
Peer-review started: July 9, 2015
First decision: August 25, 2015
Revised: November 8, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: December 18, 2015

Abstract

Portal vein thrombosis (PVT) is a frequent complication in cirrhosis, particularly in advanced stages of the disease. As for general venous thromboembolism, risk factors for PVT are slow blood flow, vessel wall damage and hypercoagulability, all features of advanced cirrhosis. Actually, the old dogma of a hemorrhagic tendency in cirrhosis has been challenged by new laboratory tools and the clinical evidence that venous thrombosis also occurs in cirrhosis. The impaired hepatic synthesis of both pro- and anticoagulants leads to a rebalanced hemostasis, more liable to be tipped towards thrombosis or even bleeding. Conventional anticoagulant drugs (low molecular weight heparin or vitamin K antagonists) may be used in cirrhosis patients with PVT, particularly in those eligible for liver transplantation, to prevent thrombosis progression thus permitting/facilitating liver transplant. However, several doubts exist on the level of anticoagulation achieved as estimated by coagulation tests, on the efficacy of treatment monitoring and on the correct timing for discontinuation in non-transplant candidates, while in transplant candidates there is expert consensus on continuing anticoagulation until transplantation. The recent introduction of direct acting oral anticoagulant drugs (DOACs) in other clinical settings generates much interest on their possible application in patients with cirrhosis and PVT. However, DOACs were not evaluated yet in patients with liver disease and cannot be recommended for the present time.

Key words: Portal vein thrombosis; Coagulopathy; Hypercoagulopathy; Direct acting oral anticoagulant drugs; Cirrhosis

Core tip: Impaired liver synthesis of both pro- and anticoagulants maintains a haemostatic balance in advanced liver disease, but this balance is more unstable than in healthy subjects and can be easily tipped towards thrombosis or bleeding. Portal vein thrombosis

(PVT) frequently occurs in advanced stages of cirrhosis and, if occlusive or extensive, may complicate or impede liver transplant. Therefore, prevention and treatment of PVT are frequent issues in cirrhosis patients, particularly in those eligible to liver transplant. Current treatments are with low molecular weight heparin or vitamin K antagonists and should be continued until transplantation in liver candidates, whereas no consensus exists regarding the duration of anticoagulation in non-transplant candidates.

Primignani M, Tosetti G, La Mura V. Therapeutic and clinical aspects of portal vein thrombosis in patients with cirrhosis. *World J Hepatol* 2015; 7(29): 2906-2912 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2906.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2906

INTRODUCTION

Portal vein thrombosis (PVT) is a rare event in the general population, but is frequent in patients with cirrhosis, particularly in the advanced stages of the disease. Actually, the prevalence of PVT parallels the progression of cirrhosis, being less than 1% in patients with compensated disease, but 8%-25% in liver transplant candidates[1-4] and further increases when the disease is complicated by the occurrence of hepatocellular carcinoma. In this review article we describe the risk factors for PVT in cirrhosis, discuss the controversial clinical impact of PVT on the natural history of cirrhosis and the current indications for PVT prevention or treatment.

RISK FACTORS AND PATHOGENESIS OF PVT IN CIRRHOSIS

Slow blood flow, vessel wall damage and hypercoagulability, the classical triad of mechanistic factors for general venous thromboembolism identified by Virchow more than 150 years ago, are the perceived risk factors also for PVT in cirrhosis. Indeed, slowing of portal blood velocity, which occurs with the progression of liver disease, has been identified as a risk factor for PVT[5].

As for venous thromboembolism hypercoagulability has been reported at increased rate in some studies, but not in others. Causes include factor V Leiden and prothrombin gene mutation, hyperhomocysteinemia, protein C and protein S deficiencies, and elevated factor Ⅷ[6,7].

More in general, the long standing belief of a "spontaneous anticoagulation" in cirrhosis, due to the reduced hepatic synthesis of coagulant factors, and recognized by the prolongation of the classical coagulation tests prothrombin time (PT) and activated partial thromboplastin time (APTT), has recently been revised in favour of a new concept of "rebalanced haemostasis" in cirrhosis. Indeed, PT and APTT, due to their design, are much more sensitive to the procoagulants, than to the naturally occurring anticoagulants protein C, protein S, and antithrombin, which are also synthesized by the liver and are decreased, often severely, in plasma from cirrhosis patients. Protein C, *in vivo*, is activated by thrombin and quenches thrombin generation in order to limit the activation of the coagulation cascade. However, for Protein C being activated, the presence of thrombomodulin, its endothelial receptor, is required. Since thrombomodulin is not present in the PT and APTT reagents, it appears that such test are unsuitable to assess the balance of pro- and anticoagulants in plasma from cirrhosis patients and to predict bleeding events[8-10]. Recently, global coagulation tests able to account for both pro- and anticoagulants, such as the thrombin generation test, indicate that plasma from patients with cirrhosis generates equal amounts of thrombin as compared to plasma from healthy subjects, if measured in the presence of thrombomodulin[11]. However, since also platelets, besides their role in primary haemostasis, support thrombin generation by assembling activated coagulation factors on their surface, a normal thrombin generation is still found if a sufficient platelet count of around 60 × 10^9/L is preserved[12].

Further evolution of research suggests, particularly in advanced cirrhosis, the occurrence of a procoagulant imbalance whose biomarker is the resistance to the inhibition of thrombin generation, expressed by the increased ratio of thrombin generation with/without thrombomodulin. The increased ratio between Factor Ⅷ (a strong procoagulant driver, markedly increased in cirrhosis plasma) and Protein C (a strong inhibitor of thrombin generation and of activated factor Ⅷ, severely decreased in cirrhosis plasma) appears to be the biological background of such imbalance[13].

In summary, PT or APTT can no longer be regarded as indexes of hypocoagulability in patients with cirrhosis. By converse, cirrhosis can be viewed as an acquired prothrombotic condition, a new concept that better fits with the tendency to PVT, or the increased rate of venous thromboembolism occurring in cirrhotic patients when exposed to risk factors[14].

CLINICAL IMPACT OF PVT IN CIRRHOSIS

The impact of PVT on the natural history of cirrhosis is controversial, as several studies evaluating the clinical outcome of cirrhosis patients after the diagnosis of PVT gave discordant results[4,15-18]. In fact, some studies suggest that PVT may progress to complete occlusion and/or extend to other splanchnic vessels in 40%-70% of cases. In addition, patients with PVT appear to have more than threefold increased risk of failure to control variceal bleeding[17] and a reduced post-transplant survival[4] suggesting that the presence of PVT in advanced liver disease is associated with critical outcomes. On the opposite hand, a recent multicenter study, showed, in a large series of patients, that the

incidence of PVT, most often non-occlusive, did not influence the clinical outcome and was associated with a high rate of spontaneous recanalization[19].

PROPHYLACTIC TREATMENT

The recent finding that prophylactic doses of enoxaparin, besides preventing PVT without increasing the rate of bleeding, also reduced bacterial translocation and the incidence of further decompensation in patients listed for liver transplantation[20] suggests a role for anticoagulants in cirrhosis wider than previously expected. Since activated coagulation factors have other targets, besides clotting, and promote fibrogenesis by acting on platelets, endothelial cells and stellate cells, the role of anticoagulants might extend beyond the prevention or treatment of PVT in cirrhosis, towards the prevention of progression of cirrhosis itself[21].

Larger, confirmatory studies are needed before a widespread use of enoxaparin in patients with cirrhosis permeates clinical practice. However, the growing evidence from clinical studies and the finding of normal/increased amounts of thrombin generated *in vitro* demonstrates that the so-called "auto anticoagulation" of patients with cirrhosis is a false dogma.

This new concept generates consequences in clinical practice. For instance, thromboprophylaxis for the prevention of deep vein thrombosis should be given to cirrhosis patients, as to the general population, when exposed to such risk factors as immobilization, cancer or surgery[22,23]. However, such strategy is still not generally adopted in cirrhosis, due to the perceived risk of hemorrhage.

As far as PVT is concerned, anticoagulation might be indicated for treating or preventing PVT in particular settings, such as the patients eligible or listed for liver transplant or those undergoing hepatic resection[3,24].

However, although clinical data and modern global coagulation tests indicate that patients with cirrhosis have a rebalanced hemostasis or even a prothrombotic phenotype, particularly in advanced stages of the disease, this balance may be weak and can be tipped towards thrombosis or haemorrhage by ensuing comorbidities, as bacterial infections or renal failure. In addition, patients with cirrhosis may have severe thrombocytopenia, which adds risk to anticoagulation therapy.

Therefore, several warnings must be considered before implementing a more liberal use of anticoagulants in patients with liver cirrhosis and the risks and benefit of anticoagulation in these patients must be carefully weighted. These warnings regard safety, monitoring and, to some extent, the proper indication of anticoagulation in cirrhosis patients with PVT.

STRATEGIES OF TREATMENT IN PATIENTS WITH OVERT PVT

Concerning efficacy and safety of anticoagulation in cirrhosis patients with established PVT, the available data refer to five case series including, overall, 163 subjects, mostly with partial PVT[4,24-27] (Table 1). At month-6 of therapy, complete or partial recanalization occurred in 33%-45%, while thrombus progression developed in less than 10%. Factors predicting recanalization were recent onset (< 6 mo) of the thrombus and partial PVT. Treatment prolongation after six months was associated with a higher rate of recanalization and a lower incidence of thrombosis progression or recurrence. Most patients had past bleeding or high-risk varices, and received endoscopic therapy with or without beta-blockers prior to anticoagulation. Bleeding occurred in 5% of the patients. After stopping anticoagulation, the benefit declined rapidly, as PVT recurred in up to 40% of patients. Overall, these data indicate a favorable risk/benefit ratio.

However, although these good safety data for portal hypertension-related bleeding in patients treated with anticoagulants, the number of patients enrolled in clinical studies is still low to allow firm conclusions on the safety of prolonged anticoagulation treatment, and few data on non-portal hypertension-related bleedings are available. In addition, more data on the safety of anticoagulation in patients with advanced stages of cirrhosis are required.

The use of vitamin K anatagonists and low molecular weight heparins

As far as the anticoagulant to choose for patients with cirrhosis is concerned, vitamin K antagonists (VKA) and low molecular weight heparin (LMWH) are those currently used, although several doubts exist on the required doses, on the level of anticoagulation achieved as estimated by coagulation tests, and on the efficacy of treatment monitoring. In fact, LMWH monitoring may be complicated by the low levels of antithrombin frequently occurring in these patients[25] and by analytical troubles affecting the anti-factor Xa measurement in patients with liver disease[28,29]. As for VKA in cirrhosis patients, one may consider that the PT-INR was originally intended for patients on VKA to harmonize the PT results from different laboratories and thromboplastins, but is less valid for patients with cirrhosis as their coagulopathy is different from that caused by VKA[30-33]. In addition, the frequently encountered baseline PT prolongation in cirrhotic patients casts doubts on whether the level of anticoagulation achieved is correctly represented by the PT.

This notwithstanding, VKA and LMWH appear to be equally effective and relatively safe in patients with cirrhosis, although long-term regimens have not been evaluated yet. LMWH, although less practical because of the need for subcutaneous injections, can be used until transplantation. Importantly, and at difference with VKA, it does not interfere with the MELD score.

Conversely, VKA can be given orally, but interfere with the MELD score. Anticoagulation can be reversed rapidly at the time of transplantation by the administration of

Table 1 Studies on management of portal vein thrombosis in cirrhotic patients

Ref.	Study type	PVT	Number anticoagulate patients	Number controls	Typeanticoagulant	Duration anticoagulation	Repermeation/stabilization/ progression of thrombosis in anticoagulate patients	Repermeation/stabilization/ progression of thrombosis in control patients	Bleeding complications in anticoagulate patients	Bleeding complications in controls
Francoz *et al*[4]	Proscpective; Case control	29	19	10	VKA (target INR 2-3)	Mean 8.1 mo	8/10/1	0/4/6	1 variceal bleeding after EBL	NA
Amitrano *et al*[25]	Prospective	28	28	-	Enoxaparin 200 UI/kg per day	6 mo in repermeation, until end follow up in partial responders	21/5/2	-	1 anemia in PHG	-
Senzolo *et al*[24]	Prospective; Case control	56	35	21	Nadroparin 95 antiXa U/kg twice a day	6 mo after complete repermeation, until the end of follow up in other patients	21/7/5	1/5/15	1 variceal bleeding; 1 cerebral haemorrage; 1 haematuria; 1 epistaxis	5 variceal bleeding
Delgado *et al*[26]	Retrospective	55	55	-	VKA (8 patients); VKA → LMWH (21 patients); LMWH (26 patients)	Median 6.8 mo (range 1-56 mo)	33/22/0	-	6 variceal bleeding; 1 obscure gastrointestinal bleeding; 1 lower gastrointestinal bleeding; 1 oral bleeding after dental extraction	-

PVT: Portal vein thrombosis; VKA: Vitamin K antoagonists; LMWH: Low molecular weight heparin; NA: Not available; PHG: Portal hypertensive gastropathy; EBL: Endoscopic bend ligation; INR: Internatiol normalized ratio.

fresh frozen plasma. A platelet count < 50 × 10^9/L and the use of VKA were the only factors more frequently observed in patients with a bleeding episode suspected to be related to anticoagulation therapy[26].

Direct oral anticoagulant agents: Where we are

Many of the warnings related to LMWH and VKA could be overcome by the recently introduced direct acting oral anticoagulant drugs (DOACs), which inhibit thrombin (Dabigatran) or activated factor X (Rivaroxaban, Apixaban and Edoxaban). These drugs have the advantages of oral administration, fixed dose and no need of laboratory monitoring[34]. Moreover, their mechanism of action is independent of antithrombin, which is necessary for LMWH be effective, but may be severely impaired in cirrhosis patients. Finally, DOACs do not interfere with the MELD score. However for now, anticoagulation induced by DOACs is not quickly reversible and this may be a concern in case of bleeding. Another potential issue is renal function; DOACs, especially dabigatran, have predominantly renal excretion and impairment of renal function, frequently observed in patients with cirrhosis, could cause drug accumulation[35].

Notwithstanding, DOACs, which are currently used to prevent venous thromboembolism in orthopedic surgery and, at therapeutic dosages, in atrial fibrillation and deep vein thrombosis[36-38], appear to be as effective as LMWH or warfarin, with less bleeding complications. However, although the overall lower bleeding rate in patients treated with DOACs *vs* VKA, the incidence of gastrointestinal bleeding appears to be slightly but definitively increased, with an absolute risk of 2.6% *vs* 2%[39]. This might be a matter of concern in cirrhosis patients for the future. For now, whether DOACs are safe and effective in cirrhosis is unknown, as patients with abnormal liver function tests were excluded from the studies on DOACs.

Indication to anticoagulation: Critical issues

A further point refers to the proper indication of anticoagulation in patients with PVT and cirrhosis. Several issues need consideration: The grade (partial or occlusive) and

extent of PVT, its clinical presentation and the assumed consequence on the outcome of cirrhosis, and, notably, whether the patient is eligible or not for liver transplant.

Firstly, PVT occurring in cirrhosis patients is often partial and asymptomatic, accidentally detected at ultrasound evaluation during follow-up. For this PVT type there is no evidence from prospective studies[19,40,41] of a causal relationship between its occurrence and worsening of the disease. In addition, the outcome of partial PVT, when assessed prospectively, appears to be highly variable, with either spontaneous progression or regression[41]. In other instances, abdominal pain, gastrointestinal bleeding, development or worsening of ascites or hepatic encephalopathy are associated with the onset of PVT. In addition, intestinal infarction may occur. Such clinical presentation, most often related to occlusive PVT, mainly when extended proximally and deeply into the superior mesenteric vein, obviously affects the disease outcome and requires prompt anticoagulation.

A third issue regards the eligibility of the patient to liver transplant. Because of improved surgical techniques and perioperative management, liver transplant is no longer a contraindication, even in cases of extensive PVT. However, PVT causes technical difficulties in the setting of transplantation, with a negative impact on the outcome and, in some instances, may represent a definitive contraindication for transplantation.

Furthermore, the real impact of PVT on the access to the waiting list for liver transplantation is presently unknown. Therefore, the current or future eligibility of the patient to liver transplant must be considered when deciding to prescribe anticoagulants or not.

All these issues were discussed at the recent Baveno VI workshop in a session devoted to vascular diseases of the liver, where the risks and benefits of anticoagulation were thoroughly balanced. It was agreed to consider anticoagulation in potential transplant candidates with thrombosis of the main portal vein trunk or progressive PVT, in order to permit or facilitate liver transplantation and reduce post-transplant mortality and morbidity. In addition, in untreated potential transplant candidates with PVT, it was agreed to recommend an imaging follow-up every three months and anticoagulation in case of progression. As for the duration of anticoagulation, there was consensus on maintaining anticoagulation until transplantation to prevent re-thrombosis.

Conversely, for non-candidates to LT, no recommendation regarding anticoagulation treatment could be made, but it was stated that anticoagulation could be considered in selected cases, such as patients with thrombosis extended to the superior mesenteric vein or with known "strong" prothrombotic conditions[42].

Transjugular intrahepatic porto-systemic shunt: An alternative to anticoagulation?

Similarly to what is described for acute extrahepatic portal vein obstruction in non-cirrhotic patients[43], theoretically, an endovascular approach could be useful to manage PVT also in the context of cirrhosis. However, the experience in the setting of patients with cirrhosis is very limited and hampered by the additional inconvenience that the detection of PVT in this clinical setting is generally incidental and it is difficult to establish the age of the thrombus. The best described endovascular approach is the transjugular intrahepatic porto-systemic shunt (TIPS). It might represent an alternative to anticoagulation. Indeed, TIPS may be feasible in patients with PVT, particularly if the intra-hepatic branches of portal vein are patent[44,45]. Luca *et al*[45] reported a case series of 70 patients with non-tumoral PVT treated with TIPS for the management of complications due to portal hypertension. More than half of patients achieved complete recanalization and 30% a marked decrease in thrombosis, whereas no improvement occurred in only 13%. This success rate was similar to that observed for anticoagulation. Therefore TIPS may be an option in patients with contraindication to anticoagulation treatment.

CONCLUSION

Despite a long-standing faith, liver cirrhosis is not associated with hypocoagulability. Instead, hypercoagulability, particularly in advanced disease, prevails. This is confirmed by the fact that both deep venous thrombosis and, particularly, PVT occur in cirrhosis. Although PVT in cirrhosis is frequently asymptomatic and may have a variable spontaneous evolution, it may require anticoagulation treatment in patients eligible for liver transplant or in case of thrombus progression. Either LMWH or VKA can be used, appear equally effective and share a relatively good safety profile, but larger studies, involving patients with advanced disease are needed to confirm these findings. DOACs, although promising because of their mechanism of action, have not been evaluated in patients with liver disease and cannot currently be recommended.

Anticoagulants, besides their effect on clotting, appear to decrease fibrogenesis. Such finding, if confirmed in future studies, will expand the role of anticoagulants in the clinical management of cirrhosis.

REFERENCES

1 **Tripodi A**, Anstee QM, Sogaard KK, Primignani M, Valla DC. Hypercoagulability in cirrhosis: causes and consequences. *J Thromb Haemost* 2011; **9**: 1713-1723 [PMID: 21729237]

2 **Okuda K**, Ohnishi K, Kimura K, Matsutani S, Sumida M, Goto N, Musha H, Takashi M, Suzuki N, Shinagawa T. Incidence of portal vein thrombosis in liver cirrhosis. An angiographic study in 708 patients. *Gastroenterology* 1985; **89**: 279-286 [PMID: 4007419]

3 **Yamashita Y**, Bekki Y, Imai D, Ikegami T, Yoshizumi T, Ikeda T, Kawanaka H, Nishie A, Shirabe K, Maehara Y. Efficacy of postoperative anticoagulation therapy with enoxaparin for portal vein thrombosis after hepatic resection in patients with liver cancer. *Thromb Res* 2014; **134**: 826-831 [PMID: 25156238 DOI: 10.1016/j.thromres.2014.07.038]

4 **Francoz C**, Belghiti J, Vilgrain V, Sommacale D, Paradis V, Condat B, Denninger MH, Sauvanet A, Valla D, Durand F. Splanchnic vein thrombosis in candidates for liver transplantation:

usefulness of screening and anticoagulation. *Gut* 2005; **54**: 691-697 [PMID: 15831918]
5 **Zocco MA**, Di Stasio E, De Cristofaro R, Novi M, Ainora ME, Ponziani F, Riccardi L, Lancellotti S, Santoliquido A, Flore R, Pompili M, Rapaccini GL, Tondi P, Gasbarrini GB, Landolfi R, Gasbarrini A. Thrombotic risk factors in patients with liver cirrhosis: correlation with MELD scoring system and portal vein thrombosis development. *J Hepatol* 2009; **51**: 682-689 [PMID: 19464747]
6 **Amitrano L**, Guardascione MA, Brancaccio V, Margaglione M, Manguso F, Iannaccone L, Grandone E, Balzano A. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. *J Hepatol* 2004; **40**: 736-741 [PMID: 15094219]
7 **Amitrano L**, Brancaccio V, Guardascione MA, Margaglione M, Iannaccone L, D'Andrea G, Marmo R, Ames PR, Balzano A. Inherited coagulation disorders in cirrhotic patients with portal vein thrombosis. *Hepatology* 2000; **31**: 345-348 [PMID: 10655256]
8 **Ewe K**. Bleeding after liver biopsy does not correlate with indices of peripheral coagulation. *Dig Dis Sci* 1981; **26**: 388-393 [PMID: 7249879]
9 **Boks AL**, Brommer EJ, Schalm SW, Van Vliet HH. Hemostasis and fibrinolysis in severe liver failure and their relation to hemorrhage. *Hepatology* 1986; **6**: 79-86 [PMID: 3943792]
10 **Ng VL**. Liver disease, coagulation testing, and hemostasis. *Clin Lab Med* 2009; **29**: 265-282 [PMID: 19665678 DOI: 10.1016/j.cll.2009.05.001]
11 **Tripodi A**, Salerno F, Chantarangkul V, Clerici M, Cazzaniga M, Primignani M, Mannuccio Mannucci P. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. *Hepatology* 2005; **41**: 553-558 [PMID: 15726661]
12 **Tripodi A**, Primignani M, Chantarangkul V, Clerici M, Dell'Era A, Fabris F, Salerno F, Mannucci PM. Thrombin generation in patients with cirrhosis: the role of platelets. *Hepatology* 2006; **44**: 440-445 [PMID: 16871542]
13 **Tripodi A**, Primignani M, Chantarangkul V, Dell'Era A, Clerici M, de Franchis R, Colombo M, Mannucci PM. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology* 2009; **137**: 2105-2111 [PMID: 19706293]
14 **Søgaard KK**, Horváth-Puhó E, Grønbaek H, Jepsen P, Vilstrup H, Sørensen HT. Risk of venous thromboembolism in patients with liver disease: a nationwide population-based case-control study. *Am J Gastroenterol* 2009; **104**: 96-101 [PMID: 19098856 DOI: 10.1038/ajg.2008.34]
15 **Englesbe MJ**, Schaubel DE, Cai S, Guidinger MK, Merion RM. Portal vein thrombosis and liver transplant survival benefit. *Liver Transpl* 2010; **16**: 999-1005 [PMID: 20677291 DOI: 10.1002/lt.22105]
16 **Maruyama H**, Okugawa H, Takahashi M, Yokosuka O. De novo portal vein thrombosis in virus-related cirrhosis: predictive factors and long-term outcomes. *Am J Gastroenterol* 2013; **108**: 568-574 [PMID: 23381015 DOI: 10.1038/ajg.2012.452]
17 **D'Amico G**, De Franchis R. Upper digestive bleeding in cirrhosis. Post-therapeutic outcome and prognostic indicators. *Hepatology* 2003; **38**: 599-612 [PMID: 12939586]
18 **Augustin S**, Altamirano J, González A, Dot J, Abu-Suboh M, Armengol JR, Azpiroz F, Esteban R, Guardia J, Genescà J. Effectiveness of combined pharmacologic and ligation therapy in high-risk patients with acute esophageal variceal bleeding. *Am J Gastroenterol* 2011; **106**: 1787-1795 [PMID: 21625271 DOI: 10.1038/ajg.2011.173]
19 **Nery F**, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou PE, Plessier A, Roulot D, Chaffaut C, Bourcier V, Trinchet JC, Valla DC. Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study. *Hepatology* 2015; **61**: 660-667 [PMID: 25284616 DOI: 10.1002/hep.27546]
20 **Villa E**, Cammà C, Marietta M, Luongo M, Critelli R, Colopi S, Tata C, Zecchini R, Gitto S, Petta S, Lei B, Bernabucci V, Vukotic R, De Maria N, Schepis F, Karampatou A, Caporali C, Simoni L, Del Buono M, Zambotto B, Turola E, Fornaciari G, Schianchi S, Ferrari A, Valla D. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* 2012; **143**: 1253-1260.e1-4 [PMID: 22819864]
21 **Anstee QM**, Dhar A, Thursz MR. The role of hypercoagulability in liver fibrogenesis. *Clin Res Hepatol Gastroenterol* 2011; **35**: 526-533 [PMID: 21570930]
22 **Lisman T**, Kamphuisen PW, Northup PG, Porte RJ. Established and new-generation antithrombotic drugs in patients with cirrhosis - possibilities and caveats. *J Hepatol* 2013; **59**: 358-366 [PMID: 23548197]
23 **Aldawood A**, Arabi Y, Aljumah A, Alsaadi A, Rishu A, Aldorzi H, Alqahtani S, Alsultan M, Felemban A. The incidence of venous thromboembolism and practice of deep venous thrombosis prophylaxis in hospitalized cirrhotic patients. *Thromb J* 2011; **9**: 1 [PMID: 21244669]
24 **Senzolo M**, M Sartori T, Rossetto V, Burra P, Cillo U, Boccagni P, Gasparini D, Miotto D, Simioni P, Tsochatzis E, A Burroughs K. Prospective evaluation of anticoagulation and transjugular intrahepatic portosystemic shunt for the management of portal vein thrombosis in cirrhosis. *Liver Int* 2012; **32**: 919-927 [PMID: 22435854]
25 **Amitrano L**, Guardascione MA, Menchise A, Martino R, Scaglione M, Giovine S, Romano L, Balzano A. Safety and efficacy of anticoagulation therapy with low molecular weight heparin for portal vein thrombosis in patients with liver cirrhosis. *J Clin Gastroenterol* 2010; **44**: 448-451 [PMID: 19730112]
26 **Delgado MG**, Seijo S, Yepes I, Achécar L, Catalina MV, García-Criado A, Abraldes JG, de la Peña J, Bañares R, Albillos A, Bosch J, García-Pagán JC. Efficacy and safety of anticoagulation on patients with cirrhosis and portal vein thrombosis. *Clin Gastroenterol Hepatol* 2012; **10**: 776-783 [PMID: 22289875]
27 **Werner KT**, Sando S, Carey EJ, Vargas HE, Byrne TJ, Douglas DD, Harrison ME, Rakela J, Aqel BA. Portal vein thrombosis in patients with end stage liver disease awaiting liver transplantation: outcome of anticoagulation. *Dig Dis Sci* 2013; **58**: 1776-1780 [PMID: 23314858 DOI: 10.1007/s10620-012-2548-y]
28 **Bechmann LP**, Sichau M, Wichert M, Gerken G, Kröger K, Hilgard P. Low-molecular-weight heparin in patients with advanced cirrhosis. *Liver Int* 2011; **31**: 75-82 [PMID: 20958919 DOI: 10.1111/j.1478-3231.2010.02358.x]
29 **Potze W**, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Porte RJ, Lisman T. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. *Br J Haematol* 2013; **163**: 666-673 [PMID: 24219333]
30 **Tripodi A**, Chantarangkul V, Mannucci PM. The international normalized ratio to prioritize patients for liver transplantation: problems and possible solutions. *J Thromb Haemost* 2008; **6**: 243-248 [PMID: 17988228]
31 **Trotter JF**, Olson J, Lefkowitz J, Smith AD, Arjal R, Kenison J. Changes in international normalized ratio (INR) and model for endstage liver disease (MELD) based on selection of clinical laboratory. *Am J Transplant* 2007; **7**: 1624-1628 [PMID: 17511686]
32 **Tripodi A**, Chantarangkul V, Primignani M, Fabris F, Dell'Era A, Sei C, Mannucci PM. The international normalized ratio calibrated for cirrhosis (INR(liver)) normalizes prothrombin time results for model for end-stage liver disease calculation. *Hepatology* 2007; **46**: 520-527 [PMID: 17659574]
33 **Bellest L**, Eschwège V, Poupon R, Chazouillères O, Robert A. A modified international normalized ratio as an effective way of prothrombin time standardization in hepatology. *Hepatology* 2007; **46**: 528-534 [PMID: 17654598]
34 **Yeh CH**, Hogg K, Weitz JI. Overview of the new oral anticoagulants: opportunities and challenges. *Arterioscler Thromb Vasc Biol* 2015; **35**: 1056-1065 [PMID: 25792448 DOI: 10.1161/ATVBAHA.115.303397]
35 **Steiner T**, Böhm M, Dichgans M, Diener HC, Ell C, Endres M, Epple C, Grond M, Laufs U, Nickenig G, Riess H, Röther J, Schellinger PD, Spannagl M, Veltkamp R. Recommendations for

the emergency management of complications associated with the new direct oral anticoagulants (DOACs), apixaban, dabigatran and rivaroxaban. *Clin Res Cardiol* 2013; **102**: 399-412 [PMID: 23669868 DOI: 10.1007/s00392-013-0560-7]

36 **Ruff CT**, Giugliano RP, Braunwald E, Hoffman EB, Deenadayalu N, Ezekowitz MD, Camm AJ, Weitz JI, Lewis BS, Parkhomenko A, Yamashita T, Antman EM. Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. *Lancet* 2014; **383**: 955-962 [PMID: 24315724]

37 **van Es N**, Coppens M, Schulman S, Middeldorp S, Büller HR. Direct oral anticoagulants compared with vitamin K antagonists for acute venous thromboembolism: evidence from phase 3 trials. *Blood* 2014; **124**: 1968-1975 [PMID: 24963045]

38 **Castellucci LA**, Cameron C, Le Gal G, Rodger MA, Coyle D, Wells PS, Clifford T, Gandara E, Wells G, Carrier M. Clinical and safety outcomes associated with treatment of acute venous thromboembolism: a systematic review and meta-analysis. *JAMA* 2014; **312**: 1122-1135 [PMID: 25226478]

39 **Holster IL**, Valkhoff VE, Kuipers EJ, Tjwa ET. New oral anticoagulants increase risk for gastrointestinal bleeding: a systematic review and meta-analysis. *Gastroenterology* 2013; **145**: 105-112. e15 [PMID: 23470618]

40 **Englesbe MJ**, Kubus J, Muhammad W, Sonnenday CJ, Welling T, Punch JD, Lynch RJ, Marrero JA, Pelletier SJ. Portal vein thrombosis and survival in patients with cirrhosis. *Liver Transpl* 2010; **16**: 83-90 [PMID: 20035521]

41 **John BV**, Konjeti R, Aggarwal A, Lopez R, Atreja A, Miller C, Zein NN, Carey WD. Impact of untreated portal vein thrombosis on pre and post liver transplant outcomes in cirrhosis. *Ann Hepatol* 2013; **12**: 952-958 [PMID: 24114826]

42 **de Franchis R**. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: 26047908 DOI: 10.1016/j.jhep.2015.05.022]

43 **Hollingshead M**, Burke CT, Mauro MA, Weeks SM, Dixon RG, Jaques PF. Transcatheter thrombolytic therapy for acute mesenteric and portal vein thrombosis. *J Vasc Interv Radiol* 2005; **16**: 651-661 [PMID: 15872320]

44 **Senzolo M**, Burra P, Patch D, Burroughs AK. Tips for portal vein thrombosis (pvt) in cirrhosis: not only unblocking a pipe. *J Hepatol* 2011; **55**: 945-946; author reply 947-948 [PMID: 21419182 DOI: 10.1016/j.jhep.2011.02.027]

45 **Luca A**, Miraglia R, Caruso S, Milazzo M, Sapere C, Maruzzelli L, Vizzini G, Tuzzolino F, Gridelli B, Bosch J. Short- and long-term effects of the transjugular intrahepatic portosystemic shunt on portal vein thrombosis in patients with cirrhosis. *Gut* 2011; **60**: 846-852 [PMID: 21357252]

P- Reviewer: Denk GU, Tsai WL **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.i29.2913

World J Hepatol 2015 December 18; 7(29): 2913-2919
ISSN 1948-5182 (online)

MINIREVIEWS

Clinical applications of squamous cell carcinoma antigen-immunoglobulins M to monitor chronic hepatitis C

Andrea Martini, Andrea Gallotta, Patrizia Pontisso, Giorgio Fassina

Andrea Martini, Patrizia Pontisso, Department of Medicine, University of Padua, 35122 Padua, Italy

Andrea Gallotta, Giorgio Fassina, Xeptagen S.p.A., VEGA Science Park, 30175 Marghera, Venice, Italy

Author contributions: Martini A designed the study, acquired the data and drafted the article; Gallotta A made critical comments and reviewed the manuscript; Pontisso P and Fassina G conceived the study and made the final critical revision of the manuscript; all authors have seen and approved the final version of the manuscript.

Conflict-of-interest statement: Fassina G (Chief Scientific Officer) and Gallotta A (Senior Scientist) are employees of Xeptagen S.p.A.

Correspondence to: Dr. Giorgio Fassina, Xeptagen S.p.A., VEGA Science Park, Building Auriga, Via delle Industrie 9, 30175 Marghera, Venice, Italy. fassina@xeptagen.com
Telephone: +39-041-5093910

Received: July 10, 2015
Peer-review started: July 12, 2015
First decision: September 8, 2015
Revised: September 30, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: December 18, 2015

Abstract

Hepatitis C virus (HCV) is the main cause of chronic liver disease and cirrhosis in Western countries. Over time, the majority of cirrhotic patients develop hepatocellular carcinoma (HCC), one of the most common fatal cancers worldwide - fourth for incidence rate. A high public health priority need is the development of biomarkers to screen for liver disease progression and for early diagnosis of HCC development, particularly in the high risk population represented by HCV-positive patients with cirrhosis. Several studies have shown that serological determination of a novel biomarker, squamous cell carcinoma antigen-immunoglobulins M (SCCA-IgM), might be useful to identify patients with progressive liver disease. In the initial part of this review we summarize the main clinical studies that have investigated this new circulating biomarker on HCV-infected patients, providing evidence that in chronic hepatitis C SCCA-IgM may be used to monitor progression of liver disease, and also to assess the virological response to antiviral treatment. In the last part of this review we address other, not less important, clinical applications of this biomarker in hepatology.

Key words: Hepatitis C virus; Treatment; Prognosis; Squamous cell carcinoma antigen-immunoglobulins M; Cirrhosis

Core tip: A high public health priority need is the development of biomarkers to screen for liver disease progression in hepatitis C virus (HCV)-positive patients. Serological squamous cell carcinoma antigen-immunoglobulins M has shown the ability to identify patients with progressive liver disease and patients at higher risk of hepatocellular carcinoma development. In this review we summarize the main clinical studies performed using this new circulating biomarker for monitoring cirrhosis progression in HCV-positive patients and to evaluate virological response to antiviral treatment.

Martini A, Gallotta A, Pontisso P, Fassina G. Clinical applications of squamous cell carcinoma antigen-immunoglobulins M to monitor chronic hepatitis C. *World J Hepatol* 2015; 7(29): 2913-2919 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2913.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2913

INTRODUCTION

Liver cirrhosis is an increasing cause of morbidity and mortality in Europe and the United States. It is the fourth most common cause of death in adults worldwide and the major reason for more than 5500 liver transplants in Europe each year[1]. The main causes of cirrhosis in Western countries are infection with hepatitis C virus (HCV), alcohol abuse, and, increasingly, non-alcoholic fatty liver disease (NAFLD)[2]. In sub-Saharian Africa and in most parts of Asia, infection with hepatitis B virus (HBV) represents the most common cause of cirrhosis[2]. The prevalence of this advanced liver disease is difficult to assess and probably higher than reported, because the initial stages are asymptomatic until cirrhosis with clinical decompensation occurs, therefore the disorder is often undiagnosed[2]. In line with these findings, about 90% of individuals with viral hepatitis in Europe are not aware of their status[1]. Moreover, the prevalence of NAFLD is 2%-44% in the European population and even higher (42.6%-69.5%) in people with type 2 diabetes[1].

Hepatocellular carcinoma (HCC), one of the main complications of cirrhosis, and the leading cause of death among these patients, is the sixth most common neoplasm and the third most frequent cause of cancer death[3]. Whereas the survival of patients with most malignancies has enhanced over the last decade, 5-year survival rate of patients with HCC has not improved sufficiently and remains less than 10%[4]. The poor outcome of patients with HCC is related to the late detection of the cancer, with the majority of patients diagnosed at advanced stages of disease[4]. It has been demonstrated that HCC surveillance of population at risk increases survival, because of detection of tumours amenable to curative therapies[5-7]; in fact, surveillance is recommended by international guidelines[8].

A major problem with HCC detection and surveillance is the lack of reliable biomarkers. Table 1 summarizes the sensitivity and specificity of the serological markers currently available for HCC diagnosis.

Alpha-fetoprotein (AFP) is the most widely used serum marker for HCC diagnosis and surveillance; however, not all HCCs secrete AFP (about 32%-59% of patients with the tumour have normal AFP levels)[4]. Furthermore, AFP may be elevated in patients with chronic liver disease in the absence of HCC, making this biomarker inadequate for surveillance tests[4]. Indeed, European and American guidelines consider AFP too inaccurate to survey patients at risk of HCC and recommend the use of ultrasound (US) alone[9,10]. US sensitivity depends on many factors, including the quality of the US machine, the experience of the examiner, and also the patient[11]. In patients with liver cirrhosis, regenerative nodules may be hard to distinguish from HCC using US, and the sensitivity of this imaging technique to detect early HCC lies between 32% and 65%[11]. For this reason, some authors, as well as Asian guidelines, suggest the use of AFP in HCC surveillance[12,13].

From 1990's, especially in Japan, new biomarkers for HCC diagnosis and surveillance have been explored. Among these, des-γ carboxy-prothrombin, an abnormal prothrombin protein, has been considered[14], but the results indicate that its sensitivity is highly dependent on tumour size[15]. The clinical utility of lens culinaris agglutinin-reactive fraction of AFP-L3 in early prediction of HCC development in patients with chronic HBV or HCV infection was also recently evaluated[16]. It was shown that several factors (gender, age, race, and presence of more advanced liver disease) are independent predictors of increased levels of this biomarker, which also lacks in sensitivity, specificity, and predictive values required for routine HCC surveillance[17].

Another biomarker that has been developed in recent years is Osteopontin, a molecule expressed by transformed malignant cells, also evaluated for colon and pancreatic cancer[18]. The majority of the studies analyzing osteopontin for the diagnosis of HCC was retrospective and included a range of 30 to 179 patients with HCC. The reported sensitivity of osteopontin for HCC was 86%, with a specificity of 86%, resulting in a diagnostic accuracy comparable to that of AFP. Further validation studies are needed to use this marker in daily clinical routine[18]. On the basis of the above considerations, a reliable biomarker to complement US in detecting early HCC still represents a crucial unmet need.

In recent years relevant emphasis has been ascribed to innate or natural immunity, which acts as the first line of defense, and also as the link between acquired immunity and immunological memory[19]. Poly-reactive natural auto-antibodies [immunoglobulins M (IgM)] can bind, with low affinity and high avidity, different markers that are expressed during cancer growth[20]. The presence of IgM-linked immune-complexes with diagnostic value has been found recently in different human tumours, including colon[21] and prostate cancer[22], and also in other pathologic conditions[23]. For liver disease, the diagnostic value of squamous cell carcinoma antigen-IgM (SCCA-IgM) immune-complex in serum has been demonstrated in several studies[24,25].

In this review we summarize the role of SCCA-IgM as a novel promising tool to monitor liver disease evolution and response to antiviral treatment in patients with chronic hepatitis C; we also analyze the value of this biomarker for the diagnosis and prognosis of HCC. An overview of the main observations on SCCA-IgM behaviour in clinical settings is presented in Table 2.

Table 1 Sensitivity and specificity (%) of various biomarkers for hepatocellular carcinoma diagnosis

Biomarker	Sensitivity	Specificity
SCCA-IgM[57]	52-89	50-82
AFP[58]	41-65	80-94
Osteopontin[58]	87	82
DCP[58]	23-89	95
AFP-L3[58]	37-60	90-92

AFP: Alpha-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen-immunoglobulins M.

SCCA

SCCA belongs to the clade B subset of the serpins family[26]. SCCA1 (SERPINB3) and its isoform SCCA2 (SERPINB4) are over-expressed in squamous cell carcinoma (SCC) of the uterine cervix, lung, head and neck, rectal colon, pancreatic and liver tumors[27-30]. The isoform that has been better evaluated in literature is SERPINB3, which has showed functional connection with tumorigenesis. This isoform was found to prevent cell death through its binding to complex 1 of the mitochondrial respiratory chain or *via* suppression of c-JUN, as a response to different types of stress, such as UV, radiation, chemotherapy, tumour necrosis factor-alpha and natural killer cells[31-34]. Moreover, its inflammatory and pro-tumorigenic role has been revealed demonstrating its ability to enhance interleukin-6 effects through nuclear factor κB pathway in response to Rat Sarcoma Viral Oncoprotein (coding RAS gene) stimuli[29].

SCCA1 and 2 are undetectable in normal hepatocytes, but their expression progressively increases from chronic liver disease to dysplastic nodules and HCC[35]. In particular, SERPINB3 is more expressed in high-grade dysplastic nodules and in HCC than in large regenerative nodules, suggesting a role in hepatocarcinogenesis[36]. Furthermore, this serpin was identified in the majority of hepatoblastomas, with the highest levels in tumours of more advanced stage[37]. In HCC, high expression of SERPINB3 is significantly associated with early tumour recurrence, and shows a better prognostic significance than other clinical and histological variables[38]. These important clinical findings were confirmed at the molecular level: SCCA expression in liver tumor has been correlated with liver regeneration activity (expressed by MIB-I-labeling index)[39], and increased proliferation was also documented in hepatoma cell lines over-expressing SERPINB3 and in a mouse model transgenic for this serpin[39,40]. Recent data indicate that SERPINB3 is highly expressed in the hepatic stem/progenitor cell compartment of both fetal and adult livers[41]; moreover, after induction by HIF2-alpha in an hypoxic environment[42], SERPINB3 was shown to be crucial for tumour invasiveness and metastasis, since it promotes epithelial-mesenchymal transition[39] and transforming growth factor-beta production[43] (Figure 1).

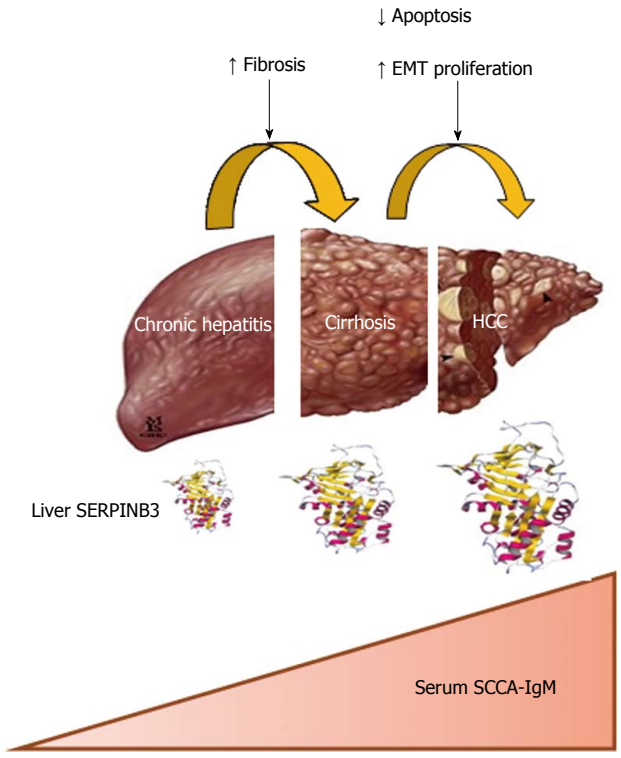

Figure 1 Schematic representation of SERPINB3 behavior in the liver and of serological squamous cell carcinoma antigen-immunoglobulins M levels in chronic liver disease. SCCA-IgM: Squamous cell carcinoma antigen-immunoglobulins M; HCC: Hepatocellular carcinoma; EMT: Epithelial-mesenchymal transition.

SCCA-IGM IN HCV-POSITIVE PATIENTS

Recently, an ELISA assay has been developed to detect serological SCCA isoforms (SERPINB3 and SERPINB4) complexed with natural IgM[24]. The clinical usefulness of monitoring SCCA-IgM immune-complexes in chronic liver disease has been evaluated in several studies. In 2008 Biasiolo *et al*[44] observed that SCCA-IgM was detectable, at presentation, in 33% of untreated patients with histologically proven chronic hepatitis, but not in healthy control subjects. After a median period of six years, the same patients underwent a second liver biopsy and an increased level of the immune-complex was observed in 75% of cases with progressive disease (defined as an increase in fibrosis score $\geqslant$ 2 during follow-up in untreated patients). On the other hand, SCCA-IgM levels were substantially stable in patients with no disease progression during the same interval, and no difference in the level of the biomarker was detected in regard to the etiology of chronic liver disease[44].

In chronic HCV infection the presence of non-alcoholic steatohepatitis (NASH) at the histological level reflects a more severe clinical and pathological state than steatosis alone, being associated with a more rapid progression of fibrosis[45]. Recently, the relationship between SCCA-IgM and NASH was investigated in 91 patients with chronic hepatitis C: In patients with histological diagnosis of NASH the immune-complex levels were elevated and

Table 2 Overview of the main observations on squamous cell carcinoma antigen-immunoglobulins M behaviour in clinical settings

Ref.	Main observation
SCCA-IgM in monitoring chronic liver disease	
Biasiolo *et al*[44]	Significant increase of SCCA-IgM levels over time in 75% of untreated patients with chronic hepatitis and with histologically proven liver disease progression (fibrosis score increase ⩾ 2) after six years of follow-up
Martini *et al*[46]	In patients with chronic hepatitis C, SCCA-IgM was found an independent predictor of histologically proven non alcoholic steatohepatitis
SCCA-IgM and antiviral treatment	
Giannini *et al*[48]	In chronic hepatitis C treatment with standard therapy, only patients who achieved sustained viral response showed a significant decrease in median values of SCCA-IgM up to one year of follow-up
Fransvea *et al*[49]	Reduction of SCCA-IgM levels during the first month of standard antiviral therapy was an independent predictor of sustained viral response
Martini *et al*[46]	Significant reduction of SCCA-IgM, lasting up to 6 mo of follow-up, was observed only in HCV-positive patients with sustained response to standard therapy
SCCA-IgM in diagnosis and prognosis of HCC	
Pontisso *et al*[51]	Significant increase over time of SCCA-IgM only in patients with early cirrhosis (histologically proven) who developed HCC within four years of follow up
Buccione *et al*[52]	In HCV-positive patients with overt cirrhosis, SCCA-IgM negativity (cut off ⩽ 200 AU/mL) accurately identified patients at low risk of liver cancer development in the subsequent year
Beneduce *et al*[24]	SCCA-IgM showed higher sensitivity for the diagnosis of HCC, compared to AFP
Pozzan *et al*[53]	In patients with HCC, SCCA-IgM levels were found an independent predictor of survival. A reduction in SCCA-IgM levels was correlated with response to HCC treatment

HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; AFP: Alpha-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen-immunoglobulins M.

associated with more severe steatosis (> 33%), while in HCV-negative patients with steatosis and NASH SCCA-IgM was barely detectable. These results were probably due to a higher production of IgM in HCV positive patients, followed by an amplification of the ELISA signal as a result of a lower threshold required for B-cell activation after the engagement of CD81 by the HCV-E2 protein[46].

The association between SCCA-IgM and NASH in HCV positive patients was confirmed at univariate and multivariate logistic regression analysis. Among the various clinical aspects that were considered, only HCV genotype 3 was identified as an additional independent variable significantly associated with NASH[46]. Furthermore, a close correlation between the intensity of SCCA-1 expression in the liver and SCCA-IgM levels in the serum was documented in serum and liver samples from the same patients. Indeed, in cases of negative serological SCCA-IgM, SCCA-1 detection in the corresponding liver biopsy was weak, even in the presence of steatosis. On the other hand, the serpin was highly expressed in patients with elevated values of SCCA-IgM in serum[46].

SCCA-IGM AND ANTIVIRAL TREATMENT

Combination therapy of pegylated interferon-alpha (PEG-IFNα) and ribavirin results in complete viral eradication in about 50% of patients with chronic HCV infection. However, a substantial number of patients show no significant response to therapy or develop viral relapse after the cessation of IFN-based therapy[47].

The first evidence of the behavior of SCCA-IgM during antiviral treatment with PEG-IFN and ribavirin was obtained from a longitudinal study in 2010[48]. Giannini *et al*[48] demonstrated that in patients with HCV-related cirrhosis who achieved sustained virological response (SVR) after standard treatment with PEG-IFN and ribavirin, there was a significant decrease in serum levels of SCCA-IgM at the end of treatment, and up to one year of follow-up, when compared to baseline. In null responders (NR) baseline values of serological SCCA-IgM were not statistically different from SVR patients, but during follow-up SCCA-IgM levels did not show significant changes compared to baseline[48]. In 2012, in a multicentre prospective study, 103 patients with HCV chronic infection undergoing antiviral treatment with PEG-IFN and ribavirin were enrolled to test the efficacy of SCCA-IgM as a marker of response[49]. This study confirmed that the reduction of SCCA immune-complexes was significantly different between patients that showed SVR and those who did not. Moreover, the decreased serological concentration of the biomarker was an independent predictor of SVR in regard to HCV genotype and age[49]. In addition, the behavior of SCCA-IgM in relation to antiviral therapy was recently confirmed in 91 patients with chronic hepatitis C. In the subgroup of patients who reached SVR and had the baseline positivity to SCCA-IgM, the serological values significantly decreased after six months of treatment and remained persistently low even at six months of follow-up after treatment. In NR patients, no significant variation in SCCA-IgM serum values was observed after six months of treatment or at six months after the end of therapy[46].

These studies clearly demonstrate that the termination of HCV-associated liver damage determines a progressive decline of SCCA-IgM serological levels, therefore this biomarker could be used as a surrogate marker to monitor active disease resolution.

SCCA-IGM AND HCC

One of the most important and yet unmet clinical needs

in hepatology is the availability of serological markers to identify patients with chronic hepatitis and cirrhosis at higher risk of HCC development. In liver cirrhosis the rate of HCC progression is 3%-4% every year[50]; the identification of the subgroup of patients with possible HCC development within the next few years would allow the development of a personalized clinical management characterized by more effective early therapeutic interventions. In order to explore this possibility, SCCA-IgM was analyzed in a retrospective, longitudinal study that was preliminary conducted in a cohort of HCV-infected patients with early stage of cirrhosis, defined on the basis of histological findings[51]. This population was divided in two groups with similar clinical characteristics and no significant difference in the absolute value of the immune-complex at baseline. The first group included 16 cirrhotic patients who developed HCC during a median follow up of 4 years, while the second group included 17 control patients with cirrhosis who did not develop HCC during the same period. The progressive increase of SCCA-IgM over time was remarkable in cirrhotic patients who eventually developed HCC, while figures remained unchanged in the majority of the cirrhotic patients without evidence of liver cancer during the same time interval. The increase of AFP, measured in parallel in the same serum samples, was not significantly different in patients who developed HCC and in those without liver tumor progression. Accordingly, the predictive value of SCCA-IgM variation was found to be significantly better than that of AFP for predicting the progression to HCC (AUC: 0.821 *vs* 0.654)[51].

These data were in line with another retrospective study performed by Buccione *et al*[52]. The aim of this study was to evaluate whether the levels of SCCA-IgM in serum could identify HCV patients with clinical signs of cirrhosis at risk of HCC development. The study involved 57 cirrhotic patients, during a median period of 48 mo. The baseline value of serological SCCA-IgM was nearly 4-fold higher in patients who developed HCC than in those who did not, and the SCCA-IgM value $\leqslant$ 200 AU/mL accurately identified patients at low risk of liver cancer in the subsequent year, with a negative predictive value of 97%[52]. These results suggest that in patients with evident cirrhosis the assessment of this biomarker can improve the diagnostic process: The subgroup of patients at higher risk of liver tumor development, that need a constant monitoring, can be identified based on SCCA-IgM positivity.

In regard to the diagnostic value of SCCA-IgM for HCC, a cross sectional study performed by Beneduce *et al*[24] have demonstrated the positivity of this biomarker in the vast majority of HCC serum samples (70% sensitivity *vs* 42% sensitivity of AFP), whereas all healthy control samples were negative. Although no correlation with HCC etiology was found, the authors observed that the amount of circulating SCCA-IgM at different stages of liver disease reflected the extent of SCCA over-expression detected by immunohistochemistry in liver specimens. Moreover, SCCA-IgM positivity did not overlap with that of AFP, suggesting that the combination of these two biomarkers could improve the sensitivity for detecting HCC without compromising the diagnostic specificity[24].

In summary, the prognostic role of SCCA-IgM has been explored in patients with chronic hepatitis and cirrhosis, documenting a higher risk of fibrosis progression and liver tumor development.

Until recently, no data were available on the prognostic role of this biomarker in HCC prognosis. This aspect was addressed in a recent study by Pozzan *et al*[53], who retrospectively analyzed the serum of 327 patients, including patients with cirrhosis and HCC. In this study the ability of SCCA-IgM to predict liver cancer prognosis was proved for the first time. Indeed, the negativity of this biomarker was able to identify HCC patients with longer overall and progression-free survival. Median survival was 48 mo for patients with low SCCA-IgM (< 130 AU/mL) and 26 mo for those with high SCCA-IgM (> 130 AU/mL). The levels of the biomarker at four weeks were stable or increased in treated patients with stable disease or tumor, and reduced in patients with complete response; patients with partial response showed an intermediate behavior. In the same study, AFP was not able to predict complete response. The significant impact of SCCA-IgM determination in defining patient prognosis was confirmed also by data showing that SCCA-IgM levels and tumor size were the only identified independent predictors of overall survival[53]. Although these findings must be confirmed in further studies, they are supported by recent data demonstrating that liver tumors with high SCCA-1 tissue expression exhibit higher early recurrence after surgical resection[38].

FUTURE PERSPECTIVES

Recent innovations in antiviral therapy for HCV have resulted in a remarkable improvement in SVR rates, better acceptability, and decreased duration of treatment compared to IFN and ribavirin-based therapy[54]. The improvement in the antiviral efficacy of the new drug regimens promises higher cure rates with fewer side effects and shorter times of treatment compared to the old standard of care, but it is more expensive and requires major investments[55]. Although recent data have demonstrated that treatment of chronic HCV infection with one of the new oral drug regimens can reduce HCV-related complications and is cost-effective in most patients[56], treating all eligible patients could have an enormous economic impact for both private and public resources[56]. As a consequence, one of the emerging needs is to identify patients that will benefit the most from the new antiviral regimens[56]. Up to now, no biomarkers have been proposed to define the subset of HCV-infected patients with more aggressive disease. In a context of inadequate resources, SCCA-IgM could be used as a support tool to prioritize patients who will benefit the most from these new drugs.

In conclusion, although further studies are needed to confirm the data, serological SCCA-IgM is emerging as a very useful tool in different clinical settings of liver disease.

REFERENCES

1 **Blachier M**, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; **58**: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]

2 **Tsochatzis EA**, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014; **383**: 1749-1761 [PMID: 24480518 DOI: 10.1016/S0140-6736(14)60121-5]

3 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]

4 **Lok AS**, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010; **138**: 493-502 [PMID: 19852963 DOI: 10.1053/j.gastro.2009.10.031]

5 **Trevisani F**, De Notariis S, Rapaccini G, Farinati F, Benvegnù L, Zoli M, Grazi GL, Del PP, Di N, Bernardi M. Semiannual and annual surveillance of cirrhotic patients for hepatocellular carcinoma: effects on cancer stage and patient survival (Italian experience). *Am J Gastroenterol* 2002; **97**: 734-744 [PMID: 11922571]

6 **Zhang BH**, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004; **130**: 417-422 [PMID: 15042359]

7 **McMahon BJ**, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, Dunaway E, Williams J. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. *Hepatology* 2000; **32**: 842-846 [PMID: 11003632]

8 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051]

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 **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]

12 **Poon D**, Anderson BO, Chen LT, Tanaka K, Lau WY, Van Cutsem E, Singh H, Chow WC, Ooi LL, Chow P, Khin MW, Koo WH. Management of hepatocellular carcinoma in Asia: consensus statement from the Asian Oncology Summit 2009. *Lancet Oncol* 2009; **10**: 1111-1118 [PMID: 19880065 DOI: 10.1016/S1470-2045(09)70241-4]

13 **Lee E**, Edward S, Singal AG, Lavieri MS, Volk M. Improving screening for hepatocellular carcinoma by incorporating data on levels of α-fetoprotein, over time. *Clin Gastroenterol Hepatol* 2013; **11**: 437-440 [PMID: 23247324 DOI: 10.1016/j.cgh.2012.11.029]

14 **Li C**, Zhang Z, Zhang P, Liu J. Diagnostic accuracy of des-gamma-carboxy prothrombin versus α-fetoprotein for hepatocellular carcinoma: A systematic review. *Hepatol Res* 2014; **44**: E11-E25 [PMID: 23834468 DOI: 10.1111/hepr.12201]

15 **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]

16 **Schütte K**, Schulz C, Link A, Malfertheiner P. Current biomarkers for hepatocellular carcinoma: Surveillance, diagnosis and prediction of prognosis. *World J Hepatol* 2015; **7**: 139-149 [PMID: 25729470 DOI: 10.4254/wjh.v7.i2.139]

17 **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]

18 **Wan HG**, Xu H, Gu YM, Wang H, Xu W, Zu MH. Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2014; **38**: 706-714 [PMID: 25034355 DOI: 10.1016/j.clinre.2014.06.008]

19 **Rubin B**. Natural immunity has significant impact on immune responses against cancer. *Scand J Immunol* 2009; **69**: 275-290 [PMID: 19281540 DOI: 10.1111/j.1365-3083.2008.02220.x]

20 **Vollmers HP**, Brändlein S. Natural antibodies and cancer. *J Autoimmun* 2007; **29**: 295-302 [PMID: 17826951]

21 **Castaldi F**, Marino M, Beneduce L, Belluco C, De Marchi F, Mammano E, Nitti D, Lise M, Fassina G. Detection of circulating CEA-IgM complexes in early stage colorectal cancer. *Int J Biol Markers* 2005; **20**: 204-208 [PMID: 16398401]

22 **Beneduce L**, Prayer-Galetti T, Giustinian AM, Gallotta A, Betto G, Pagano F, Fassina G. Detection of prostate-specific antigen coupled to immunoglobulin M in prostate cancer patients. *Cancer Detect Prev* 2007; **31**: 402-407 [PMID: 18035503]

23 **Marcello A**, Wirths O, Schneider-Axmann T, Degerman-Gunnarsson M, Lannfelt L, Bayer TA. Circulating immune complexes of Abeta and IgM in plasma of patients with Alzheimer's disease. *J Neural Transm* (Vienna) 2009; **116**: 913-920 [PMID: 19415450 DOI: 10.1007/s00702-009-0224-y]

24 **Beneduce L**, Castaldi F, Marino M, Tono N, Gatta A, Pontisso P, Fassina G. Improvement of liver cancer detection with simultaneous assessment of circulating levels of free alpha-fetoprotein (AFP) and AFP-IgM complexes. *Int J Biol Markers* 2005; **19**: 155-159 [PMID: 15255549]

25 **Zuin J**, Veggiani G, Pengo P, Gallotta A, Biasiolo A, Tono N, Gatta A, Pontisso P, Toth R, Cerin D, Frecer V, Meo S, Gion M, Fassina G, Beneduce L. Experimental validation of specificity of the squamous cell carcinoma antigen-immunoglobulin M (SCCA-IgM) assay in patients with cirrhosis. *Clin Chem Lab Med* 2010; **48**: 217-223 [PMID: 20001440 DOI: 10.1515/CCLM.2010.044]

26 **Suminami Y**, Kishi F, Sekiguchi K, Kato H. Squamous cell carcinoma antigen is a new member of the serine protease inhibitors. *Biochem Biophys Res Commun* 1991; **181**: 51-58 [PMID: 1958219]

27 **Kato H**, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. *Cancer* 1977; **40**: 1621-1628 [PMID: 332328]

28 **Kato H**. Expression and function of squamous cell carcinoma antigen. *Anticancer Res* 1996; **16**: 2149-2153 [PMID: 8694535]

29 **Catanzaro JM**, Sheshadri N, Pan JA, Sun Y, Shi C, Li J, Powers RS, Crawford HC, Zong WX. Oncogenic Ras induces inflammatory cytokine production by upregulating the squamous cell carcinoma antigens SerpinB3/B4. *Nat Commun* 2014; **5**: 3729 [PMID: 24759783 DOI: 10.1038/ncomms4729]

30 **Vidalino L**, Doria A, Quarta S, Zen M, Gatta A, Pontisso P. SERPINB3, apoptosis and autoimmunity. *Autoimmun Rev* 2009; **9**: 108-112 [PMID: 19332150 DOI: 10.1016/j.autrev.2009.03.011]

31 **Katagiri C**, Nakanishi J, Kadoya K, Hibino T. Serpin squamous cell carcinoma antigen inhibits UV-induced apoptosis via suppression of c-JUN NH2-terminal kinase. *J Cell Biol* 2006; **172**: 983-990 [PMID: 16549498]

32 **Murakami A**, Suminami Y, Hirakawa H, Nawata S, Numa F, Kato H. Squamous cell carcinoma antigen suppresses radiation-induced cell death. *Br J Cancer* 2001; **84**: 851-858 [PMID: 11259103]

33 **Suminami Y**, Nagashima S, Murakami A, Nawata S, Gondo T, Hirakawa H, Numa F, Silverman GA, Kato H. Suppression of

a squamous cell carcinoma (SCC)-related serpin, SCC antigen, inhibits tumor growth with increased intratumor infiltration of natural killer cells. *Cancer Res* 2001; **61**: 1776-1780 [PMID: 11280721]

34 **Suminami Y**, Nagashima S, Vujanovic NL, Hirabayashi K, Kato H, Whiteside TL. Inhibition of apoptosis in human tumour cells by the tumour-associated serpin, SCC antigen-1. *Br J Cancer* 2000; **82**: 981-989 [PMID: 10732775]

35 **Pontisso P**, Calabrese F, Benvegnù L, Lise M, Belluco C, Ruvoletto MG, Marino M, Valente M, Nitti D, Gatta A, Fassina G. Overexpression of squamous cell carcinoma antigen variants in hepatocellular carcinoma. *Br J Cancer* 2004; **90**: 833-837 [PMID: 14970861]

36 **Guido M**, Roskams T, Pontisso P, Fassan M, Thung SN, Giacomelli L, Sergio A, Farinati F, Cillo U, Rugge M. Squamous cell carcinoma antigen in human liver carcinogenesis. *J Clin Pathol* 2008; **61**: 445-447 [PMID: 17893121]

37 **Turato C**, Buendia MA, Fabre M, Redon MJ, Branchereau S, Quarta S, Ruvoletto M, Perilongo G, Grotzer MA, Gatta A, Pontisso P. Over-expression of SERPINB3 in hepatoblastoma: a possible insight into the genesis of this tumour? *Eur J Cancer* 2012; **48**: 1219-1226 [PMID: 21737255 DOI: 10.1016/j.ejca.2011.06.004]

38 **Turato C**, Vitale A, Fasolato S, Ruvoletto M, Terrin L, Quarta S, Ramirez Morales R, Biasiolo A, Zanus G, Zali N, Tan PS, Hoshida Y, Gatta A, Cillo U, Pontisso P. SERPINB3 is associated with TGF-β1 and cytoplasmic β-catenin expression in hepatocellular carcinomas with poor prognosis. *Br J Cancer* 2014; **110**: 2708-2715 [PMID: 24809782 DOI: 10.1038/bjc.2014.246]

39 **Quarta S**, Vidalino L, Turato C, Ruvoletto M, Calabrese F, Valente M, Cannito S, Fassina G, Parola M, Gatta A, Pontisso P. SERPINB3 induces epithelial-mesenchymal transition. *J Pathol* 2010; **221**: 343-356 [PMID: 20527027 DOI: 10.1002/path.2708]

40 **Villano G**, Quarta S, Ruvoletto MG, Turato C, Vidalino L, Biasiolo A, Tono N, Lunardi F, Calabrese F, Dall'olmo L, Dedja A, Fassina G, Gatta A, Pontisso P. Role of squamous cell carcinoma antigen-1 on liver cells after partial hepatectomy in transgenic mice. *Int J Mol Med* 2010; **25**: 137-143 [PMID: 19956912]

41 **Villano G**, Turato C, Quarta S, Ruvoletto M, Ciscato F, Terrin L, Semeraro R, Paternostro C, Parola M, Alvaro D, Bernardi P, Gatta A, Pontisso P. Hepatic progenitor cells express SerpinB3. *BMC Cell Biol* 2014; **15**: 5 [PMID: 24517394 DOI: 10.1186/1471-2121-15-5]

42 **Cannito S**, Turato C, Paternostro C, Biasiolo A, Colombatto S, Cambieri I, Quarta S, Novo E, Morello E, Villano G, Fasolato S, Musso T, David E, Tusa I, Rovida E, Autelli R, Smedile A, Cillo U, Pontisso P, Parola M. Hypoxia up-regulates SERPINB3 through HIF-2α in human liver cancer cells. *Oncotarget* 2015; **6**: 2206-2221 [PMID: 25544768]

43 **Turato C**, Calabrese F, Biasiolo A, Quarta S, Ruvoletto M, Tono N, Paccagnella D, Fassina G, Merkel C, Harrison TJ, Gatta A, Pontisso P. SERPINB3 modulates TGF-beta expression in chronic liver disease. *Lab Invest* 2010; **90**: 1016-1023 [PMID: 20212457 DOI: 10.1038/labinvest.2010.55]

44 **Biasiolo A**, Chemello L, Quarta S, Cavalletto L, Bortolotti F, Caberlotto C, Beneduce L, Bernardinello E, Tono N, Fassina G, Gatta A, Pontisso P. Monitoring SCCA-IgM complexes in serum predicts liver disease progression in patients with chronic hepatitis. *J Viral Hepat* 2008; **15**: 246-249 [PMID: 18248333 DOI: 10.1111/j.1365-2893.2007.00935.x]

45 **Bedossa P**, Moucari R, Chelbi E, Asselah T, Paradis V, Vidaud M, Cazals-Hatem D, Boyer N, Valla D, Marcellin P. Evidence for a role of nonalcoholic steatohepatitis in hepatitis C: a prospective study. *Hepatology* 2007; **46**: 380-387 [PMID: 17659580]

46 **Martini A**, Fattovich G, Guido M, Bugianesi E, Biasiolo A, Ieluzzi D, Gallotta A, Fassina G, Merkel C, Gatta A, Negro F, Pontisso P. HCV genotype 3 and squamous cell carcinoma antigen (SCCA)-IgM are independently associated with histological features of NASH in HCV-infected patients. *J Viral Hepat* 2015; **22**: 800-808 [PMID: 25611978 DOI: 10.1111/jvh.12394]

47 **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]

48 **Giannini EG**, Basso M, Bazzica M, Contini P, Marenco S, Savarino V, Picciotto A. Successful antiviral therapy determines a significant decrease in squamous cell carcinoma antigen-associated (SCCA) variants' serum levels in anti-HCV positive cirrhotic patients. *J Viral Hepat* 2010; **17**: 563-568 [PMID: 19840364 DOI: 10.1111/j.1365-2893.2009.01217.x]

49 **Fransvea E**, Trerotoli P, Sacco R, Bernabucci V, Milella M, Napoli N, Mazzocca A, Renna E, Quaranta M, Angarano G, Villa E, Antonaci S, Giannelli G. SCCA-IC serum levels are predictive of clinical response in HCV chronic hepatitis to antiviral therapy: a multicentric prospective study. *J Viral Hepat* 2012; **19**: 704-710 [PMID: 22967101 DOI: 10.1111/j.1365-2893.2012.01604.x]

50 **Prorok PC**. Epidemiologic approach for cancer screening. Problems in design and analysis of trials. *Am J Pediatr Hematol Oncol* 1992; **14**: 117-128 [PMID: 1530116]

51 **Pontisso P**, Quarta S, Caberlotto C, Beneduce L, Marino M, Bernardinello E, Tono N, Fassina G, Cavalletto L, Gatta A, Chemello L. Progressive increase of SCCA-IgM immune complexes in cirrhotic patients is associated with development of hepatocellular carcinoma. *Int J Cancer* 2006; **119**: 735-740 [PMID: 16550605]

52 **Buccione D**, Fatti G, Gallotta A, Loggi E, Di Donato R, Testa L, Saitta C, Santi V, Di Micoli A, Erroi V, Frigerio M, Fazio V, Picciotto A, Biasiolo A, Degos F, Pontisso P, Raimondo G, Trevisani F. Serum Scca-IgM as a Predictor of Hepatocellular Carcinoma in Patients with Liver Cirrhosis. *OJGas* 2012; **2**: 56-61 [DOI: 10.4236/ojgas.2012.22012]

53 **Pozzan C**, Cardin R, Piciocchi M, Cazzagon N, Maddalo G, Vanin V, Giacomin A, Pontisso P, Cillo U, Farinati F. Diagnostic and prognostic role of SCCA-IgM serum levels in hepatocellular carcinoma (HCC). *J Gastroenterol Hepatol* 2014; **29**: 1637-1644 [PMID: 24635038 DOI: 10.1111/jgh.12576]

54 **Alberti A**, Piovesan S. The evolution of the therapeutic strategy in hepatitis C: features of sofosbuvir and indications. *Dig Liver Dis* 2014; **46** Suppl 5: S174-S178 [PMID: 25458777 DOI: 10.1016/j.dld.2014.09.028]

55 **Younossi Z**, Henry L. The impact of the new antiviral regimens on patient reported outcomes and health economics of patients with chronic hepatitis C. *Dig Liver Dis* 2014; **46** Suppl 5: S186-S196 [PMID: 25458773 DOI: 10.1016/j.dld.2014.09.025]

56 **Chhatwal J**, Kanwal F, Roberts MS, Dunn MA. Cost-effectiveness and budget impact of hepatitis C virus treatment with sofosbuvir and ledipasvir in the United States. *Ann Intern Med* 2015; **162**: 397-406 [PMID: 25775312 DOI: 10.7326/M14-1336]

57 **Montagnana M**, Danese E, Lippi G. Squamous cell carcinoma antigen in hepatocellular carcinoma: Ready for the prime time? *Clin Chim Acta* 2015; **445**: 161-166 [PMID: 25840050 DOI: 10.1016/j.cca.2015.03.031]

58 **Rich N**, Singal AG. Hepatocellular carcinoma tumour markers: current role and expectations. *Best Pract Res Clin Gastroenterol* 2014; **28**: 843-853 [PMID: 25260312 DOI: 10.1016/j.bpg.2014.07.018]

P- Reviewer: Hokama A, Larrubia JR **S- Editor**: Song XX
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.i29.2920

World J Hepatol 2015 December 18; 7(29): 2920-2926
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

PAI-1 4G-4G and MTHFR 677TT in non-hepatitis C virus/ hepatitis B virus-related liver cirrhosis

Linda Pasta, Francesca Pasta

Linda Pasta, Francesca Pasta, Department of Medicine, Clinica Candela, 90141 Palermo, Italy

Author contributions: Pasta L designed and performed the research, analyzed the data and wrote the paper; Pasta F performed the research, analyzed the data and wrote the paper.

Institutional review board statement: The local human research committee of AOOR of Palermo approved this retrospective study.

Informed consent statement: All participants gave their informed consent prior to their inclusion in the study, according to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

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

Data sharing statement: The technical appendix, statistical code and dataset are available from the corresponding author at: lindpas@yahoo.it.

Correspondence to: Linda Pasta, MD, Department of Medicine, Clinica Candela, Via Villareale 54, 90141 Palermo, Italy. lindpas@yahoo.it
Telephone: +39-091-587122
Fax: +39-091-589544

Received: May 29, 2015
Peer-review started: June 2, 2015
First decision: August 22, 2015
Revised: September 4, 2015
Accepted: November 23, 2015
Article in press: November 25, 2015

Published online: December 18, 2015

Abstract

AIM: To evaluate the different roles of thrombophilia in patients with and without viral etiology. The thrombophilic genetic factors (THRGFs), PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q and prothrombin 20210A, were studied as risk factors in 1079 patients with liver cirrhosis (LC), enrolled from January 2000 to January 2014.

METHODS: All Caucasian LC patients consecutively observed in a fourteen-year period were included; the presence of portal vein thrombosis (PVT) and Budd Chiari syndrome (BCS) was registered. The differences between the proportions of each THRGF with regard to the presence or absence of viral etiology and the frequencies of the THRGF genotypes with those predicted in a population by the Hardy-Weinberg equilibrium were registered.

RESULTS: Four hundred and seventeen/one thousand and seventy-six patients (38.6%) showed thrombophilia: 217 PAI-1 4G-4G, 176 MTHFR C677TT, 71 V Leiden factor and 41 prothrombin G20210 A, 84 with more than 1 THRGF; 350 presented with no viral liver cirrhosis (NVLC) and 729 with, called viral liver cirrhosis (VLC), of whom 56 patients were hepatitis C virus + hepatitis B virus. PAI-1 4G-4G, MTHFR C677TT, the presence of at least one TRHGF and the presence of > 1 THRGF, were statistically more frequent in patients with NVLC *vs* patients with VLC: All $\chi^2 > 3.85$ and $P < 0.05$. Patients with PVT and/or BCS with at least one TRHGF were 189/352 (53.7%). The Hardy-Weinberg of PAI-1 and MTHFR 677 genotypes deviated from that expected from a population in equilibrium in patients with NVLC (respectively $\chi^2 = 39.3$; $P < 0.000$ and $\chi^2 = 27.94$; $P < 0.05$), whereas the equilibrium was respected in VLC.

CONCLUSION: MTHFR 677TT was nearly twofold and PAI-1 4G-4G more than threefold more frequently found in NVLC *vs* patients with VLC; the Hardy-Weinberg equilibrium of these two polymorphisms confirms this data in NVLC. We suggest that PAI-1 4G-4G and MTHFR 677TT could be considered as factors of fibrosis and thrombosis mechanisms, increasing the inflammation response, and causing the hepatic fibrosis and augmented intrahepatic vascular resistance typical of LC. PAI-1 4G-4G and MTHFR 677TT screening of LC patients could be useful, mainly in those with NVLC, to identify patients in which new drug therapies based on the attenuation of the hepatic stellate cells activation or other mechanisms could be more easily evaluated.

Key words: PAI-1 4G-4G; MTHFR 677TT; V Leiden 506Q; Prothrombin 20210A; Liver cirrhosis; Portal vein thrombosis; Budd Chiari syndrome; Fibrogenesis

Core tip: This study on thrombophilia in 1079 patients with liver cirrhosis showed that PAI-1 4G-4G and MTHFR C677TT were statistically more frequent in 350 patients with no viral liver cirrhosis *vs* 729 patients with viral liver cirrhosis. In the same patients, PAI-1 and MTHFR 677 genotypes deviated from that expected from a population in the Hardy-Weinberg equilibrium. PAI-1 4G-4G and MTHFR 677TT could be considered as factors increasing the inflammation response mechanisms, causing fibrogenesis and augmented intrahepatic vascular resistance, typical of liver cirrhosis. New drug therapies based on the attenuation of these mechanisms could be very easily evaluated in these patients.

Pasta L, Pasta F. PAI-1 4G-4G and MTHFR 677TT in non-hepatitis C virus/hepatitis B virus-related liver cirrhosis. *World J Hepatol* 2015; 7(29): 2920-2926 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2920.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2920

INTRODUCTION

We studied the thrombophilic genetic factors (THRGFs), PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q and prothrombin 20210A, as risk factors in patients with liver cirrhosis (LC). We have published two studies on the prevalence of these THRGFs in LC. The first study included 214 patients with LC, enrolled from January 2000 to December 2007[1]. In this study, we demonstrated the significant role of PAI-1 4G-4G, MTHFR C677TT and prothrombin G20210A in patients with hepatocellular carcinoma (HCC) *vs* healthy controls, but we did not analyze the role of THRGF in patients with LC. The second study included 865 patients from June 2008 to January 2014[2]. In this study, we demonstrated the pivotal role of PAI-1 4G-4G and MTHFR 677TT in patients with alcoholic and cryptogenic LC and provided the hypothesis that thrombo and fibro-genetic mechanisms of PAI-1 4G-4G and MTHFR 677TT could have a role in the development of LC, mainly in patients without hepatitis C virus (HCV) and hepatitis B virus (HBV) etiology.

To evaluate the different role of thrombophilia, in patients with and without viral etiology, we analyzed the total number of patients with LC recruited by our group by asking the question if these THRGFs could be potential markers of liver fibrogenesis, mainly in patients without viral etiology.

We built a file with data of the individual patients with LC from the two studies described above from 2000 to 2014[1,2] and compared the results of the analysis with those from the literature that support the hypothesis of a pathogenetic role of thrombophilia in liver fibrogenesis.

MATERIALS AND METHODS

Patients

The first study included 214 patients with LC, enrolled from January 2000 to December 2007[1], and the second, 865 patients from June 2008 to January 2014[2].

Inclusion criteria: All Caucasian patients with a diagnosis of LC consecutively observed in the Medicine and Liver Department of the Emergency Hospital of Palermo were included. Exclusion criteria: Non-Caucasian patients or those with biliary cirrhosis, autoimmune cirrhosis, celiac disease, HCC and other neoplasms were excluded. The presence of portal vein thrombosis (PVT) and the extension of the thrombosis to the mesenteric or splenic vein was registered and accepted when unambiguous diagnostic evidence was detected by proper imaging techniques. All patients underwent endoscopy and the size of esophageal varices was recorded as large-medium/small-absent. All patients were asked if they had a history of episodes of gastrointestinal bleeding. The local human research committee approved this study protocol.

We analyzed the data of patients with regard to the various etiologies and the patients were also analyzed separately in two subgroups: The first with virus C and/or B and the second with alcoholic and cryptogenic cirrhosis, as our second study showed that only the latter patients showed a significant frequency of THRGFs.

THRGFs and definition of thrombophilia

To evaluate the role of PAI-1, MTHFR677, V Leiden 506Q and prothrombin 20210A mutations, genotyping of these polymorphisms was performed by PCR-RFLP, according to Patnaik *et al*[3], in both heterozygous and homozygous statuses. We defined genetic thrombophilia as the presence of at least one of the following THRGFs: PAI-1 4G-4G, MTHFR 677TT, V Leiden Q506 and prothrombin 20210A, as in our previous studies[1,2]. All patients signed an informed consent form and the study conformed to the ethical guidelines of the 1975 Helsinki

Table 1 Main demographic and clinical characteristics of patients with hepatitis C/B virus liver cirrhosis, defined virus liver cirrhosis and alcoholic and cryptogenic, aggregated as non-virus cirrhosis

	Total (%)	VLC (%)	Alcoholic (%)	Crypto (%)	NVLC (%)
Patients	1079 (100)	729 (100)	102 (100)	248 (100)	350 (100)
Age (range)	57 (19-83)	60 (24-83)	55 (19-80)	49 (19-83)	51 (19-83)
Male sex	620 (57.5)	384 (52.7)	81 (79.4)	155 (62.5)	236 (67.4)
PVT/BCS	352 (32.6)	230 (31.5)	45 (44.1)	77 (31.0)	122 (34.8)
MVT/SVT	53 (4.9)	33 (4.5)	6 (5.8)	14 (5.6)	20 (5.7)
L-M varices	445 (41.2)	574 (78.7)	48 (47.0)	150 (60.4)	245 (70.0)
N° bleeding	360 (33.3)	265 (36.3)	41 (40.1)	54 (21.7)	95 (27.1)
Child A/B/C	416/242/430	241/194/294	31/53/17	129/31/83	165/48/17
	(38.5/22.4/39.8)	(33.0/26.6/40.3)	(30.3/51.9/16.6)	(52.0/12.5/33.4)	(47.1/13.7/39.1)

No statistical differences between VLC *vs* NVLC group: All $\chi^2 > 3.85$ and $P < 0.05$. VLC: Virus liver cirrhosis; NVLC: Non-virus cirrhosis; PVT: Portal vein thrombosis; BCS: Budd Chiari syndrome; MVT: Mesenteric vein thrombosis; SVT: Splenic vein thrombosis.

Table 2 Frequencies of thrombophilic genetic factors, PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q and prothrombin 20210A, in patients with hepatitis C/B virus liver cirrhosis, alcoholic and cryptogenic, aggregated as non-virus cirrhosis

	(A) VLC (%)	Alcoholic (%)	Crypto (%)	(B) NVLC (%)	(A) *vs* (B) χ^2: *P* value; OR (95%CI)
Patients	729 (100)	102 (100)	248 (100)	350 (100)	-
PAI-1 4G-4G	95 (13.0)	33 (32.3)	89 (35.8)	122 (34.8)	68.2: 0.000; 3.6 (2.6-4.9)
MTHFR 677TT	99 (13.5)	14 (13.7)	63 (25.4)	77 (22.0)	11.7: 0.001; 1.8 (1.3-2.5)
V Leiden 506Q	41 (5.6)	9 (8.8)	22 (8.8)	30 (8.6)	3.3: 0.09; 1.5 (0.9-2.6)
Prothrombin 20210A	25 (3.4)	3 (2.9)	13 (5.2)	16 (4.5)	0.8: 0.39; 1.4 (0.7-2.7)
At least 1 THRGF	218 (29.9)	43 (42.1)	156 (62.9)	199 (56.9)	72.5: 0.000; 3.1 (2.4-4.1)
> 1 THRGF	40 (5.4)	14 (13.7)	30 (12.0)	44 (12.5)	16.5: 0.000; 2.5 (1.6-4.0)

VLC: Virus liver cirrhosis; NVLC: Non-virus cirrhosis; THRGF: Thrombophilic genetic factors.

Declaration.

Statistical analysis

We looked at the differences between the proportions of each THRGF, with regard to the presence or absence of viral etiology, using the contingency tables[4]. We only considered statistically significant differences, if the $\chi^2 > 3.84$ and P value < 0.05.

Moreover, we compared the observed frequencies of the THRGF genotypes with those predicted in a population by the Hardy-Weinberg equilibrium using a web interactive calculator[5].

RESULTS

The whole group consisted of 1079 patients: 336 patients showed PVT and 16 Budd Chiari syndrome; 53 patients showed mesenteric and/or splenic vein thrombosis associated with PVT; large-medium esophageal varices were present in 445 patients and 360 patients had had at least one gastrointestinal bleeding episode.

The main demographic and clinical characteristics of patients, declared in the previous original studies[1,2], are synthesized in Table 1. The patients were separated into two subgroups: The first with alcoholic and cryptogenic cirrhosis, *i.e.*, without viral etiology, (350 patients) called no viral cirrhosis (NVLC), and the second with virus B and/or C (729 of whom 56 patients were HCV + HBV), called viral liver cirrhosis (VLC). No statistical differences were found between NVLC *vs* VLC demographic and clinical characteristics: All $\chi^2 > 3.85$ and $P < 0.05$.

A total of 189/352 patients with PVT and/or BCS showed at least one TRHGF; in 177, PAI-1 4G-4G and/or MTHFR 677TT were present.

Table 2 shows the frequencies of the studied THRGFs in the 1079 patients with cirrhosis of various etiologies and with regard to the presence of virus. A total of 417/1079 patients (38.6%) showed thrombophilia: 217 PAI-1 4G-4G, 176 MTHFR C677TT, 71 V Leiden factor and 41 prothrombin G20210 A, 84 with more than 1 THRGF (82 patients with 2 THRGFs; 2 patients, 3).

Not one V with Leiden 506Q or Prothrombin 20210A homozygous was present. The proportion of PAI-1 polymorphisms 4G-5G and 5G-5G was, respectively, 123 and 121 in NVLC and 354 and 280 in VLC; the proportion of MTHFR polymorphisms C677T and CC677 was, respectively, 161 and 112 in NVLC and 364 and 266 in VLC.

NVLC and VLC showed at least one THRGF in 198/350 and 199/729 patients, respectively.

We tested the statistical differences of the single THRGF between patients with NVLC and VLC, with 2-way contingency table analysis. Table 2 shows the corresponding χ^2, P values and odd ratios with 95% with confidence intervals (OR, with 95%CI). V Leiden factor and prothrombin G20210 did not show statistical

differences. PAI-1 4G-4G, MTHFRC677TT, the presence of thrombophilia and the presence of > 1 THRGF were statistically more frequent in patients with NVLC *vs* patients with VLC: All χ^2 > 3.85 and P < 0.05. A total of 178/350 (50.8%) NVLC *vs* 179/729 (24.5%) VLC showed a significant proportion of PAI-1 4G-4G and/or MTHFRC677TT: χ^2 = 73.8, P value < 0.000, 95%CI: 3.2 (2.4-4.2).

The Hardy-Weinberg of PAI-1 and MTHFR 677 genotypes deviated from that expected from a population in equilibrium in patients with NVLC (respectively χ^2 = 39.3; P < 0.000 and χ^2 = 27.94; P < 0.05 respectively), whereas the equilibrium was respected in VLC. Leiden Q506 and prothrombin 20210A Hardy-Weinberg equilibrium was respected in the two groups of patients.

DISCUSSION

This study was planned to evaluate the proportions of THRGFs, PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q and prothrombin 20210A, in a large sample of patients with LC, recruited in two prospective studies[1,2]. In the second of these studies, we found that thrombo and fibro-genetic mechanisms of PAI-1 4G-4G and MTHFR 677TT could have a role in the development of LC, mainly in patients without HCV and HBV.

Many authors for many years have studied the intrinsic mechanisms of fibrogenesis in liver diseases with and without associated viruses. In our study, 417/1079 (38.6%) patients with LC showed thrombophilia. We did not find any correlation with factor V Leiden and prothrombin 20210A, even although many authors have found a correlation with hepatic fibrogenesis[6,7]. We mainly focused our attention on the role of PAI-1 4G-4G and MTHFR 677TT. The prevalence of PAI-1 4G-4G in patients with LC was more frequent than that of MTHFR 677TT in our study: 217 and 176. A total of 357/1079 (33.1%) showed the presence of PAI-1 4G-4G and/or MTHFR 677TT, with a significant difference between patients with NVLC *vs* VLC: 178/350 (50.8%) and 179/729 (24.5%).

MTHFR 677TT is nearly twofold and PAI-1 4G-4G over threefold more frequently found in NVLC *vs* patients with VLC, as shown in Table 2. Moreover, the Hardy-Weinberg equilibrium of these two polymorphisms confirms these data in NVLC.

A limitation of this study is the inability to compare our data since no comparable data have been published, mainly for PAI-1 4G-4G. This was a single center study and the patients were all Caucasian, almost exclusively from Sicily; this could lead to a high genetic frequency of these two genes on the basis of the geographical belonging, typical of populations of the islands, as in the case of Wilson's disease in Sardinia[8].

We did not estimate the correlation between the degree of liver fibrosis and the presence of thrombophilia markers; this correlation must be evaluated in future studies, including other factors such as protein C and S deficiency, antithrombin Ⅲ, increased serum levels of factor Ⅷ, resistance to thrombomodulin action, *etc*. In future studies, suitable systems to measure the speed of the portal flow, another risk factor for thrombosis development, should also be developed. Finally, the relationship between the flow velocity and the presence of thrombophilia should be studied.

Regarding PAI-1 4G-4G, there are many studies demonstrating the role of this THRGF associated with the highest serum PAI-1 activity[9] in the liver fibrosis process. PAI-1 has an active role in liver fibrosis in rats[10] through a pathogenic mechanism leading to the hepatic stellate cell (HSC) activation[11]. The relationship between ethanol, liver and PAI-1 in alcoholic liver diseases was very recently reviewed by Liu[12]; alcohol up-regulates PAI-1 and its level can be used as an index for the severity of the disease. Patients with nonalcoholic steatohepatitis showed significantly higher PAI-1 values than those with normal liver, as found by Verrijken *et al*[13]. These observations seem to be sufficient to explain why patients with PAI-1 4G-4G have an increased risk of fibrosis progression to LC development in patients without HCV or HBV.

There are many active drugs for fibrolysis, with the goal of lowering the PAI-1 synthesis. Some models of the action of these drugs on PAI 1 activity are reported below; the final objective of these drugs is the reduction of the activation of HSC caused by PAI-1. Sauchinone blocks the transforming growth factor (TGF)-β1-induced phosphorylation of Smad 2/3, the transcript levels of plasminogen activator inhibitor-1 and matrix metalloproteinase-2, as well as autophagy in HSC[14].

Spironolactone partially reverses the effects of aldosterone that promote HSC activation and the expression of TGF-β1, PAI-1 and collagen in hepatic fibrosis progression partially mediated by TGF-β1, as studied by Wang *et al*[15].

Statins lead to a profound amelioration in HSC phenotype activated by the oxidant and inflammatory pathways and counteract the stimulatory effect of tumor necrosis factor-α on secretion and expression of PAI-1. Other treatments are under evaluation for the treatment of liver fibrosis, as reported by Gracia-Sancho *et al*[16]; the last futuristic treatment is the use of nanoparticles to transport and deliver nitric oxide into the HSC[17].

Regarding MTHFR 677TT and fibrogenesis, there is much evidence that MTHFR 677TT has a role in the progression of liver diseases. Patients with MTHFR 677TT have higher serum total homocysteine, as reported by Devlin *et al*[18]. MTHFR 677TT polymorphism promotes liver fibrosis progression in patients with recurrent hepatitis C[19] and steatosis and fibrosis in patients with chronic hepatitis C[20].

Hyperhomocysteinemia determines damage of endothelial cells, reduces the flexibility of vessels and adversely affects the process of hemostasis. In addition, hyperhomocysteinemia enhances the adverse effects of risk factors such as hypertension, smoking and impaired glucose, lipid and lipoprotein metabolism, as well as

promoting the development of inflammation, as found by Baszczuk *et al*[21]. Hyperhomocysteinemia is highly prevalent in LC but not in other chronic liver diseases, mainly in patients with MTHFR 677TT; it may contribute to fibrogenesis and vascular complications of LC, as reported by Ventura *et al*[22]. The hyperhomocysteinemia causes endothelial dysfunction, as studied by Cheng *et al*[23]. According to this last study, hyperhomocysteinemia causing oxidative stress determines loss of the normal phenotype of liver sinusoidal endothelial cells (LSEC); the consequent cross-talk between LSEC and HSC induces activation of the latter ones, which in turn proliferate, migrate and increase collagen deposition around the sinusoids, contributing to fibrogenesis, architectural disruption and angiogenesis, as reported by Gracia-Sancho *et al*[16].

Regarding the therapy of MTHFR 677TT polymorphism and the consequent increase of serum homocysteine, there are no tested drugs for patients with this polymorphism apart from folic acid therapy. The administration of folic acid in a dose of 15 mg/d obtains a decrease in the concentration of homocysteine in serum, as recently demonstrated in patients with primary arterial hypertension by Baszczuk *et al*[24].

In conclusion, both PAI-1 4G-4G and MTHFR 677TT cause HSC activation, now recognized as the origin of liver fibrogenesis. This imbalance of the PAI-1 4G-4G and MTHFR 677TT allele frequency is possible evidence of the role of these two polymorphisms in the pathogenesis of LC through SHC activation, today considered the key of liver fibrogenesis.

As reported very recently by Trautwein *et al*[25], it is necessary to accelerate progress in understanding mechanisms of hepatic fibrosis and defining therapeutic targets in order to establish clinical trial designs that can accurately assess the efficacy of antifibrotic drugs.

For these reasons, we think it is important to find patients at the greatest risk for disease progression to ensure that these risk factors can be balanced between placebo and control groups in randomized controlled trials. In our study, PAI-1 4G-4G and MTHFR 677TT were present in up to 50% of patients with NVLC; we think that these genetic factors could be reliably used to stratify risk in clinical trials of new drugs aimed at obtaining the reduction of fibrosis or increase of fibrolysis, also to be evaluated in patients with HBV and/or HCV infection.

We think that drugs that cause the lowering of HSC activation could be better tested in patients with genetic markers such as PAI-1 4G-4G and MTHFR 677TT. These patients could be the tip of the iceberg in a population that produces fibrosis in the liver as well as in other organs (heart, lung, kidney and skin), as reported by Ghosh *et al*[26] with regards to PAI-1 and by Reilly *et al*[27] with regards to MTHFR 677TT.

We suggest that PAI-1 4G-4G and MTHFR 677TT may increase the inflammation response, participating in the activation of HSC and causing hepatic fibrosis and augmented intrahepatic vascular resistance in cirrhosis, as suggested by Fernandez[28]. These two genetic markers share the ultimate goal of increasing the activation of HSC, directly or by the action of LSEC on HSC.

We hope that all relevant studies can suggest new perspectives for developing strategies more effective in lowering fibrosis progression, with the common objective of the attenuation of the HSC activation and consequently liver fibrosis.

In conclusion, PAI-1 4G-4G and MTHFR 677TT could be considered as factors of thrombosis and fibrosis mechanisms that lead to the development of cirrhosis and augmented intrahepatic vascular resistance.

PAI-1 4G-4G and MTHFR 677TT screening of patients could be useful, mainly in those with alcoholic or cryptogenic cirrhosis, to identify patients in which new drug therapies based on the attenuation of the HSC activation or other mechanisms could be more easily evaluated.

The recent articles by Lee *et al*[29] and Gracia-Sancho *et al*[16] deal with the new drugs in the attempt to develop new strategies of combined therapies directed towards multi-targeted different pathophysiological mechanisms (*i.e.*, microvascular dysfunction/angiogenesis or fibrosis/microvascular dysfunction); the goal of new therapies includes efforts to inhibit fibrogenesis and promote resolution of fibrosis. The evaluation of the genetic profile of thrombophilia (obviously as complete as possible) of patients with chronic liver diseases could be considered a noninvasive method to assess the dynamics of fibrogenesis and fibrolysis on a genetic basis.

As an immediate clinical application of the results of our study according to the principles of the translational medicine[29], we think it is advisable to screen patients with chronic liver disease for a genetic predisposition to liver fibrogenesis, as well as in patients with hepatic virus diseases, where these genetic markers can lead to a lower response to the antiviral drugs.

In conclusion, it could be recommended that a complete analysis of the risk of progression of liver fibrosis should include all thrombophilia factors.

In patients with MTHFR 677TT, folic acid supplementation should be prescribed. Patients with PAI-1 4G-4G and perhaps MTHFR 677TT represent a subset of patients in which trials of statins, anti-aldosterone, antioxidants and other drugs should be tested to obtain more rapid results, although none of these drugs are approved yet. Combination therapies should include their association with antiviral treatment and non-selective beta-blockers. In patients with portal vein thrombosis, severe portal hypertension or deep vein thrombosis, thromboprophylaxis with low molecular weight heparins should be recommended, according to Rodriguez-Castro *et al*[30].

COMMENTS

Background

In this study, 417/1079 (38.6%) patients with liver cirrhosis (LC) showed

thrombophilia and PAI-1 4G-4G and MTHFR C677TT were statistically more frequent in 350 patients with no viral LC *vs* 729 patients with viral LC. In the same patients, PAI-1 and MTHFR 677 genotypes deviated from that expected from a population in the Hardy-Weinberg equilibrium.

Research frontiers

Many authors for many years have studied the intrinsic mechanisms of fibrogenesis in liver diseases with and without associated viruses. They suggest that PAI-1 4G-4G and MTHFR 677TT could be considered as factors of fibrosis and thrombosis mechanisms, increasing the inflammation response and causing the hepatic fibrosis and augmented intrahepatic vascular resistance typical of LC.

Innovations and breakthroughs

PAI-1 4G-4G and MTHFR 677TT could be considered as factors increasing the response inflammation mechanisms, causing fibrogenesis and augmented intrahepatic vascular resistance typical of LC.

Applications

PAI-1 4G-4G and MTHFR 677TT screening of hepatic chronic liver disease patients could be useful, mainly in those with no virus-related diseases, to identify patients in which new drug therapies based on the attenuation of the hepatic stellate cell activation or other mechanisms could be evaluated more easily.

Terminology

Genetic thrombophilia is involved in fibrosis and thrombosis mechanisms, increasing the inflammation response and causing the hepatic fibrosis and augmented intrahepatic vascular resistance typical of chronic liver diseases. In this study, the authors defined genetic thrombophilia as the presence of at least one of the following thrombophilic genetic factors: PAI-1 4G-4G, MTHFR 677TT, V Leiden Q506 and prothrombin 20210A.

Peer-review

There are many studies demonstrating the role of PAI-1 4G-4G associated with the highest serum PAI-1 activity in the liver fibrosis process. Regarding MTHFR 677TT and fibrogenesis, patients with MTHFR 677TT had a higher serum total homocysteine and there is much evidence that MTHFR 677TT has a role in the progression of fibrosis in liver diseases.

REFERENCES

1 **D'Amico M**, Pasta L, Sammarco P. MTHFR C677TT, PAI1 4G-4G, V Leiden Q506, and prothrombin G20210A in hepatocellular carcinoma with and without portal vein thrombosis. *J Thromb Thrombolysis* 2009; **28**: 70-73 [PMID: 18618228 DOI: 10.1007/s11239-008-0246-6]

2 **D'Amico M**, Pasta F, Pasta L. Thrombophilic genetic factors PAI-1 4G-4G and MTHFR 677TT as risk factors of alcohol, cryptogenic liver cirrhosis and portal vein thrombosis, in a Caucasian population. *Gene* 2015; **568**: 85-88 [PMID: 25987440 DOI: 10.1016/j.gene.2015.05.034]

3 **Patnaik M**, Dlott JS, Fontaine RN, Subbiah MT, Hessner MJ, Joyner KA, Ledford MR, Lau EC, Moehlenkamp C, Amos J, Zhang B, Williams TM. Detection of genomic polymorphisms associated with venous thrombosis using the invader biplex assay. *J Mol Diagn* 2004; **6**: 137-144 [PMID: 15096570 DOI: 10.1016/s1525-1578(10)60502-8]

4 **Rosner B**. Hypothesis testing: categorical data. Fundamentals of Biostatistics. 6th ed. Belmont, CA: Duxbury/Thomson Brooks/Cole, 2006: 385-447

5 **Rodriguez S**, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009; **169**: 505-514 [PMID: 19126586 DOI: 10.1093/aje/kwn359]

6 **Perez-Pujol S**, Aras O, Escolar G. Factor v leiden and inflammation. *Thrombosis* 2012; **2012**: 594986 [PMID: 22666576 DOI: 10.1155/2012/594986]

7 **Maharshak N**, Halfon P, Deutsch V, Peretz H, Berliner S, Fishman S, Zelber-Sagi S, Rozovski U, Leshno M, Oren R. Increased fibrosis progression rates in hepatitis C patients carrying the prothrombin G20210A mutation. *World J Gastroenterol* 2011; **17**: 5007-5013 [PMID: 22174551 DOI: 10.3748/wjg.v17.i45.5007]

8 **Gialluisi A**, Incollu S, Pippucci T, Lepori MB, Zappu A, Loudianos G, Romeo G. The homozygosity index (HI) approach reveals high allele frequency for Wilson disease in the Sardinian population. *Eur J Hum Genet* 2013; **21**: 1308-1311 [PMID: 23486543 DOI: 10.1038/ejhg.2013.43]

9 **Wong TY**, Poon P, Szeto CC, Chan JC, Li PK. Association of plasminogen activator inhibitor-1 4G/4G genotype and type 2 diabetic nephropathy in Chinese patients. *Kidney Int* 2000; **57**: 632-638 [PMID: 10652041 DOI: 10.1046/j.1523-1755.2000.t01-1-00884.x]

10 **Zhang LP**, Takahara T, Yata Y, Furui K, Jin B, Kawada N, Watanabe A. Increased expression of plasminogen activator and plasminogen activator inhibitor during liver fibrogenesis of rats: role of stellate cells. *J Hepatol* 1999; **31**: 703-711 [PMID: 10551395 DOI: 10.1016/s0168-8278(99)80351-1]

11 **AbdulHameed MD**, Tawa GJ, Kumar K, Ippolito DL, Lewis JA, Stallings JD, Wallqvist A. Systems level analysis and identification of pathways and networks associated with liver fibrosis. *PLoS One* 2014; **9**: e112193 [PMID: 25380136 DOI: 10.1371/journal.pone.0112193]

12 **Liu J**. Ethanol and liver: recent insights into the mechanisms of ethanol-induced fatty liver. *World J Gastroenterol* 2014; **20**: 14672-14685 [PMID: 25356030 DOI: 10.3748/wjg.v20.i40.14672]

13 **Verrijken A**, Francque S, Mertens I, Prawitt J, Caron S, Hubens G, Van Marck E, Staels B, Michielsen P, Van Gaal L. Prothrombotic factors in histologically proven nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology* 2014; **59**: 121-129 [PMID: 24375485 DOI: 10.1002/hep.26510]

14 **Lee JH**, Jang EJ, Seo HL, Ku SK, Lee JR, Shin SS, Park SD, Kim SC, Kim YW. Sauchinone attenuates liver fibrosis and hepatic stellate cell activation through TGF-β/Smad signaling pathway. *Chem Biol Interact* 2014; **224C**: 58-67 [PMID: 25451574 DOI: 10.1016/j.cbi.2014.10.005]

15 **Wang S**, Zhang Z, Zhu X, Wu H, Gao H, Yang C. Effect of aldosterone and its antagonist on the expression of PAI-1 and TGF-β1 in rat hepatic stellate cells. *Int J Clin Exp Med* 2014; **7**: 4677-4685 [PMID: 25663965]

16 **Gracia-Sancho J**, Maeso-Díaz R, Fernández-Iglesias A, Navarro-Zornoza M, Bosch J. New cellular and molecular targets for the treatment of portal hypertension. *Hepatol Int* 2015; **9**: 183-191 [PMID: 25788198 DOI: 10.1007/s12072-015-9613-5]

17 **Duong HT**, Dong Z, Su L, Boyer C, George J, Davis TP, Wang J. The use of nanoparticles to deliver nitric oxide to hepatic stellate cells for treating liver fibrosis and portal hypertension. *Small* 2015; **11**: 2291-2304 [PMID: 25641921 DOI: 10.1002/smll.201402870]

18 **Devlin AM**, Clarke R, Birks J, Evans JG, Halsted CH. Interactions among polymorphisms in folate-metabolizing genes and serum total homocysteine concentrations in a healthy elderly population. *Am J Clin Nutr* 2006; **83**: 708-713 [PMID: 16522921]

19 **Adinolfi LE**, Ingrosso D, Cesaro G, Cimmino A, D'Antò M, Capasso R, Zappia V, Ruggiero G. Hyperhomocysteinemia and the MTHFR C677T polymorphism promote steatosis and fibrosis in chronic hepatitis C patients. *Hepatology* 2005; **41**: 995-1003 [PMID: 15834927]

20 **Ertl T**, Sulyok E, Ezer E, Sárkány I, Thurzó V, Csaba IF. The influence of metoclopramide on the composition of human breast milk. *Acta Paediatr Hung* 1991; **31**: 415-422 [PMID: 1790024]

21 **Baszczuk A**, Kopczyński Z. [Hyperhomocysteinemia in patients with cardiovascular disease]. *Postepy Hig Med Dosw* (Online) 2014; **68**: 579-589 [PMID: 24864108 DOI: 10.5604/17322693.1102340]

22 **Ventura P**, Rosa MC, Abbati G, Marchini S, Grandone E, Vergura P, Tremosini S, Zeneroli ML. Hyperhomocysteinaemia in chronic liver diseases: role of disease stage, vitamin status and

methylenetetrahydrofolate reductase genetics. *Liver Int* 2005; **25**: 49-56 [PMID: 15698398 DOI: 10.1111/j.1478-3231.2005.01042.x]

23 **Cheng Z**, Jiang X, Pansuria M, Fang P, Mai J, Mallilankaraman K, Gandhirajan RK, Eguchi S, Scalia R, Madesh M, Yang X, Wang H. Hyperhomocysteinemia and hyperglycemia induce and potentiate endothelial dysfunction via μ-calpain activation. *Diabetes* 2015; **64**: 947-959 [PMID: 25352635 DOI: 10.2337/db14-0784]

24 **Baszczuk A**, Kopczyński Z, Kopczyński J, Cymerys M, Thielemann A, Bielawska L, Banaszewska A. Impact of administration of folic acid on selected indicators of inflammation in patients with primary arterial hypertension. *Postepy Hig Med Dosw* (Online) 2015; **69**: 429-435 [PMID: 25897102 DOI: 10.5604/17322693.1148336]

25 **Trautwein C**, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: Concept to treatment. *J Hepatol* 2015; **62**: S15-S24 [PMID: 25920084 DOI: 10.1016/j.jhep.2015.02.039]

26 **Ghosh AK**, Vaughan DE. PAI-1 in tissue fibrosis. *J Cell Physiol* 2012; **227**: 493-507 [PMID: 21465481 DOI: 10.1002/jcp.22783]

27 **Reilly R**, McNulty H, Pentieva K, Strain JJ, Ward M. MTHFR 677TT genotype and disease risk: is there a modulating role for B-vitamins? *Proc Nutr Soc* 2014; **73**: 47-56 [PMID: 24131523 DOI: 10.1017/S0029665113003613]

28 **Fernandez M**. Molecular pathophysiology of portal hypertension. *Hepatology* 2015; **61**: 1406-1415 [PMID: 25092403 DOI: 10.1002/hep.27343]

29 **Lee YA**, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. *Gut* 2015; **64**: 830-841 [PMID: 25681399 DOI: 10.1136/gutjnl-2014-306842]

30 **Rodriguez-Castro KI**, Simioni P, Burra P, Senzolo M. Anticoagulation for the treatment of thrombotic complications in patients with cirrhosis. *Liver Int* 2012; **32**: 1465-1476 [PMID: 22734713 DOI: 10.1111/j.1478-3231.2012.02839.x]

P- Reviewer: Mihaila RG **S- Editor**: Qiu S
L- Editor: Roemmele 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