ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2015 December 28; 7(30): 2927-2998

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 30 December 28, 2015

World Journal of Hepatology
Volume 7 Number 30 December 28, 2015

Contents

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

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

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

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

PUBLICATION DATE
December 28, 2015

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

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

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i30.2927

World J Hepatol 2015 December 28; 7(30): 2927-2932
ISSN 1948-5182 (online)

EDITORIAL

Role of endoscopy in the conservative management of biliary complications after deceased donor liver transplantation

Andrea Lisotti, Pietro Fusaroli, Giancarlo Caletti

Andrea Lisotti, Unità Operativa di Gastroenterologia, Ospedale Nuovo di Imola, 40026 Imola, Italy

Andrea Lisotti, Pietro Fusaroli, Giancarlo Caletti, Department of Medical and Surgical Sciences, University of Bologna, 40138 Bologna, Italy

Andrea Lisotti, Pietro Fusaroli, Giancarlo Caletti, Gastroenterology Unit, Hospital of Imola, 40026 Imola, Italy

Author contributions: Lisotti A and Fusaroli P wrote the paper; Caletti G revised the final draft of the manuscript for important intellectual content.

Conflict-of-interest statement: All Authors declare no conflict of interest for this study and for the preparation of this manuscript.

Correspondence to: Andrea Lisotti, PhD, Unità Operativa di Gastroenterologia, Ospedale Nuovo di Imola, via Montericco 9, 40026 Imola, Italy. lisotti.andrea@gmail.com
Telephone: +39-0542-662407
Fax: +39-0542-662409

Received: June 9, 2015
Peer-review started: June 19, 2015
First decision: September 18, 2015
Revised: October 21, 2015
Accepted: December 9, 2015
Article in press: December 11, 2015
Published online: December 28, 2015

Abstract

The clinical outcome of patients receiving liver transplantation could be significantly affected by biliary complications, including strictures, leaks, stones and bilomas; early diagnosis and treatment of these conditions lead to markedly reduction in morbidity and mortality. Therapeutic gold standard is represented by conservative approaches, both endoscopic and percutaneous, based on the type of biliary reconstruction, the local availability of the procedures and specific expertise. In patients with previous transplantation, the difficult biliary access and the possible presence of concomitant complications (mainly strictures) further restrict the efficacy of the endoscopic and percutaneous treatments; on the other hand, surgery should generally be avoided because of the even increased morbidity and mortality due to technical and clinical issues. Here we review the most common biliary complications occurring after liver transplantation and discuss available treatment options including future perspectives such as endoscopic ultrasound-guided biliary access in patients with Roux-en-Y choledocho-jejunostomy or extracorporeal shock wave lithotripsy for difficult stones.

Key words: Endoscopic ultrasonography; Endoscopic ultrasound; Percutaneous trans-hepatic drainage; Endoscopic retrograde cholangiopancreatography; Biliary drainage

Core tip: The clinical outcome of patients receiving liver transplantation could be significantly affected by biliary complications, including strictures, leaks, stones and bilomas; early diagnosis and treatment of these conditions allow to markedly reduction in morbidity and mortality. With the continuous increase of endo-

scopic knowledge and expertise, the interventional management of these conditions is constantly evolving toward a conservative approach. In this manuscript are summarized current evidences regarding conservative approaches to biliary complication, with an overview on future management and research areas.

Lisotti A, Fusaroli P, Caletti G. Role of endoscopy in the conservative management of biliary complications after deceased donor liver transplantation. *World J Hepatol* 2015; 7(30): 2927-2932 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i30/2927.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i30.2927

INTRODUCTION

Orthotopic liver transplantation (LT) has become the standard treatment for end-stage liver disease and hepatocellular carcinoma[1]. Despite continuous surgical improvement, biliary complications are still considered one of the most common issue after transplantation: 10%-40% of cases report duct stricture (anastomotic and non-anastomotic), leaks, stones-sludge or cast, sphincter of oddi dysfunction (SOD), biloma, hemobilia, ductopenia (due to chronic ischemia or rejection), cholestatic recurrency of the underlying liver disease and, finally, biliary cast syndrome[2].

The type of biliary reconstruction, ischemia and reperfusion injury, hepatic artery thrombosis, cytomegalovirus infection and primary sclerosing cholangitis are the principal risk factors for the development of post-LT biliary morbidity[3,4].

In this difficult setting, conservative approaches are usually preferred: Endoscopic treatments include sphincterotomy, plastic or metallic stenting or multi-stenting, balloon dilation, stone clearance using various devices (*i.e.,* balloon catheter, Dormia basket), placement of naso-biliary tube; placement of external or internal-external drainage, stenting and balloon dilation are the most common percutaneous treatment options. Finally, surgery is usually avoided and confined after failure of conventional approaches[5,6].

The occurrence of biliary complications after LT could lead to recurrent hospitalizations and, even, to graft failure; the early detection and management of those conditions could reduce the increased costs with a significant improvement in post-transplant quality of life and survival[7,8].

The clinical presentation could be ambiguous, ranging from severe acute manifestations (cholangitis) to asymptomatic liver function tests (LFT) abnormalities. Presence of biliary complication should be suspected in every case with unexpected increase in LFT (AST, ALT, gamma-GT, ALP or bilirubin); patients could also report non-specific symptoms, such as fever, fatigue, abdominal pain (right-upper quadrant) or fever. No validated diagnostic algorithm have been proposed for the investigation of suspected biliary complications, however it is widely accepted to perform trans-abdominal ultrasound (t-US) with Doppler study as first-line test, for the exclusion of hepatic artery stenosis-obstruction and evaluation of biliary tree. In the case of suspected vascular complication, a computed tomography-scan with vascular reconstruction and a therapeutic hepatic angiography have to be performed. On the other hand, the patient should undergo liver biopsy to exclude rejection or hepatitis C recurrence.

After exclusion of both vascular and parenchymal conditions that could justify the manifestation, magnetic resonance cholangio-pancreatography (MRCP) or even a cholangiogram through a T-tube are considered the diagnostic standard for biliary tract complications[9]. Invasive approaches, such as endoscopic retrograde cholangiography (ERC) or percutaneous trans-hepatic cholangiography (PTC) should be considered treatment options rather than diagnostic tools. Overall, first-line treatment approach have to be decided based on local expertise and residual biliary tree anatomy: Endoscopy (ERC) should be preferred in the case of duct-to-duct biliary reconstruction while percutaneous treatment (PTC) should be reserved to patients with Roux-en-Y choledoco-jejunostomy or after failure of endoscopic attempts. Surgery has to be considered a rescue therapy, after failure of all other treatment options. The incidence and principal risk factors for biliary complications are summarized in Table 1.

Biliary stricture

The development of stricture is the most common biliary complication after LT. Early strictures are mainly related to technical issues and observed more frequent in the case of living donor LT (2.5 folds) than deceased donor LT[10]. Late strictures, occurring usually 6-9 mo after LT, are secondary to ischemia, vascular insufficiency, immune injuries or other complications (*i.e.,* leak or cholangitis). Biliary strictures could be classified upon their anatomic localization into anastomotic strictures (AS) and non-AS (NAS).

Anastomotic strictures: AS are single, short and circumferential; AS occur usually within 1 year after LT and are more commonly observed in patients who underwent Roux-en-Y choledocho- or hepatico-jejunostomy rather than duct-to-duct reconstruction. Thus, the direct anastomosis between biliary tree and the intestinal limb expose the biliary system to the intestinal microbiota. Bile leak is (after removal of T-tube) represents another independent risk factor for the development of AS.

Clinical manifestations range from asymptomatic liver function test abnormalities to jaundice, pain or fever (in the case of cholangitis). Intrahepatic bile duct dilation could be observed on t-US, however in post-transplant setting, it is not uncommon to find a clinically significant biliary stricture without upstream dilation, due to increased periductal fibrosis. Liver biopsy could show the presence of bile duct proliferation and bilirubin deposition. MRCP or direct cholangiography (through T-tube) represent the gold standard for the diagnosis of AS.

Table 1 A detailed clinical presentation and suggested therapeutic approaches for each condition

Biliary complication	Incidence	Risk factors
Stricture		
Anastomotic	4%-12%	Ischemia, surgical complications, duct-to-duct anastomosis
Non-anastomotic	1%-10%	Hepatic artery thrombosis, cold ischemia time, immune conditions (AB0 mismatch), recurrence of underlying PSC, cytotoxic injuries (hydrophobic bile acids)
Leak or fistula	2%-25%	Surgical issues, T-tube removal
Obstruction (stone, cast, sludge, clots)	1%-6%	Stricture, kinking, infections
Cholangitis	10%	Concomitant biliary complications (stricture, obstruction) or maneuvers (ERC, PTC)
Sphincter of Oddi dysfunction	2%-7%	Efferent denervation of papillary region
Hemobilia	[1]	Endoscopic or percutaneous biliary maneuvers, liver biopsy
Compression		
Cystic duct mucocele	Rare	Mucus-producing cells in cystic duct remnant
Periductal Neurinoma	Rare	Unknown
Periductal lymphoma	Rare	Unknown (immunesuppresive therapy?)
Kinking	1.6%	Redundant donor or recipient CBD remnant
Biliary cast syndrome		Hilar stricture, untreated obstruction
Ductopenia (vanishing bile-duct syndrome)	Rare	Drugs (antibiotics, chemotherapy), chronic rejection, ischemia, untreated stricture

[1]Only case reports are available in literature. PSC: Primary sclerosing cholangitis; CBD: Common bile duct; ERC: Endoscopic retrograde cholangiography; PTC: Percutaneous trans-hepatic cholangiography.

In patients with duct-to-duct reconstruction, ERC represents the first-line treatment approach for biliary stricture, leading to successful resolution of AS in up-to 70% of patients[11-13]. After deep biliary cannulation and biliary sphincterotomy, endoscopic balloon-dilatation is able to reduce AS in a significant amount of cases; however, balloon-dilation alone is burdened by a significant rate of stricture recurrence (up to 60%); therefore, stricture dilation alone is not a reliable treatment. In the last 15 years, increasing evidences suggest that balloon dilation followed-up by biliary stenting appears to provide a more durable effect: Placing one or more 10 French plastic prosthesis reduced stricture recurrence to about 30% of cases. Stent patency ranges from 2 to 4 mo, due to presence of casts, debris and clots; it is indicated to exchange plastic stents every 3 mo for the first 9-12 mo, placing larger ones or multiple stents, until the resolution of the stenosis. We suggest evaluating the presence of residual stricture assessing the resistance to the transit of inflated balloon catheter through the anastomosis[14-18]. A recent systematic review reported a 80%-95% of stricture resolution after endoscopic placement of fully covered self-expanding metal stents (SEMS); however a study reporting the use of SEMS in patients with post-LT AS reported a significant rate of complications (up to 38%, mostly cholangitis) with worse clinical outcome (68% of stricture resolution)[19,20].

In patients with Roux-en-Y choledocho-jejunostomy the altered anatomy of the upper gastrointestinal tract the endoscopic access to the biliary tree is usually unfeasible. Some authors reported successful ERC using scope designed for the exploration of small bowel (single or double-balloon enteroscopy)[21]; however, in these patients, percutaneous approach with stricture dilation and stenting is usually performed[22,23].

Recent technical advances suggest that endoscopic ultrasound (EUS)-guided approach (*i.e.*, trans-gastric intrahepatic access) with antegrade treatments could be useful in this difficult-to-treat population[24].

In the case of failure of endoscopic approach (failure to traverse the stricture with a guidewire, refractory stricture or residual AS despite several ERC) surgical re-intervention could be considered: Resection and duct-to-duct re-anastomosing or conversion to Roux-en-Y hepatico-jejunostomy usually lead to AS resolution, eliminating the need of multiple intervention requiring hospitalization. Surgery is widely considered a second-line treatment (after failure of endoscopic or percutaneous interventions); however, in selected cases such as late tight stricture, bilioenteric anastomosis is the first-line approach in order to reduce futile intervention and to delay curative option[25].

NAS: Post-LT strictures could be localized at any level of the biliary tree. Early (within the first 12 mo) NAS have been related to chronic ischemic injuries and are more usually detected in the common bile duct, common hepatic duct or bifurcation; on the other hand, late-onset (> 12 mo) NAS are related to immunological factors and have been detected in a diffuse pattern affecting the intrahepatic small bile ducts. Overall incidence of NAS ranges from 1% to 10%[13].

Cholangiographic features are similar to those observed in patients with primary sclerosing cholangitis. The diffuse presence of multiple strictures is responsible for development of sludge deposition, casts and even acute recurrent cholangitis. Presence of NAS is associated to poor prognosis and reduced graft survival.

The goals of NAS treatments are the same of AS ones; biliary drainage and stricture dilation have to be reach through all the available non-surgical approaches (endoscopic, percutaneous, even combined). The presence of concomitant multiple strictures and the difficult localization (small intrahepatic ducts) account for the reduced treatment outcomes. Endoscopic sphincterotomy, balloon-dilation (4-6 mm, smaller than AS) and stenting (10-11.5 Fr, replaced every 3 mo) are similar approaches; patients with NAS usually require

more ERC interventions but only 50% of them achieve long-term successful clinical results[26].

Patients with extrahepatic NAS with good graft residual function could undergo surgical resection construction of a Roux-en-Y hepatico-jejunostomy. In the case of failure, up to 50% of the patients with NAS, require retransplantation[27].

Leak

Bile leakage could be observed in the early post-LT (within 3 mo) from the anastomosis, the cystic duct stump, from the insertion of the T-tube or, in the case of living donor LT or split-LT, from the cutting surface of the liver graft. Overall estimated incidence was 8.2%[26].

The mainstay of treatment of a bile leak is the reduction and decompression of biliary tree; in the case of refractory leakage, biliary drainage could be necessary to healing process. In the case of early occurrence, if the T-tube was already *in situ*, bile leak could be managed conservatively by leaving the T-tube open without further intervention. In the case of small leak, ERC with sphincterotomy is able to resolve the leakage. In the case of persistence of endoscopic sphincterotomy, placement of biliary plastic stent is able to resolve 90%-95% of early bile leaks. Usually, the biliary stent was placed with 2 mo and then removed (shorter period could not be adequate for healing process due to immunosuppressive therapy and could be justified only in the case of suspected obstruction).

In patients with Roux-en-Y anatomy, percutaneous or even surgical approach is usually necessary; we hypothesized that the decompression of the biliary tree through EUS-guided access could be an intriguing field of development for future research directions[28-30].

Biloma: Continuous bile leak within the liver or abdominal cavity could result in a uniloculated biliary collection; biloma could compress the biliary tree, vessels or could even be superinfected, leading to clinical manifestations.

Treatment: small biloma usually are self-limiting and resolve spontaneously; in complex collection, endoscopic sphincterotomy and stenting could be necessary to heal the underlying fistula. In the case of infected collection, drainage is necessary. If ERC was not sufficient to drain the biloma both percutaneous and EUS approach have been demonstrated efficacy and safe[31].

Surgical drainage has to be considered only as rescue therapy, after failure of all conservative approaches.

Biliary stones and other filling defects

Presence of biliary stones, sludge, cast, blood clots or even migrated stents could be observed in a significant amount of LT-patients (up to 10%); biliary stones are the most common filling defects observed in this setting[13].

The concomitant presence of biliary tree anatomy alterations, strictures, acute cholangitis, increased bile viscosity and stasis, drug-induced lithogenesis (*i.e.,* cyclosporine), bile acid depletion and cholesterol supersaturation are all concomitant risk factors for post-transplant CBD stones.

Endoscopic removal of CBD stone is the first-line treatment option. Stone number, size and shape, presence of impacted stones, concomitant biliary tree anatomy and presence of distal narrowing are the most common causes of ERCP failure. Moreover, in patients with previous LT, the difficult biliary access and the possible presence of concomitant biliary complications (mainly strictures) further restrict the efficacy of the interventions[32].

In patients with duct-to-duct anastomosis, the underlying presence of concomitant complications (stricture, kinking, *etc.*) should be treated accordingly. In patients with Roux-en-Y bilioenteric anastomosis, a first approach with an enteroscope, when available, should be attempted; in the case of failure, percutaneous approach is indicated. We also suggest, as future field of research, the comparison between PTC and EUS-guided biliary access, drainage and stone clearance.

In the case of difficult CBD stones (*i.e.,* large stones, triangular-shaped, or discrepancy between stone size and narrowed CBD diameter) we reported the safety and efficacy of ESWL. Among six patients with difficult choledocholithiasis, after failure of either endoscopic or percutaneous approaches, ESWL led to complete resolution of biliary complications in 5 patients (> 80%) with no procedure-related adverse events. One patients underwent surgical hepatico-jejunostomy because of tight anastomotic stricture, despite multiple endoscopic balloon-dilation and multi-stenting[32].

SOD

Papillary obstruction could be found in 2%-7% of LT patients[33]. Efferent denervation CBD remnant and ampulla but also chronic injuries with fibrotic stricture lead to hypertonic sphincter function or to obstruction. The insidious manifestation (usually characterized by elevated enzymes with or without biliary tree dilation) could justify a delayed diagnosis. Endoscopic sphincterotomy is usually effective with long-term clinical resolution. Temporary biliary stenting could be considered in the case of presence of fibrotic tissue and scarring[34].

Bile duct kinking

Redundant bile duct is defined as a reconstructed CBD (duct-to-duct anastomosis) longer than the recipient CBD creating a kinking (sigmoid-shaped loop) that, in the absence of other complications (*i.e.,* stricture), leads to cholestasis due to reduced bile outflow[35].

A single experience evaluated specifically the incidence of redundant CBD; the authors reported an incidence of 1.6%. Clinical presentation is characterized by asymptomatic cholestasis; but in our experience, presence of redundant bile duct could lead to development of CBD stones and even acute cholangitis[32].

Endoscopic stenting (long single plastic stent) lead to clinical resolution in up to 80% of patients; in the remaining, surgical Roux-en-Y hepatico-jejunostomy leads to resolution of clinical cholestasis. Also in this setting, the first-line endoscopic approach could lead to

symptoms resolution and spare unnecessary surgical intervention[35].

Recent technical and technological advances

As discussed above, minimally invasive approaches are indicated as first-line techniques for the management of biliary complications after deceased donor LT; we also discussed the possible technical difficulty encountered during deep biliary cannulation in the case of stricture, altered anatomy and other conditions in this setting. When guidewire cannot pass an angulated stenosis, the rendezvous technique can be used to overcome the issue.

The rendezvous technique is a useful and safe method for the access to biliary tree, replacement of biliary prosthesis and stent in the case of difficult biliary stricture after LT with duct-to-duct anastomosis. Various Authors reported various rendezvous techniques that could be suggested for the management of biliary complications after failure of endoscopic and percutaneous approaches[36-39].

Peroral cholangioscopy has been introduced over the past years to allow direct observation of the biliary tree and even to tissue acquisition. The introduction of new generation cholangioscopes reduced previous limitations (*i.e.,* low scope resolution, low maneuverability requiring two endoscopists, unavailable accessories) and led to the new innovative applications. Some Authors, for example, suggested the use of Spyglass for difficult biliary cannulation in the case of severe stricture and use peroral cholangioscopy to evaluate and treat anastomotic stricture after liver transplantation[40,41].

CONCLUSION

The clinical outcome of patients receiving liver transplantation could be significantly affected by biliary complications, including strictures, leaks, stones or debris, bilomas and SODs; early diagnosis and treatment of these conditions allow to markedly reduction in morbidity and mortality[42]. Therapeutic gold standard is represented by conservative approaches, both endoscopic and percutaneous, based on the type of biliary reconstruction, the local availability of the procedures and specific expertise[43]. With continuous improvements in surgical, endoscopic and echoendoscopic techniques[44], the management of biliary complications constantly evolves; what has not changed over time is the pivotal role of the early detection and management, in order to reduce the clinical burden and to improve long-term outcome (graft function and patient survival). We hope that in the next future, with the availability of new expertise, knowledge and specifically designed devices, the endoscopic management of biliary complications will further improve the quality of LT-management, with a reduction of cost related to surgery and hospitalization.

REFERENCES

1 **Martin P**, DiMartini A, Feng S, Brown R, Fallon M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* 2014; **59**: 1144-1165 [PMID: 24716201 DOI: 10.1002/hep.26972]

2 **Dubbeld J**, van Hoek B, Ringers J, Metselaar H, Kazemier G, van den Berg A, Porte RJ. Biliary complications after liver transplantation from donation after cardiac death donors: an analysis of risk factors and long-term outcome from a single center. *Ann Surg* 2015; **261**: e64 [PMID: 24401915]

3 **Lu D**, Xu X, Wang J, Ling Q, Xie H, Zhou L, Yan S, Wang W, Zhang M, Shen Y, Zheng S. The influence of a contemporaneous portal and hepatic artery revascularization protocol on biliary complications after liver transplantation. *Surgery* 2014; **155**: 190-195 [PMID: 24238120 DOI: 10.1016/j.surg.2013.06.056]

4 **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]

5 **Shin M**, Joh JW. Section 10. Endoscopic management of biliary complications in adult living donor liver transplantation. *Transplantation* 2014; **97** Suppl 8: S36-S43 [PMID: 24849832 DOI: 10.1097/01.tp.0000446274.13310.b9]

6 **Ko GY**, Sung KB. Section 11. Radiological intervention approaches to biliary complications after living donor liver transplantation. *Transplantation* 2014; **97** Suppl 8: S43-S46 [PMID: 24849833 DOI: 10.1097/01.tp.0000446275.51428.65]

7 **El-Meteini M**, Hamza A, Abdalaal A, Fathy M, Bahaa M, Mukhtar A, Abouelfetouh F, Mostafa I, Shaker M, Abdelwahab S, El-Dorry A, El-Monayeri M, Hobballah A, Sabry H. Biliary complications including single-donor mortality: experience of 207 adult-to-adult living donor liver transplantations with right liver grafts. *HPB* (Oxford) 2010; **12**: 109-114 [PMID: 20495654]

8 **Kato H**, Kawamoto H, Tsutsumi K, Harada R, Fujii M, Hirao K, Kurihara N, Mizuno O, Ishida E, Ogawa T, Fukatsu H, Yamamoto K, Yagi T. Long-term outcomes of endoscopic management for biliary strictures after living donor liver transplantation with duct-to-duct reconstruction. *Transpl Int* 2009; **22**: 914-921 [PMID: 19497069 DOI: 10.1111/j.1432-2277.2009.00895.x]

9 **Boraschi P**, Donati F. Postoperative biliary adverse events following orthotopic liver transplantation: assessment with magnetic resonance cholangiography. *World J Gastroenterol* 2014; **20**: 11080-11094 [PMID: 25170197 DOI: 10.3748/wjg.v20.i32.11080]

10 **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]

11 **Tarantino I**, Barresi L, Petridis I, Volpes R, Traina M, Gridelli B. Endoscopic treatment of biliary complications after liver transplantation. *World J Gastroenterol* 2008; **14**: 4185-4189 [PMID: 18636664 DOI: 10.3748/wjg.14.4185]

12 **Girotra M**, Soota K, Klair JS, Dang SM, Aduli F. Endoscopic management of post-liver transplant biliary complications. *World J Gastrointest Endosc* 2015; **7**: 446-459 [PMID: 25992185 DOI: 10.4253/wjge.v7.i5.446]

13 **Karimian N**, Westerkamp AC, Porte RJ. Biliary complications after orthotopic liver transplantation. *Curr Opin Organ Transplant* 2014; **19**: 209-216 [PMID: 24752062 DOI: 10.1097/MOT.0000000000000082]

14 **Poley JW**, Lekkerkerker MN, Metselaar HJ, Kuipers EJ, Bruno MJ. Clinical outcome of progressive stenting in patients with anastomotic strictures after orthotopic liver transplantation. *Endoscopy* 2013; **45**: 567-570 [PMID: 23580410]

15 **Poley JW**, Cahen DL, Metselaar HJ, van Buuren HR, Kazemier G, van Eijck CH, Haringsma J, Kuipers EJ, Bruno MJ. A prospective group sequential study evaluating a new type of fully covered self-expandable metal stent for the treatment of benign biliary strictures (with video). *Gastrointest Endosc* 2012; **75**: 783-789 [PMID: 22325806 DOI: 10.1016/j.gie.2011.10.022]

16 **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]
17 **Cantù P**, Tenca A, Donato MF, Rossi G, Forzenigo L, Piodi L, Rigamonti C, Agnelli F, Biondetti P, Conte D, Penagini R. ERCP and short-term stent-trial in patients with anastomotic biliary stricture following liver transplantation. *Dig Liver Dis* 2009; **41**: 516-522 [PMID: 18838317 DOI: 10.1016/j.dld.2008.08.002]
18 **Chaput U**, Scatton O, Bichard P, Ponchon T, Chryssostalis A, Gaudric M, Mangialavori L, Duchmann JC, Massault PP, Conti F, Calmus Y, Chaussade S, Soubrane O, Prat F. Temporary placement of partially covered self-expandable metal stents for anastomotic biliary strictures after liver transplantation: a prospective, multicenter study. *Gastrointest Endosc* 2010; **72**: 1167-1174 [PMID: 20970790 DOI: 10.1016/j.gie.2010.08.016]
19 **Devière J**, Nageshwar Reddy D, Püspök A, Ponchon T, Bruno MJ, Bourke MJ, Neuhaus H, Roy A, González-Huix Lladó F, Barkun AN, Kortan PP, Navarrete C, Peetermans J, Blero D, Lakhtakia S, Dolak W, Lepilliez V, Poley JW, Tringali A, Costamagna G. Successful management of benign biliary strictures with fully covered self-expanding metal stents. *Gastroenterology* 2014; **147**: 385-395; quiz e15 [PMID: 24801350 DOI: 10.1053/j.gastro.2014.04.043]
20 **Kao D**, Zepeda-Gomez S, Tandon P, Bain VG. Managing the post-liver transplantation anastomotic biliary stricture: multiple plastic versus metal stents: a systematic review. *Gastrointest Endosc* 2013; **77**: 679-691 [PMID: 23473000 DOI: 10.1016/j.gie.2013.01.015]
21 **Inamdar S**, Slattery E, Sejpal DV, Miller LS, Pleskow DK, Berzin TM, Trindade AJ. Systematic review and meta-analysis of single-balloon enteroscopy-assisted ERCP in patients with surgically altered GI anatomy. *Gastrointest Endosc* 2015; **82**: 9-19 [PMID: 25922248 DOI: 10.1016/j.gie.2015.02.013]
22 **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* 2011; **16**: 5-13 [PMID: 21716179]
23 **Odemis B**, Oztas E, Yurdakul M, Torun S, Suna N, Kayacetin E. Interesting rendezvous location in a liver transplantation patient with anastomosis stricture. *World J Gastroenterol* 2014; **20**: 15916-15919 [PMID: 25400478 DOI: 10.3748/wjg.v20.i42.15916]
24 **Siripun A**, Sripongpun P, Ovartlarnporn B. Endoscopic ultrasound-guided biliary intervention in patients with surgically altered anatomy. *World J Gastrointest Endosc* 2015; **7**: 283-289 [PMID: 25789101 DOI: 10.4253/wjge.v7.i3.283]
25 **Davidson BR**, Rai R, Nandy A, Doctor N, Burroughs A, Rolles K. Results of choledochojejunostomy in the treatment of biliary complications after liver transplantation in the era of nonsurgical therapies. *Liver Transpl* 2000; **6**: 201-206 [PMID: 10719021 DOI: 10.1002/lt.500060215]
26 **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]
27 **Verdonk RC**, Buis CI, Porte RJ, Haagsma EB. Biliary complications after liver transplantation: a review. *Scand J Gastroenterol Suppl* 2006; (**243**): 89-101 [PMID: 16782628 DOI: 10.1080/00365520600664375]
28 **Morelli J**, Mulcahy HE, Willner IR, Baliga P, Chavin KD, Patel R, Payne M, Cotton PB, Hawes R, Reuben A, Cunningham JT. Endoscopic treatment of post-liver transplantation biliary leaks with stent placement across the leak site. *Gastrointest Endosc* 2001; **54**: 471-475 [PMID: 11577309 DOI: 10.1067/mge.2001.117762]
29 **Llach J**, Bordas JM, Elizalde JI, Enrico C, Ginès A, Pellisé M, Mondelo F, Piqué JM. Sphincterotomy in the treatment of biliary leakage. *Hepatogastroenterology* 2002; **49**: 1496-1498 [PMID: 12397716]
30 **Saab S**, Martin P, Soliman GY, Machicado GA, Roth BE, Kunder G, Han SH, Farmer DG, Ghobrial RM, Busuttil RW, Bedford RA. Endoscopic management of biliary leaks after T-tube removal in liver transplant recipients: nasobiliary drainage versus biliary stenting. *Liver Transpl* 2000; **6**: 627-632 [PMID: 10980063]
31 **Tonozuka R**, Itoi T, Tsuchiya T, Sofuni A, Ishii K, Ikeuchi N, Umeda J, Tanaka R, Mukai S, Gotoda T, Moriyasu F. EUS-guided drainage of hepatic abscess and infected biloma using short and long metal stents (with videos). *Gastrointest Endosc* 2015; **81**: 1463-1469 [PMID: 25843615 DOI: 10.1016/j.gie.2015.01.023]
32 **Lisotti A**, Caponi A, Gibiino G, Muratori R. Safety and efficacy of extracorporeal shock-wave lithotripsy in the management of biliary stones after orthotopic liver transplantation. *Dig Liver Dis* 2015; **47**: 817-818 [PMID: 26143107 DOI: 10.1016/j.dld.2015.06.001]
33 **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]
34 **Fernandez-Simon A**, Royg D, Sendino O. Sphincter of Oddi dysfunction after liver transplantation: experience in a high-volume transplant center. *Hepatology* 2014; **60**: 545A [DOI: 10.1002/hep.27415]
35 **Torres V**, Martinez N, Lee G, Almeda J, Gross G, Patel S, Rosenkranz L. How do we manage post-OLT redundant bile duct? *World J Gastroenterol* 2013; **19**: 2501-2506 [PMID: 23674851 DOI: 10.3748/wjg.v19.i16.2501]
36 **Mönkemüller KE**, Linder JD, Fry LC. Modified rendezvous technique for biliary cannulation. *Endoscopy* 2002; **34**: 936 [PMID: 12430084 DOI: 10.1055/s-2002-35304]
37 **Cantù P**, Parzanese I, Melada E, Rossi G, Conte D, Penagini R. A new duodenal rendezvous technique for biliary cannulation in patients with T-tube after orthotopic liver transplantation (with video). *Gastrointest Endosc* 2015; Epub ahead of print [PMID: 26234695 DOI: 10.1016/j.gie.2015.06.050]
38 **Yoshiya S**, Shirabe K, Matsumoto Y, Ikeda T, Soejima Y, Yoshizumi T, Uchiyama H, Ikegami T, Harimoto N, Maehara Y. Rendezvous ductoplasty for biliary anastomotic stricture after living-donor liver transplantation. *Transplantation* 2013; **95**: 1278-1283 [PMID: 23492991 DOI: 10.1097/TP.0b013e31828a9450]
39 **Chang JH**, Lee IS, Chun HJ, Choi JY, Yoon SK, Kim DG, You YK, Choi MG, Choi KY, Chung IS. Usefulness of the rendezvous technique for biliary stricture after adult right-lobe living-donor liver transplantation with duct-to-duct anastomosis. *Gut Liver* 2010; **4**: 68-75 [PMID: 20479915 DOI: 10.5009/gnl.2010.4.1.68]
40 **Wright H**, Sharma S, Gurakar A, Sebastian A, Kohli V, Jabbour N. Management of biliary stricture guided by the Spyglass Direct Visualization System in a liver transplant recipient: an innovative approach. *Gastrointest Endosc* 2008; **67**: 1201-1203 [PMID: 18308314 DOI: 10.1016/j.gie.2007.10.055]
41 **Theodoropoulou A**, Vardas E, Voudoukis E, Tavernaraki A, Tribonias G, Konstantinidis K, Paspatis GA. SpyGlass Direct Visualization System facilitated management of iatrogenic biliary stricture: a novel approach in difficult cannulation. *Endoscopy* 2012; **44** Suppl 2 UCTN: E433-E434 [PMID: 23258490 DOI: 10.1055/s-0032-1325857]
42 **Rizk RS**, McVicar JP, Emond MJ, Rohrmann CA, Kowdley KV, Perkins J, Carithers RL, Kimmey MB. Endoscopic management of biliary strictures in liver transplant recipients: effect on patient and graft survival. *Gastrointest Endosc* 1998; **47**: 128-135 [PMID: 9512276 DOI: 10.1016/S0016-5107(98)70344-X]
43 **Zimmerman MA**, Baker T, Goodrich NP, Freise C, Hong JC, Kumer S, Abt P, Cotterell AH, Samstein B, Everhart JE, Merion RM. Development, management, and resolution of biliary complications after living and deceased donor liver transplantation: a report from the adult-to-adult living donor liver transplantation cohort study consortium. *Liver Transpl* 2013; **19**: 259-267 [PMID: 23495079 DOI: 10.1002/lt.23595]
44 **Fabbri C**, Luigiano C, Lisotti A, Cennamo V, Virgilio C, Caletti G, Fusaroli P. Endoscopic ultrasound-guided treatments: are we getting evidence based--a systematic review. *World J Gastroenterol* 2014; **20**: 8424-8448 [PMID: 25024600 DOI: 10.3748/wjg.v20.i26.8424]

P- Reviewer: Gupta S, Hsieh CB, Kaya M, Thomopoulos KC
S- Editor: Qiu S **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.i30.2933

World J Hepatol 2015 December 28; 7(30): 2933-2939
ISSN 1948-5182 (online)

EDITORIAL

Clinical value of gadoxetic acid-enhanced magnetic resonance imaging in surgery for hepatocellular carcinoma - with a special emphasis on early hepatocellular carcinoma

Masanori Matsuda

Masanori Matsuda, First Department of Surgery, Yamanashi University School of Medicine, Yamanashi 409-3898, Japan

Author contributions: Matsuda M solely contributed to this work.

Conflict-of-interest statement: The author has no conflict of interests.

Correspondence to: Masanori Matsuda, MD, PhD, First Department of Surgery, Yamanashi University School of Medicine, 1110 Shimokato, Chuo-city, Yamanashi 409-3898, Japan. masam@yamanashi.ac.jp
Telephone: +81-55-2737390
Fax: +81-55-2737390

Received: May 24, 2015
Peer-review started: May 25, 2015
First decision: August 16, 2015
Revised: October 7, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: December 28, 2015

Abstract

Gadoxetic acid- or gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging (EOB-MRI) achieves excellent lesion detection and characterization for both hypervascular hepatocellular carcinoma (HCC) in arterial phase imaging and hypovascular early HCC (small well-differentiated HCC of the vaguely nodular type) in hepatobiliary phase imaging, and has become an indispensable imaging modality in the treatment of HCC. Early HCCs have been detected more frequently since the introduction of EOB-MRI into daily clinical practice. Early HCC is known to progress to conventional hypervascular HCC, and many risk factors have been identified for the hypervascularization of early HCC including the diameter of the tumor, presence of fat, and imaging findings of EOB-MRI. The rate of the development of hypervascular HCC was previously reported to be high in patients with chronic liver disease and early HCC. The presence of early HCC is regarded as a predictor for the recurrence of HCC following hepatic resection. On the other hand, although early HCC itself is currently not regarded as a target lesion for hepatic resection, early HCC at high risk of hypervascularity needs to be treated by local ablation therapy. If concomitant early HCC with progressed HCC is at high risk of hypervascularization and the functional liver reserve of a patient is sufficient, its simultaneous treatment at the time of hepatic resection for progressed HCC is recommended. Further studies on larger numbers of patients are needed before this strategy is adopted.

Key words: Hepatocarcinogenesis; Gadoxetic acid-enhanced magnetic resonance imaging; Hepatobiliary phase; Hypervascularization; Early hepatocellular carcinoma; Organic anion transporting polypeptide; Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging; Hepatic resection

Core tip: Gadoxetic acid- or gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging has excellent lesion detection and

characterization for both hypervascular hepatocellular carcinomas (HCC) in arterial phase imaging and hypovascular early HCC in hepatobiliary phase imaging, and has become an indispensable imaging modality in the treatment of HCC. Early HCC is known to progress to conventional hypervascular HCC. Although early HCC itself is currently not considered to be a target lesion for hepatic resection, if concomitant early HCC with progressed HCC is at high risk of hypervascularization, its simultaneous treatment at the time of hepatic resection is recommended.

Matsuda M. Clinical value of gadoxetic acid-enhanced magnetic resonance imaging in surgery for hepatocellular carcinoma - with a special emphasis on early hepatocellular carcinoma. *World J Hepatol* 2015; 7(30): 2933-2939 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i30/2933.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i30.2933

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most malignant tumors worldwide, and hepatic resection still represents the most effective treatment; however, the recurrence rate of HCC is very high even after curative resection. The postoperative 5-year recurrence rate was previously reported to be higher than 70%, with 80% to 95% of recurrence being confined to the liver[1-3].

A proper preoperative evaluation of intrahepatic tumor progression by imaging modalities and appropriate hepatic resection, in addition to the early diagnosis of recurrent HCC followed by treatment[4,5], are needed in order to achieve a favorable prognosis after hepatic resection.

A new magnetic resonance imaging (MRI) contrast medium, gadoxetic acid, or gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), which has the properties of both an extracellular gadolinium chelate and liver-specific (hepatocyte-targeting) contrast material, has recently become available. The injection of a bolus of Gd-EOB-DTPA allows tumor vascularity to be assessed using arterial phase imaging and enables hepatobiliary phase imaging approximately 20 min after its administration, with approximately 50% of the contrast material being taken up by hepatocytes[6-11]. Gd-EOB-DTPA-enhanced MRI (EOB-MRI), which includes a gradient dual echo sequence and diffusion-weighted imaging, has been recommended for the pretherapeutic evaluation of patients with HCC[12]. We previously showed that EOB-MRI was the most useful imaging technique for evaluating small HCC, including early HCC[11].

Early HCCs have been detected more frequently in daily clinical practice since the introduction of EOB-MRI in Japan.

The treatment of early HCCs including hepatic resection will become a very important issue in the near future.

Current understanding on the clinical value of EOB-MRI in surgery for HCC and the significance of newly diagnosed early HCC from several clinical viewpoints have been outlined in this review. The prospect of treatment strategies for newly diagnosed early HCC concomitantly with progressed HCC has also been discussed.

ROLE OF EOB-MRI IN THE DIAGNOSIS AND MANAGEMENT OF HCC

Ichikawa *et al*[13] analyzed the findings of multicenter phase Ⅲ studies in order to evaluate the safety and efficacy of EOB-MRI for the detection and characterization of focal liver lesions. They showed that EOB-MRI was safe and improved the detection and characterization of focal hepatic lesions over that with unenhanced MRI. EOB-MRI also appeared to be more beneficial than spiral computed tomography (CT), especially for the detection of smaller lesions or HCC underlying cirrhotic liver. Therefore, they concluded that EOB-MRI enabled excellent lesion detection and characterization for both hypervascular HCCs in arterial phase imaging and hypovascular HCCs in hepatobiliary phase imaging[13]. EOB-MRI is recommended every 3-4 mo in selected cases of HCC ultrahigh-risk groups, and at least once during the first visit in all HCC ultrahigh-risk groups[14].

Therefore, we routinely perform EOB-MRI together with other imaging modalities prior to hepatic resection for HCC. EOB-MRI is also used in postoperative follow-ups for patients with HCC after hepatic resection.

HEPATOCARCINOGENESIS AND HISTOLOGICAL FINDINGS OF EARLY HCC

Two types of human hepatocarcinogenesis are now considered: *De novo* hepatocarcinogenesis and multistep carcinogenesis from a low-grade dysplastic nodule (DN) to a high-grade DN followed by early HCC and hypervascular HCC (progressed HCC)[15-17].

However, difficulties have been associated with the precise histological diagnosis of early HCC and accurate differentiation between early HCC and DN because of similarities in their pathological features[18].

The International Consensus Group for Hepatocellular Neoplasia (ICGHN), which was composed of 34 world-renowned pathologists and two clinicians, finally announced a consensus on the pathological criteria of early HCC (small well-differentiated HCC of the vaguely nodular type) after significant debate in 2009. In this report, early HCC was characterized by various combinations of the following major histological features: (1) a cell density more than 2-fold higher than that of the surrounding tissue, with a higher nuclear/cytoplasm ratio and irregularly thin trabecular pattern; (2) varying

numbers of portal tracts within the nodule (intratumoral portal tracts); (3) pseudoglandular pattern; (4) diffuse fatty change; and (5) varying numbers of unpaired arteries.

Since all of these features may be found in high-grade DN, stromal invasion remains the most helpful objective pathological finding for differentiating early HCC from DN[19].

DIAGNOSIS OF EARLY HCC BY EOB-MRI

Among the various imaging modalities currently used, the role of EOB-MRI has become increasingly important in the diagnosis of early HCC.

We conducted a review on the imaging findings obtained from multi-imaging modalities of early HCC cases diagnosed according to the pathological criteria of the ICGHN by only including surgically resected nodules in order for pathologists to thoroughly investigate the whole nodule. These multi-imaging modalities included EOB-MRI, contrast-enhanced CT, CT during arterioportography, and CT during hepatic arteriography. EOB-MRI is the only imaging modality that has sufficient resolution for the detection and classification of early HCC. The most significant imaging feature in the diagnosis of early HCC was hypointensity on hepatobiliary phase images of EOB-MRI; all cases of early HCC that were detected on the hepatobiliary phase images of EOB-MRI showed hypointensity, while all of the images of DN showed isointensity or hyperintensity relative to the liver parenchyma. The findings of the diagnostic performance analysis showed that EOB-MRI had excellent sensitivity (97%) for detecting early HCC and exceptional specificity (100%) for distinguishing early HCC from DN[11,20].

RISK FACTORS FOR HYPERVASCULARIZATION OF EARLY HCC

Hypovascular nodules that appear hypointense on hepatobiliary phase EOB-MRI (hypovascular hypointense nodules) may progress to conventional hypervascular HCC[21]. Therefore, identifying the risk factors for the hypervascularization of these nodules, most of which are early HCCs, is important for decision making on the timing of treatment.

We previously reported that nodules that were more than 10 mm in diameter and contained fat were at a higher risk of developing hypervascularization[22]. In addition, a maximum diameter of more than 10 mm[23] or 15 mm or greater[24], increased growth rate, hyperintensity on T1-weighted images[25], hyperintensity on T2-weighted and diffusion-weighted images[26], and a tumor volume doubling time of less than 542 d[27] were identified as risk factors for hypervascularization in early HCC.

PRESENCE OF A HYPOVASCULAR HYPOINTENSE NODULE AS A PREDICTOR OF THE OCCURRENCE OF HYPERVASCULAR HCC

In order to determine whether the presence of a hypovascular hypointense nodule was a risk factor for hypervascular HCC in patients with chronic liver disease, we retrospectively selected 41 patients with pathologically confirmed hypervascular HCC and 41 age- and gender-matched controls and evaluated risk factors for hypervascular HCC. A multivariate analysis revealed that serum albumin levels (OR = 0.19, 95%CI: 0.06-0.57; $P = 0.0024$), a history of hypervascular HCC (OR = 8.62 95%CI: 2.71-32.8; $P = 0.0001$), and the presence of a hypovascular hypointense nodule (OR = 4.18, 95%CI: 1.18-17.2; $P = 0.0256$) were significant risk factors for hypervascular HCC. We concluded that the risk of developing HCC was high in patients with chronic liver disease showing a hypovascular hypointense nodule[28].

Komatsu *et al*[29] selected 127 patients with chronic hepatitis B or C and no history of HCC, including 68 with liver cirrhosis, divided them into those with (non-clean liver group, $n = 18$) and without (clean liver group, $n = 109$) hypovascular hypointense nodules, and investigated whether the risk of hepatocarcinogenesis was higher in patients with these nodules. Seventeen patients (10 in the non-clean liver group and seven in the clean liver group) developed typical HCC. The cumulative 3-year rates of HCC development were 55.5% in the non-clean liver group and 6.4% in the clean liver group ($P < 0.001$), and those at different sites from the initial nodules were also higher in the non-clean liver group (22.2%) than in the clean liver group (6.4%) ($P = 0.003$). A multivariate analysis identified an older age ($P = 0.024$), low platelet count ($P = 0.017$), and non-clean liver ($P < 0.001$) as independent risk factors for the subsequent development of HCC[29].

USEFULNESS OF EOB-MRI AS A MODALITY TO PREDICT THE PROGNOSES AFTER HEPATIC RESECTION FOR HCC

The imaging findings of HCC detected by EOB-MRI are useful predictors of recurrence after hepatic resection. Ariizumi *et al*[30] determined the tumor margins of HCC from 61 patients preoperatively based on the hepatobiliary phase images of EOB-MR and found that a non-smooth tumor margin in the hepatobiliary phase of EOB-MRI predicted microscopic portal vein invasion, intrahepatic metastasis, and early recurrence after hepatic resection in patients with HCC.

The presence of non-hypervascular hypointense

hepatic nodules detected during the hepatobiliary phase of EOB-MRI was identified as another predictor of the recurrence of HCC after hepatic resection. Toyoda *et al*[31] prospectively examined 77 patients who underwent hepatic resection for primary, non-recurrent, hypervascular HCC after hepatic resection and compared postoperative recurrence rates according to the presence of non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of EOB-MRI. They showed that recurrence rates after hepatic resection were higher in patients with non-hypervascular hypointense nodules (RR = 1.9396, 95%CI: 1.3615-2.7222), the presence of non-hypervascular hypointense nodules was an independent factor associated with postoperative recurrence (RR = 2.1767, 95%CI: 1.5089-3.1105) in addition to HCC differentiation and portal vein invasion, and intrahepatic recurrence was mainly multicentric in origin[31]. This group subsequently reported that the presence of concurrent non-hypervascular hypointense hepatic nodules in the hepatobiliary phase of pretreatment EOB-MRI in patients with early-stage typical HCC was an indicator of the higher likelihood of recurrence after treatments including hepatic resection and radiofrequency ablation (RFA), and may be a marker for an unfavorable outcome[32].

The signal intensity of HCC in the hepatobiliary phase of EOB-MRI was found to be another prognostic maker of HCC after hepatic resection.

Kitao *et al*[33] classified 180 surgically resected hypervascular HCCs in 180 patients as either hypointense (n = 158) or hyperintense (n = 22) relative to the signal intensity of the background liver in the hepatobiliary phase of EOB-MRI and compared clinical and pathological features as well as recurrence and survival rates after hepatic resection between the two groups. The grade of differentiation was higher (P = 0.028) and portal vein invasion was less frequent in hyperintense HCCs (13.6%) than in hypointense HCCs (36.7%) (P = 0.039). Serum levels of alpha-fetoprotein (AFP), the Lens culinaris agglutinin reactive fraction of AFP, and prothrombin induced by vitamin K absence or antagonist Ⅱ (PIVKA-Ⅱ) were lower in hyperintense than in hypointense HCCs (P = 0.003, 0.004 and 0.026, respectively). Immunohistochemical AFP and PIVKA-Ⅱ expression levels were lower in hyperintense than in hypointense HCCs (both P < 0.001) and organic anion transporting polypeptide 8 (OATP8, synonymous with OATP1B3) expression was significantly lower in hypointense HCCs than in hyperintense HCCs (P < 0.001). The recurrence rate was lower in hyperintense than in hypointense HCCs (P = 0.039). They concluded that hyperintense HCC in the hepatobiliary phase of EOB-MRI was less aggressive than hypointense HCCs[33].

SURGICAL TREATMENT OF EARLY HCC

It has not yet been established whether early HCC need to be treated by hepatic resection.

Takayama *et al*[34] prospectively examined 70 patients diagnosed with a single HCC of 2 cm or less in diameter who underwent curative hepatic resection and a long-term follow-up. They found that the time to recurrence was longer in the early HCC group than in the overt HCC group (3.9 years *vs* 1.7 years; P < 0.001) and there was no local recurrence. Therefore, they concluded that early HCC is a distinct clinical entity with a high rate of surgical cure. This group subsequently applied the concept of lead time to chronic liver disease, which is the length of time between screen-detected and symptom-detected disease. In order to evaluate the prolongation of survival with the treatment of early HCC, they compared the survival of patients with early and overt HCCs smaller than 2.0 cm treated with liver resection and concluded that the survival benefit of resection for early HCC was marginal because of a long lead time, and, thus, demonstrated that early HCC was not a target lesion for surgery[35].

Early HCC itself is not a target lesion for hepatic resection. However, early HCC at risk of hypervascularity needs to be treated by local ablation therapy including RFA and not surgery because early HCC seldom causes intrahepatic metastasis.

SIGNIFICANCE OF PREOPERATIVE EOB-MRI AND SIMULTANEOUS TREATMENT OF EARLY HCC AT THE TIME OF RESECTION FOR PROGRESSED HCC

Although early HCC itself is not a target lesion for hepatic resection, the treatment of concomitant early HCC with progressed HCC remains controversial.

We sometimes encounter patients who developed the recurrence of hypervascular HCC that originated from residual early HCC in a short period of time after hepatic resection (Figure 1).

Therefore, we examined 147 patients undergoing hepatic resection for HCC and determined whether preoperative imaging with EOB-MRI and the simultaneous treatment of concomitant early HCC by resection or ablation at the time of resection for progressed HCC improved the prognosis of patients following hepatic resection. Of the 147 consecutive patients undergoing their first resection for HCC, 77 received EOB-MRI before resection. Additional treatments for early HCC were more frequent in EOB-MRI patients. Recurrence-free survival was similar at 1 year (81.4% *vs* 82.1%), but improved with EOB-MRI at 3 and 5 years (62.6% and 48.7% *vs* 41.5% and 25.5%, respectively, P < 0.01). We speculated that recurrence within one year of hepatic resection was mainly due to the enlargement of preoperatively-undetectable intrahepatic micrometastasis of resected progressed HCC, while recurrence after one year was multicentric HCC that progressed from early HCC to hypervascular HCC or

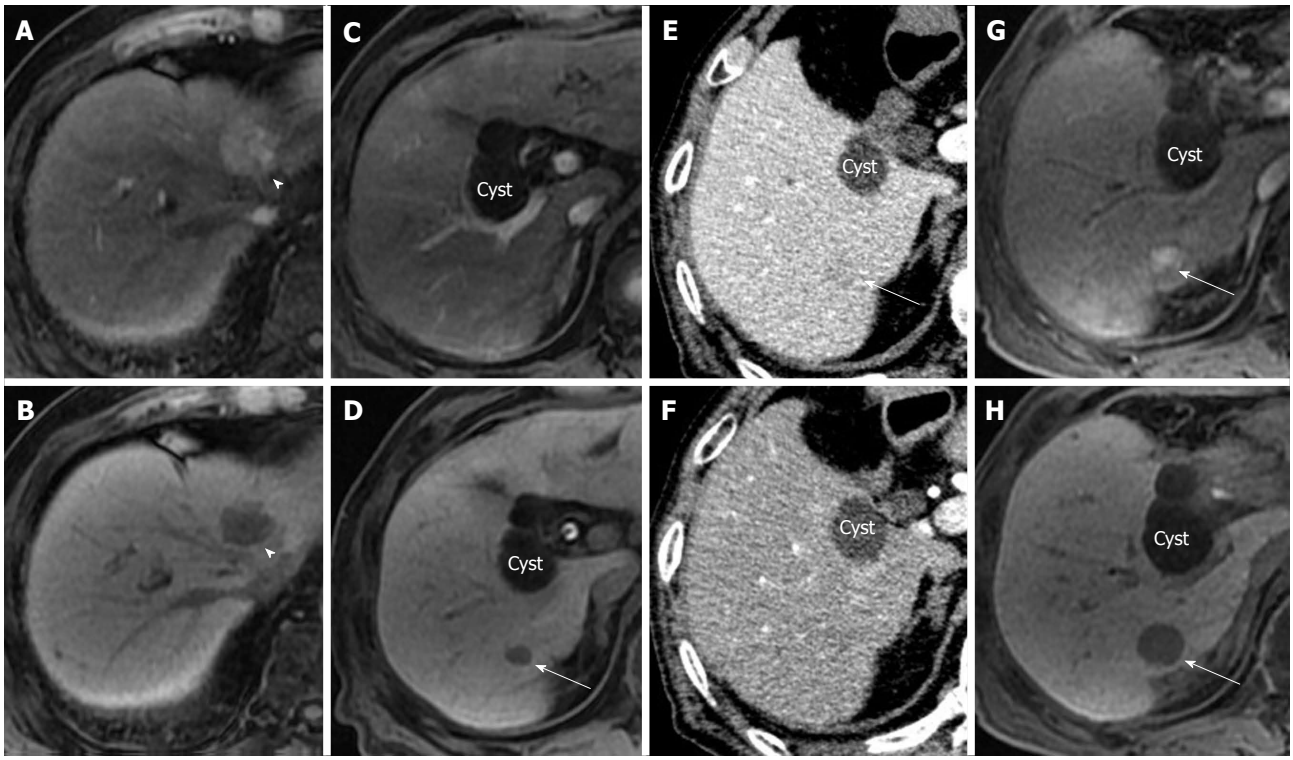

Figure 1 An 81-year-old man with hypervascular (progressed) hepatocellular carcinoma and early hepatocellular carcinoma. Preoperative EOB-MRI showed hypervascular HCC (arrowhead), 2.6 cm in diameter, in the medial section of the liver. The nodule showed hypervascularity in the hepatic arterial-dominant phase (A) and hypointensity in the hepatobiliary phase (B) of EOB-MRI. Preoperative EOB-MRI also showed a small hypointense nodule (arrow), 0.9 cm in diameter, in the posterior section of the liver in the hepatobiliary phase (D). This nodule showed no arterial enhancement in the hepatic arterial-dominant phase of EOB-MRI (C) or CTHA (F), but showed slight hypoattenuation on CTAP (E). This patient underwent left hepatic resection for hypervascular HCC. EOB-MRI taken 5 mo after hepatic resection showed an enlarged hypointense nodule in the posterior section (H) and hypervascularity of the nodule in the hepatic arterial-dominant phase (G). The recurrence of hypervascular HCC that originated from residual early HCC after hepatic resection was demonstrated. HCC: Hepatocellular carcinoma; EOB-MRI: Gadoxetic acid- or gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging; CTHA: Computed tomography during hepatic arteriography; CTAP: Computed tomography arterioportography.

was *de novo* hypervascular HCC. We estimated that the simultaneous treatment of early HCC at the time of resection for progressed HCC reduced multicentric HCC by removing early HCC, which may have progressed to hypervascular HCC. Overall survival slightly improved with EOB-MRI at all time points (1-, 3-, and 5-year survival rates: 98.7%, 90.7% and 80.8% *vs* 97.0%, 86.3% and 72.4%, respectively, $P = 0.38$). One of the reasons why preoperative EOB-MRI and the simultaneous treatment of early HCC at the time of resection prolonged recurrence-free survival but not overall survival after hepatic resection for HCC was an early diagnosis and the prompt treatment of recurrent HCC detected by our postoperative close follow-up[36].

We subsequently examined the 5-year survivors of both groups (41 with EOB-MRI and 48 without) in order to investigate the frequency of treatments for recurrent HCC until 5 years after surgery in both groups. The mean frequency of treatments for recurrent HCC until 5 years after surgery was significantly lower in patients with EOB-MRI than in those without EOB-MRI (0.83 *vs* 1.65 respectively, $P < 0.05$). Although the overall survival rate was not significantly different, reductions in the frequency of treatments for recurrent HCC may have been physically and economically beneficial for patients.

Further studies on larger numbers of patients are needed before this strategy is adopted.

CONCLUSION

EOB-MRI has excellent lesion detection and characterization for hypervascular HCC in arterial phase imaging and hypovascular early HCC in hepatobiliary phase imaging, and has become an indispensable imaging modality in the treatment of HCC. Early HCCs have been detected more frequently since the introduction of EOB-MRI into daily clinical practice. Although optimal timing for the treatment of early HCC currently remains unclear, early HCC, which has particular risk factors, progresses to conventional hypervascular HCC that requires treatment in a short period of time. Early HCC at high risk of hypervascularity need to be treated by local ablation therapy including RFA. If concomitant early HCC with progressed HCC is at a high risk of hypervascularization and the functional liver reserve of a patient is sufficient, its simultaneous treatment at the time of hepatic resection for progressed HCC is recommended. Further studies on larger numbers of patients are needed before this strategy is adopted.

Furthermore, since the risk of hypervascular HCC development or recurrence is high in patients with hypovascular hypointense nodules (almost all nodules are early HCC), close follow-ups are needed in these patients.

REFERENCES

1 **Poon RT**, Fan ST, Lo CM, Ng IO, Liu CL, Lam CM, Wong J. Improving survival results after resection of hepatocellular carcinoma: a prospective study of 377 patients over 10 years. *Ann Surg* 2001; **234**: 63-70 [PMID: 11420484 DOI: 10.1097/00000658-200107000-00010]

2 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207 [PMID: 12547409 DOI: 10.1016/S0168-8278(02)00360-4]

3 **Takayama T**. Surgical treatment for hepatocellular carcinoma. *Jpn J Clin Oncol* 2011; **41**: 447-454 [PMID: 21411469 DOI: 10.1093/jjco/hyr016]

4 **Matsuda M**, Fujii H, Kono H, Matsumoto Y. Surgical treatment of recurrent hepatocellular carcinoma based on the mode of recurrence: repeat hepatic resection or ablation are good choices for patients with recurrent multicentric cancer. *J Hepatobiliary Pancreat Surg* 2001; **8**: 353-359 [PMID: 11521181 DOI: 10.1007/s005340170008]

5 **Matsuda M**, Asakawa M, Amemiya H, Fujii H. Lens culinaris agglutinin-reactive fraction of AFP is a useful prognostic biomarker for survival after repeat hepatic resection for HCC. *J Gastroenterol Hepatol* 2011; **26**: 731-738 [PMID: 21155886 DOI: 10.1111/j.1440-1746.2010.06532.x]

6 **Vogl TJ**, Kümmel S, Hammerstingl R, Schellenbeck M, Schumacher G, Balzer T, Schwarz W, Müller PK, Bechstein WO, Mack MG, Söllner O, Felix R. Liver tumors: comparison of MR imaging with Gd-EOB-DTPA and Gd-DTPA. *Radiology* 1996; **200**: 59-67 [PMID: 8657946 DOI: 10.1148/radiology.200.1.8657946]

7 **Reimer P**, Rummeny EJ, Shamsi K, Balzer T, Daldrup HE, Tombach B, Hesse T, Berns T, Peters PE. Phase II clinical evaluation of Gd-EOB-DTPA: dose, safety aspects, and pulse sequence. *Radiology* 1996; **199**: 177-183 [PMID: 8633143 DOI: 10.1148/radiology.199.1.8633143]

8 **Ahn SS**, Kim MJ, Lim JS, Hong HS, Chung YE, Choi JY. Added value of gadoxetic acid-enhanced hepatobiliary phase MR imaging in the diagnosis of hepatocellular carcinoma. *Radiology* 2010; **255**: 459-466 [PMID: 20413759 DOI: 10.1148/radiol.10091388]

9 **Kitao A**, Zen Y, Matsui O, Gabata T, Kobayashi S, Koda W, Kozaka K, Yoneda N, Yamashita T, Kaneko S, Nakanuma Y. Hepatocellular carcinoma: signal intensity at gadoxetic acid-enhanced MR Imaging--correlation with molecular transporters and histopathologic features. *Radiology* 2010; **256**: 817-826 [PMID: 20663969 DOI: 10.1148/radiol.10092214]

10 **Tsuboyama T**, Onishi H, Kim T, Akita H, Hori M, Tatsumi M, Nakamoto A, Nagano H, Matsuura N, Wakasa K, Tomoda K. Hepatocellular carcinoma: hepatocyte-selective enhancement at gadoxetic acid-enhanced MR imaging--correlation with expression of sinusoidal and canalicular transporters and bile accumulation. *Radiology* 2010; **255**: 824-833 [PMID: 20501720 DOI: 10.1148/radiol.10091557]

11 **Sano K**, Ichikawa T, Motosugi U, Sou H, Muhi AM, Matsuda M, Nakano M, Sakamoto M, Nakazawa T, Asakawa M, Fujii H, Kitamura T, Enomoto N, Araki T. Imaging study of early hepatocellular carcinoma: usefulness of gadoxetic acid-enhanced MR imaging. *Radiology* 2011; **261**: 834-844 [PMID: 21998047 DOI: 10.1148/radiol.11101840]

12 **Ooka Y**, Kanai F, Okabe S, Ueda T, Shimofusa R, Ogasawara S, Chiba T, Sato Y, Yoshikawa M, Yokosuka O. Gadoxetic acid-enhanced MRI compared with CT during angiography in the diagnosis of hepatocellular carcinoma. *Magn Reson Imaging* 2013; **31**: 748-754 [PMID: 23218794 DOI: 10.1016/j.mri.2012.10.028]

13 **Ichikawa T**, Saito K, Yoshioka N, Tanimoto A, Gokan T, Takehara Y, Kamura T, Gabata T, Murakami T, Ito K, Hirohashi S, Nishie A, Saito Y, Onaya H, Kuwatsuru R, Morimoto A, Ueda K, Kurauchi M, Breuer J. Detection and characterization of focal liver lesions: a Japanese phase III, multicenter comparison between gadoxetic acid disodium-enhanced magnetic resonance imaging and contrast-enhanced computed tomography predominantly in patients with hepatocellular carcinoma and chronic liver disease. *Invest Radiol* 2010; **45**: 133-141 [PMID: 20098330 DOI: 10.1097/RLI.0b013e3181caea5b]

14 **Kudo M**, Matsui O, Sakamoto M, Kitao A, Kim T, Ariizumi S, Ichikawa T, Kobayashi S, Imai Y, Izumi N, Fujinaga Y, Arii S. Role of gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging in the management of hepatocellular carcinoma: consensus at the Symposium of the 48th Annual Meeting of the Liver Cancer Study Group of Japan. *Oncology* 2013; **84** Suppl 1: 21-27 [PMID: 23428854 DOI: 10.1159/000345885]

15 **Kojiro M**. Pathology of Hepatocellular Carcinoma. United States: Blackwell Publishing Ltd., MA, 2006

16 **Kudo M**. Multistep human hepatocarcinogenesis: correlation of imaging with pathology. *J Gastroenterol* 2009; **44** Suppl 19: 112-118 [PMID: 19148804 DOI: 10.1007/s00535-008-2274-6]

17 **Kitao A**, Zen Y, Matsui O, Gabata T, Nakanuma Y. Hepatocarcinogenesis: multistep changes of drainage vessels at CT during arterial portography and hepatic arteriography--radiologic-pathologic correlation. *Radiology* 2009; **252**: 605-614 [PMID: 19703890 DOI: 10.1148/radiol.2522081414]

18 **Sakamoto M**, Hirohashi S, Shimosato Y. Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. *Hum Pathol* 1991; **22**: 172-178 [PMID: 1848205 DOI: 10.1016/0046-8177(91)90039-R]

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

20 **Ichikawa T**, Sano K, Morisaka H. Diagnosis of Pathologically Early HCC with EOB-MRI: Experiences and Current Consensus. *Liver Cancer* 2014; **3**: 97-107 [PMID: 24945000 DOI: 10.1159/000343865]

21 **Kobayashi S**, Matsui O, Gabata T, Koda W, Minami T, Ryu Y, Kozaka K, Kitao A. Relationship between signal intensity on hepatobiliary phase of gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA)-enhanced MR imaging and prognosis of borderline lesions of hepatocellular carcinoma. *Eur J Radiol* 2012; **81**: 3002-3009 [PMID: 22748558 DOI: 10.1016/j.ejrad.2012.03.029]

22 **Motosugi U**, Ichikawa T, Sano K, Sou H, Onohara K, Muhi A, Amemiya F, Enomoto N, Matsuda M, Fujii H, Araki T. Outcome of hypovascular hepatic nodules revealing no gadoxetic acid uptake in patients with chronic liver disease. *J Magn Reson Imaging* 2011; **34**: 88-94 [PMID: 21608068 DOI: 10.1002/jmri.22630]

23 **Takechi M**, Tsuda T, Yoshioka S, Murata S, Tanaka H, Hirooka M, Mochizuki T. Risk of hypervascularization in small hypovascular hepatic nodules showing hypointense in the hepatobiliary phase of gadoxetic acid-enhanced MRI in patients with chronic liver disease. *Jpn J Radiol* 2012; **30**: 743-751 [PMID: 23001373 DOI: 10.1007/s11604-012-0120-5]

24 **Kumada T**, Toyoda H, Tada T, Sone Y, Fujimori M, Ogawa S, Ishikawa T. Evolution of hypointense hepatocellular nodules observed only in the hepatobiliary phase of gadoxetate disodium-enhanced MRI. *AJR Am J Roentgenol* 2011; **197**: 58-63 [PMID: 21701011 DOI: 10.2214/AJR.10.5390]

25 **Higaki A**, Ito K, Tamada T, Teruki S, Yamamoto A, Higashi H, Kanki A, Sato T, Noda Y. High-risk nodules detected in the hepatobiliary phase of Gd-EOB-DTPA-enhanced MR imaging in cirrhosis or chronic hepatitis: incidence and predictive factors for hypervascular transformation, preliminary results. *J Magn*

Reson Imaging 2013; **37**: 1377-1383 [PMID: 23172841 DOI: 10.1002/jmri.23933]

26 **Kim YK**, Lee WJ, Park MJ, Kim SH, Rhim H, Choi D. Hypovascular hypointense nodules on hepatobiliary phase gadoxetic acid-enhanced MR images in patients with cirrhosis: potential of DW imaging in predicting progression to hypervascular HCC. *Radiology* 2012; **265**: 104-114 [PMID: 22891358 DOI: 10.1148/radiol.12112649]

27 **Hyodo T**, Murakami T, Imai Y, Okada M, Hori M, Kagawa Y, Kogita S, Kumano S, Kudo M, Mochizuki T. Hypovascular nodules in patients with chronic liver disease: risk factors for development of hypervascular hepatocellular carcinoma. *Radiology* 2013; **266**: 480-490 [PMID: 23362095 DOI: 10.1148/radiol.12112677]

28 **Ichikawa S**, Ichikawa T, Motosugi U, Sano K, Morisaka H, Enomoto N, Matsuda M, Fujii H, Araki T. Presence of a hypovascular hepatic nodule showing hypointensity on hepatocyte-phase image is a risk factor for hypervascular hepatocellular carcinoma. *J Magn Reson Imaging* 2014; **39**: 293-297 [PMID: 23633285 DOI: 10.1002/jmri.24164]

29 **Komatsu N**, Motosugi U, Maekawa S, Shindo K, Sakamoto M, Sato M, Tatsumi A, Miura M, Amemiya F, Nakayama Y, Inoue T, Fukasawa M, Uetake T, Ohtaka M, Sato T, Asahina Y, Kurosaki M, Izumi N, Ichikawa T, Araki T, Enomoto N. Hepatocellular carcinoma risk assessment using gadoxetic acid-enhanced hepatocyte phase magnetic resonance imaging. *Hepatol Res* 2014; **44**: 1339-1346 [PMID: 24528803 DOI: 10.1111/hepr.12309]

30 **Ariizumi S**, Kitagawa K, Kotera Y, Takahashi Y, Katagiri S, Kuwatsuru R, Yamamoto M. A non-smooth tumor margin in the hepatobiliary phase of gadoxetic acid disodium (Gd-EOB-DTPA)-enhanced magnetic resonance imaging predicts microscopic portal vein invasion, intrahepatic metastasis, and early recurrence after hepatectomy in patients with hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci* 2011; **18**: 575-585 [PMID: 21360083 DOI: 10.1007/s00534-010-0369-y]

31 **Toyoda H**, Kumada T, Tada T, Niinomi T, Ito T, Sone Y, Kaneoka Y, Maeda A. Non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI are a risk factor for recurrence of HCC after hepatectomy. *J Hepatol* 2013; **58**: 1174-1180 [PMID: 23376360 DOI: 10.1016/j.jhep.2013.01.030]

32 **Toyoda H**, Kumada T, Tada T, Sone Y, Maeda A, Kaneoka Y. Non-hypervascular hypointense nodules on Gd-EOB-DTPA-enhanced MRI as a predictor of outcomes for early-stage HCC. *Hepatol Int* 2015; **9**: 84-92 [PMID: 25788383 DOI: 10.1007/s12072-014-9553-5]

33 **Kitao A**, Matsui O, Yoneda N, Kozaka K, Kobayashi S, Koda W, Gabata T, Yamashita T, Kaneko S, Nakanuma Y, Kita R, Arii S. Hypervascular hepatocellular carcinoma: correlation between biologic features and signal intensity on gadoxetic acid-enhanced MR images. *Radiology* 2012; **265**: 780-789 [PMID: 23175543 DOI: 10.1148/radiol.12120226]

34 **Takayama T**, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, Kosuge T, Okada S, Takayasu K, Yamasaki S. Early hepatocellular carcinoma as an entity with a high rate of surgical cure. *Hepatology* 1998; **28**: 1241-1246 [PMID: 9794907 DOI: 10.1002/hep.510280511]

35 **Midorikawa Y**, Takayama T, Shimada K, Nakayama H, Higaki T, Moriguchi M, Nara S, Tsuji S, Tanaka M. Marginal survival benefit in the treatment of early hepatocellular carcinoma. *J Hepatol* 2013; **58**: 306-311 [PMID: 23063418 DOI: 10.1016/j.jhep.2012.09.026]

36 **Matsuda M**, Ichikawa T, Amemiya H, Maki A, Watanabe M, Kawaida H, Kono H, Sano K, Motosugi U, Fujii H. Preoperative gadoxetic Acid-enhanced MRI and simultaneous treatment of early hepatocellular carcinoma prolonged recurrence-free survival of progressed hepatocellular carcinoma patients after hepatic resection. *HPB Surg* 2014; **2014**: 641685 [PMID: 24701029 DOI: 10.1155/2014/641685]

P- Reviewer: Morana G, Schmeding M, Tarazov PG
S- Editor: Gong ZM **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.i30.2940

World J Hepatol 2015 December 28; 7(30): 2940-2954
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

2015 Advances in Cirrhosis

Nutritional assessment in cirrhotic patients with hepatic encephalopathy

Fernando Gomes Romeiro, Laís Augusti

Fernando Gomes Romeiro, Laís Augusti, Gastroenterology Division, Department of Internal Medicine - Botucatu Medical School, UNESP-Univ Estadual Paulista, Botucatu 18608-917, São Paulo, Brazil

Author contributions: Romeiro FG designed the study, obtained the data, drafted and corrected the final article; Augusti L obtained and organized the data, drafted and corrected the article. Both authors read and approved the final manuscript.

Supported by São Paulo Research Foundation (FAPESP), CAPES and PROPe/UNESP, Nos. 2013/15121-8 and 2013/11761-2.

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

Correspondence to: Dr. Fernando Gomes Romeiro, Gastroenterology Division, Department of Internal Medicine - Botucatu Medical School, UNESP-Univ Estadual Paulista, Distrito de Rubião Jr. s/n, Botucatu 18608-917, São Paulo, Brazil. fgromeiro@fmb.unesp.br
Telephone: +55-14-38801173
Fax: +55-14-38801667

Received: April 28, 2015
Peer-review started: May 6, 2015
First decision: September 8, 2015
Revised: September 23, 2015
Accepted: December 13, 2015
Article in press: December 14, 2015
Published online: December 28, 2015

Abstract

Hepatic encephalopathy (HE) is one of the worst complications of liver disease and can be greatly influenced by nutritional status. Ammonia metabolism, inflammation and muscle wasting are relevant processes in HE pathophysiology. Malnutrition worsens the prognosis in HE, requiring early assessment of nutritional status of these patients. Body composition changes induced by liver disease and limitations superimposed by HE hamper the proper accomplishment of exams in this population, but evidence is growing that assessment of muscle mass and muscle function is mandatory due to the role of skeletal muscles in ammonia metabolism. In this review, we present the pathophysiological aspects involved in HE to support further discussion about advantages and drawbacks of some methods for evaluating the nutritional status of cirrhotic patients with HE, focusing on body composition.

Key words: Hepatic encephalopathy; Liver cirrhosis; Malnutrition; Anthropometry; Muscle strength; Electric impedance; Nutrition assessment; Dual-energy X-ray absorptiometry

Core tip: Ammonia metabolism, inflammation and muscle wasting are relevant processes in hepatic encephalopathy (HE) pathophysiology and malnutrition worsens the prognosis in this condition, requiring early assessment of nutritional status in these patients. Body composition changes induced by liver disease and limitations superimposed by HE make difficult to accomplish exams properly in this population, but there is a growing evidence that assessment of muscle mass and muscle function is mandatory due to the role of skeletal muscles in ammonia metabolism. In this article,

we review HE pathophysiology and discuss the main methods of nutritional assessment, suggesting the best approaches in HE patients.

Romeiro FG, Augusti L. Nutritional assessment in cirrhotic patients with hepatic encephalopathy. *World J Hepatol* 2015; 7(30): 2940-2954 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i30/2940.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i30.2940

INTRODUCTION

Hepatic encephalopathy (HE), a neurological dysfunction affecting primarily the brain, is caused by acute or chronic liver insufficiency and/or by the presence of portosystemic shunting. This syndrome embraces distinct forms of neurological symptoms from subclinical presentations to hepatic coma[1]. Overt HE afflicts 30%-45% of cirrhotic patients, whereas minimal HE may affect more than half of the cases with advanced cirrhosis[2-4]. The estimated incidence in decompensated cirrhosis is 8% per year and the majority of patients with HE episodes are received in emergency care services[5]. In an observational study, the mean hospitalization length of these patients was between 5.7 and 7.1 d[2].

Among cirrhosis complications, HE mortality is higher than the rate caused by variceal bleeding or ascites. After overt episodes, death rates vary between 42% and 64% in one year according to HE grade[6-8]. The relevance of this condition was demonstrated even in patients without overt HE, for whom a mortality index combining electroencephalogram findings and the model of end-stage liver disease score (MELD) had a higher accuracy for predicting 12- and 18-mo survival than the MELD score alone[9].

The role of nutrition in HE physiopathology is well established, and nutritional treatment is an important step in order to improve quality of life (QOL) and survival of patients with cirrhosis and HE. Since HE is a late complication of advanced liver disease, it is not surprising that cirrhotic patients with HE are susceptible to nutrition disorders[10]. Hence, there are many reasons that nutrition and HE cause a great impact on each other.

Ammonia metabolism is probably the most studied of the mechanisms responsible for the nutritional effects on HE, because ammonia production is highly influenced by dietary components. Consequently, many nutritional treatments have been studied in order to modulate ammonia production, to increase survival rates and to improve QOL of cirrhotic patients stricken by HE.

Nutrition status is directly associated with survival of cirrhotic patients[11-14]. Moreover, when these patients also have HE this association can be even more important. Although there are many studies focusing on the nutrition role in cirrhosis, this article specifically aimed to discuss practical nutritional strategies that can be applied to evaluate the body composition of HE patients. With the purpose of giving pathophysiological support to this discussion, the main factors related to HE etiology are presented as ammonia production, inflammation, and muscle wasting - whereas other factors are briefly addressed.

PATHOPHYSIOLOGY OF HE AND NUTRITIONAL STATUS INFLUENCE ON THE MAIN PROCESSES INVOLVED

The pathophysiology of HE is multifactorial and there is a general agreement that ammonia and inflammation act synergistically to cause astrocyte swelling and brain edema[15]. Since body composition affects ammonia metabolism and has a clear impact on cirrhosis and HE, the main pathophysiological point discussed herein is ammonia metabolism.

Ammonia metabolism

Even though ammonia levels are not strictly proportional to neurological impairment, ammonia is of great relevance in HE. High blood ammonia levels are not sufficient to establish the diagnosis of HE in cirrhotic patients; however, a normal value requires diagnostic reevaluation[1]. It means that ammonia blood levels are still valuable when they are related to additional clinical information, but isolated ammonia levels should never be considered sufficient to make a diagnosis. It is also important to consider that the values are influenced by physical activity, the source of the blood collected (arterial or venous), the prandial status and the diet pattern before the test.

In humans, there are three main sources of ammonia: The intestines, the kidneys and the muscles. The total daily production in adults is around 1000 mmol of ammonia[16]. The kidney production is increased in cirrhotic patients during hemodynamic disturbances[17]. The role of intestines in ammonia production is fairly the most frequently cited aspect in prior studies about HE, making the colon one of the main treatment targets.

Studies on non-absorbable disaccharides were the initial evidence that orally ingested substances could be useful in HE treatment. Lactulose and lactitol are the most studied among them in clinical trials of HE treatment. Lactulose is a good example of how a non-absorbable component could be relevant in this setting. Beyond the cathartic property, achieved by increasing intestinal transit, lactulose is also converted to lactic acid and acetic acid, which have the potential of converting ammonia to ammonium. Another interesting effect of disaccharides is that the colon acidification favors the proliferation of nonammoniagenic bacteria[18]. According to this point of view, lactulose also has a prebiotic property, and these effects could be achieved, at least in part, by nutritional treatments.

Colonic production of ammonia is a target in HE treatment, but it is important to notice that three systems are able to convert ammonia into other substances. The liver is the chief organ, and urea synthesis is the main metabolic route for ammonia clearance in normal conditions[19]. Skeletal muscles are a secondary system that can convert ammonia to glutamine, as is the brain tissue. Given the osmotic effect of glutamine, continuous conversion of ammonia to glutamine inside the astrocytes can cause cellular edema. Astrocytes constitute a significant part of the cerebral volume, so any degree of edema in these cells can be relevant to the brain and can lead to decreased excitatory neurotransmission, which is a characteristic feature of HE[20].

Accordingly, in advanced cirrhosis, the liver can be insufficient to accomplish the ammonia clearance as needed. Hence, the only way to avoid glutamine formation inside the neural cells is to convert ammonia to glutamine in skeletal muscles. Conversely, cirrhosis is a catabolic disease, and patients with advanced cirrhosis are commonly affected by muscular wasting, which decreases the amount of muscle tissue available to make this conversion. One of the main reasons for this catabolic state is that changes in the cirrhotic liver impair the glucose metabolism, so that when these patients are in a fasting state they have to use muscular proteins as an energy source. In many of these patients, HE arises as a late complication, developed when their muscles have been affected for a long duration, leading to muscle depletion and malnutrition. It can be difficult to counteract this process in HE patients, and prevention is clearly the best choice to preserve muscle mass in this setting.

It is estimated that nearly half of the total ammonia can be metabolized in muscles through glutamine synthesis. However, glutamine synthesis and ammonia uptake are low during rest[19]. Some studies have hypothesized that in the rest state the muscle might not be significant to ammonia metabolism in healthy subjects, but it could be more important in liver disease[20]. Since muscle is the best means to increase ammonia clearance in cirrhotic patients with HE, physical activity could be very relevant in this setting. However, the role of muscle activity as a way to prevent HE was not verified in clinical trials.

Due to the role of muscle mass in cirrhotic patients, especially those with HE, early nutritional assessment must be performed properly. The early nutritional diagnosis must be followed by an appropriate dietary treatment in order to reduce the process of muscle wasting related to this liver disease. Even so, ammonia metabolism is not the only aspect that should be considered.

Inflammation

The inflammatory process is another significant issue in HE pathophysiology that can also be a target for different forms of treatment, including nutritional strategies. Inflammatory cytokines affect the blood-brain barrier and increase the ammonia diffusion in astrocytes[21]. Cirrhosis is characterized by subclinical increasing of inflammatory cytokines, but it can be difficult to show the correlation between HE and inflammation through peripheral blood cytokine tests[22]. In a double-blind randomized trial comparing the effects of two antibiotics, our group showed for the first time that hospitalization length was associated with C reactive protein levels during HE treatment. This finding illustrates the inflammation impact not only when the patient arrives at the hospital but also during HE treatment[23].

The level of inflammation products found through fecal calprotectin tests is higher in patients with cirrhosis and HE, indicating that the gastrointestinal tract (GIT) is the main source of inflammation in these patients[24]. A further study suggested that there is a global mucosal-immune interface change in these patients that affects the entire GIT[25]. Cirrhosis is often associated to slow gastrointestinal transit and mucosal edema, allowing the passage of bacterial products through the epithelial barrier. These products keep a continuous flow of bacterial particles through the portal vein, promoting the release of tumor necrosis factor inside the liver and causing a long-lasting cellular damage into the organ[26].

Obviously, nutritional management is not sufficient to avoid inflammation caused by acute infections, and reducing this sustained inflammatory state in cirrhotic patients is a challenge. However, when the main source of inflammation is the GIT, nutritional treatments can be useful to limit this process. Cirrhotic patients with HE are frequently stricken by small intestinal bacterial overgrowth, which leads to alterations in intestinal microbiota profile[27,28]. In this context, probiotics, prebiotics and symbiotics ingested can increase GIT transit and alter the microbiota involved in GIT inflammation to achieve a better bacterial profile, similar to the effects obtained by lactulose.

To demonstrate this effect in a clinical trial, Liu *et al*[27] evaluated the results obtained by a symbiotic preparation on minimal HE in patients with cirrhosis. They found that the symbiotic treatment favored the fecal proliferation of *Lactobacilli*, thus reducing the amount of the previous bacterial strains, which were more associated to inflammation. This modulation of GIT microbiota was concomitant to a remarkable decrease in serum ammonia and a significant improvement of HE in half of the subjects. Remarkably, the symbiotic treatment was also associated with endotoxemia reduction, improving the Child-Pugh class in nearly 50% of cases. Treatment with fermentable fiber alone was similarly helpful to many patients. Thus, the authors concluded that treatment with symbiotics or fermentable fiber would constitute an alternative to lactulose for the management of minimal HE in cirrhotic patients[27].

Other mechanisms involved in HE and also in different types of encephalopathy

Besides the fact that ammonia and inflammation

are the most appraised components involved in HE pathophysiology, the evidence supporting the role of oxidative stress and hyponatremia in HE is growing. The increase of blood-brain barrier permeability related to hyponatremia and oxidative stress has been associated to HE development[17]. Hyponatremia in cirrhotic patients is often caused by fluid retention, leading to hypervolemic (dilutional) hyponatremia. Conventional therapy in this setting is a challenge because fluid restriction and loop diuretics are frequently inefficacious[29]. It has been considered as a third hit in HE pathophysiology, because it can worsen the cerebral edema caused by hyperammonemia and inflammation. The management of these conditions in the emergency room is beyond the scope of this review, but nutritional strategies can be convenient in order to avoid or correct some electrolytic disturbances.

Electrolytes and fluid imbalances are commonly associated with several illnesses and have a great impact in cirrhosis and HE. Potassium and zinc deficits can be a consequence of restricted diets, and some enriched supplements can be useful for avoiding or treating them. Hypokalemia should be promptly corrected in patients with HE because it increases ammonia production and excretion by the kidneys[30]. Additionally, zinc deficiency is not rare in HE, and the early diagnosis should be done in this setting[31]. It should always be suspected in the presence of HE associated with malnutrition, especially when a poor diet is maintained for a long duration, as in the case of severe alcohol addiction. In theory, the lack of dietary proteins could be a contributing factor, because they are a significant source of zinc. Zinc deficiency can lead to HE, diarrhea, muscle cramps and skin lesions, complications that can be avoided by the prompt initiation of the disturbance correction.

Many HE patients have high manganese levels, possibly reproducing the effects of hepatocellular failure, as well as impaired biliary flow and the existence of porto-systemic venous shunts[32]. Manganese accumulation in brain tissue is found in severe cases of HE and acquired hepatocerebral degeneration[33,34]. Other electrolyte imbalances found in patients with cirrhosis and HE include hypocalcaemia and hypomagnesaemia, but their role in HE is not so clear. Finally, these patients present severe amino acid imbalances that lead to depletion in branched chain amino acids, which can be supplemented to achieve improvement in HE[35,36].

NUTRITIONAL STATUS IN HE

Among cirrhotic patients, 75% of those who develop HE have moderate to severe malnutrition, which affects their energy reserves and muscle mass[1]. Due to the muscular involvement in ammonia metabolism, malnutrition is associated with a higher incidence of HE[37-39]. The best means to define malnutrition in cirrhosis is protein calorie malnutrition, in which both lean and fat tissue can be depleted[39,40]. Although this reduction in both tissues is recognized as cachexia, the predominant loss of muscle mass in cirrhosis suggests that sarcopenia, or loss of skeletal muscle mass, is the first nutritional deficiency[40].

Concurrently, overweight has been cited in cirrhotic patients as another matter of concern. Berzigotti *et al*[41] evaluated 161 cirrhotic patients over a mean follow-up of 59 mo or until cirrhosis decompensation. The incidence of complications in patients was 15% in those with normal body mass index (BMI), 31% in those overweight and 43% in obese patients. In cirrhosis, obesity can be associated with loss of muscle mass, an ambiguous state of excess adipose tissue and muscle wasting denominated sarcopenic obesity, which has accumulated risks for each of the two phenotypes of body composition[41-44].

Thus, the presence of cirrhosis and HE affects nutritional status by many mechanisms, as described in Table 1.

As HE is frequently associated with advanced cirrhosis and this combination of severe conditions can have a substantial effect on food ingestion, the effects are clear in the clinical setting. In a multicentric trial assessing cirrhotic inpatients with jaundice, the spontaneous caloric intakes in the control group were approximately 20-25 kcal/kg per day, considered by the authors to constitute more than expected[45]. A large study aimed to document the impact of malnutrition and nutritional practice in 396 cirrhotic inpatients registered the caloric intake as 35.1 ± 10.0 kcal/kg per day in Child A, 29.0 ± 7.3 kcal/kg per day in Child B and 24.0 ± 8.0 kcal/kg per day in Child C subjects. Of note, changes in dietary and protein intake during hospitalization were related to mortality[46]. In a study of 60 outpatients, in which the majority of whom had compensated liver disease, the caloric intake was between 24 and 40 kcal/kg per day[47]. Another study assessing 300 cirrhotic outpatients obtained a mean value of 32 kcal/kg per day[48]. Unfortunately, the HE rates are not clearly documented, so it is difficult to identify the food intake patterns in this condition.

To analyze this question, our group assessed 60 outpatients with cirrhosis and HE. The majority of them had grade 1 HE (34 patients), while 23 presented minimal HE and only two subjects had grade 2 HE. The mean caloric ingestion was 20.5 ± 8.61 kcal/kg per day (unpublished data). These values were clearly below the recommendations for cirrhotic patients with HE, which range from 35 to 40 kcal/kg per day[32,49]. It is difficult to know whether the presence of HE is the main reason for the disagreement between our data and other results of caloric ingestion obtained in studies that did not evaluate only patients with HE, given the potential relevance of many other differences between the populations assessed. To clarify this hypothesis, we encourage researchers to implement new studies of caloric ingestion in HE patients to contribute with additional data on this topic.

Given all the mechanisms that influence malnutrition in HE, there are no doubts that it is necessary to evaluate

Table 1 Possible causes of malnutrition in patients with cirrhosis and hepatic encephalopathy

Possible causes	Clinical manifestation
Reduced ingestion of foods	Anorexia
	Early satiety
	Ascites
	Confusion and/or excessive somnolence
	Frequent hospitalizations
Impaired absorption of nutrients	Alterations in enterohepatic circulation
	Impaired biliary excretion
	Small intestinal bacterial overgrowth
	Portosystemic shunts
Metabolic disturbances	Protein hypercatabolism/BCAA depletion
	Decreased glycogen stores and gluconeogenesis
	Insulin resistance and enhanced ketogenesis
	Increased lipolysis and fatty acid oxidation
Other factors	Restricted diets (*e.g.*, low sodium diets)
	Protein loss during large volume paracentesis
	Abdominal distention during lactulose therapy

BCAA: Branched chain amino acids.

the nutritional status of cirrhotic patients who develop HE. However, it can be a challenge because patients with HE often have altered body composition, including variations in fluid balance and protein catabolism induced by the liver disease[1,32,50,51]. Moreover, they can be particularly difficult to evaluate through exams requiring extensive patient collaboration.

Some authors have suggested that changes in water homeostasis and compartmentalization can exist even before fluid accumulation is detected, and when these patients have ascites, pleural effusion or edema their evaluation *via* traditional methods can be even less accurate for assessing body composition[52,53]. Additionally, changes in lean mass and fat ratio can also reduce the accuracy of some methods of nutritional evaluation, as in sarcopenic, obese and also sarcopenic obese patients[41,43,44]. These alterations in body composition require more attention to assess patients with advanced liver disease, which can affect some measures obtained by the traditional methods.

Finally, metabolic changes lead to lessening in protein and fat reserves in 50%-75% of cirrhotic patients[1,50]. Furthermore, the degree of depletion in these reserves is related to prognosis and is a risk factor for developing HE, thus necessitating the evaluation of body composition in this population[39].

METHODS TO EVALUATE THE NUTRITIONAL STATUS OF CIRRHOTIC PATIENTS WHO DEVELOPED HE

Detailed body composition assessment is essential in HE patients. It is the first step to define the pattern of tissue loss and to establish nutritional treatment strategies[54]. Therefore, nutritional assessment of cirrhotic patients with HE must combine a good dietary history, body composition data and laboratory exams[1,32,49,55]. In this review, we will focus on body composition evaluation.

Most techniques to assess body composition are focused on differentiating fat mass from fat-free mass, while some methods presume that fat-free mass has constant characteristics, such as hydration fraction and density. Thus, techniques such as anthropometry and bioelectrical impedance could lead to over- or under-estimation of body composition findings when these assumptions are invalid[56]. Despite that, anthropometric and bioelectrical impedance data were associated with prognosis in cirrhotic patients, and their potential lack of accuracy is difficult to quantify.

Herein, we analyze the usefulness of the most widely utilized techniques in the assessment of nutritional status in cirrhotic patients who developed HE, presenting some of the advantages and drawbacks of each method. Given that patients with severe HE can be difficult to evaluate through exams requiring extensive patient collaboration, we suggest some of them that could be suitable in a practical scenario, adding more complex techniques in the initial evaluation and/or to bring more accuracy. Table 2 summarizes some of the findings in this setting.

Subjective Global Assessment

Subjective Global Assessment (SGA) is one of the most widely used methods to evaluate nutritional status in patients during their hospital stay. It gathers information about food intake, weight changes, gastrointestinal symptoms and physical examinations, which are intended to evaluate subcutaneous fat, muscular atrophy, edema and ascites[57].

Although SGA is useful as a screening tool to be applied upon hospital arrival, it is not sufficient to evaluate cirrhotic patients with HE on account of some methodological limitations. First, SGA depends on personal information that can be difficult to obtain from patients with cognitive impairment or somnolence. Second, the only anthropometric measure utilized is the body weight, which is often changed by ascites and edema[50,51]. Therefore, the guidelines of the International Society for HE and Nitrogen Metabolism (ISHEN) highlight that SGA can underestimate malnutrition occurrence in cirrhotic patients and does not predict outcome accurately[32].

Anthropometry

Anthropometric measurements are objective methods to evaluate the nutritional status. They are rapid, non-invasive and low-cost techniques, specifically suited to assess somatometric characteristics. These measurements have been considered the most useful procedures to assess nutritional status in cirrhotic patients[58]. Nonetheless, they also have limitations when applied in patients with HE and cirrhosis. For instance, ascites and edema can influence body weight and BMI values,

Table 2 Advantages and disadvantages of the main methods of nutritional assessment for cirrhotic patients with hepatic encephalopathy

Method	Advantages	Disadvantages
SGA	Quick application Low cost Can identify patients under risk of malnutrition upon hospital arrival Can be applied in hospital rooms	Requires patient comprehension and collaboration Subjectivity (the only objective measure used is weight) Can underestimate malnutrition Cannot be used as a follow-up method
Anthropometry	Quick application Low cost Demands little collaboration Can be applied in hospital rooms Some measures (CAMA, MAMC, APMT) are less influenced by water retention and overweight/obesity MAMC is widely recommended for liver disease patients MAMC and TSF are associated with outcomes in cirrhotic patients and are related to the presence of HE	Some measures (body weight, body mass index, AC, TSF) can be highly influenced by water retention and overweight/obesity Interobserver variation decreases the data reproducibility Can underestimate malnutrition
Handgrip strength	Quick application Low cost Can be applied in hospital rooms Identify impaired muscle function Is not influenced by either water retention or overweight/obesity Is an independent predictor of cirrhosis decompensation	Cannot identify muscle wasting anatomically Is not so suitable for evaluating cirrhotic women, because skeletal muscle function correlates with muscle mass only in men
Bioelectrical impedance analysis	Quick application Can be applied in hospital rooms when portable equipment is used PA and BCM are associated with outcomes in cirrhotic patients	Controversial applicability in patients with fluid retention Requires patient removal to the equipment room when non-portable equipment is used Can underestimate malnutrition
Dual-energy X-ray absorptiometry	Adequate accuracy to identify muscle depletion Excellent reproducibility Can also identify bone mass reduction as a screening tool Gives detailed analyses of body composition (segmental results), obtaining measures that have prognostic impact in cirrhotic patients FFMI is an independent predictor of HE AMMI can be used to diagnose sarcopenia	High cost Requires patient removal to the equipment room Exposure to ionizing radiation makes routine use less attractive as a follow up method
Computed tomography scan	Adequate accuracy to identify muscle depletion Excellent reproducibility Can be performed retrospectively from images previously obtained Can also identify hepatic nodules, portosystemic shunts and other abnormalities Skeletal muscle thickness in cross-sectional images has prognostic impact in cirrhotic patients L3 SMI can be used to diagnose sarcopenia	High cost Requires patient removal to the equipment room Exposure to ionizing radiation makes routine use less attractive as a follow-up method

The bedside techniques are presented at the top and can be valuable even in conditions of restricted access to technology. The more complex methods are shown at the bottom, and should also be used when technology is unrestrained, providing more accuracy. Methods used only for research purposes and those not applied to patients with hepatic encephalopathy are not included. AC: Arm circumference; AMMI: Appendicular muscle mass index; APMT: Adductor pollicis muscle thickness; BCM: Body cell mass; CAMA: Corrected arm muscle area; FFMI: Fat-free mass index; HE: Hepatic encephalopathy; L3 SMI: Third lumbar vertebrae skeletal muscle index; MAMC: Mid-arm muscle circumference; PA: Phase angle; TSF: Triceps skinfold; SGA: Subjective Global Assessment.

underestimating the prevalence of malnutrition among these patients[51]. Although there is a specific classification of BMI for cirrhotic patients that categorizes the presence of ascites, this reference is not fully applied in clinical trials, and presents a clear limitation of having a single cutoff value to diagnose malnourished patients, precluding the diagnosis of eutrophic or overweight patients[59]. Most studies still use the dry weight, which is estimated by subtracting the weight of ascites and edema that is known from information obtained during clinical assessment, weight values formerly registered, ascites volume drained and references previously established[32].

Skinfolds and body circumferences are less affected

by water retention than BMI. Skinfolds are mainly used to estimate the body fat. Fiore *et al*[60], evaluating 40 cirrhotic subjects without overt fluid retention, found that the percentage of body fat assessed by skinfolds had a difference of less than 5% in comparison to the values obtained by dual-energy X-ray absorptiometry (DEXA). The authors evaluated body composition measures in patients who did not present any degree of edema or ascites. Moreover, most subjects had compensated cirrhosis (Child-Pugh A/B/C = 24/16/0) while the rate of patients with HE is not mentioned in the article, hampering extrapolation of the findings to patients with HE and/or more advanced liver disease.

Two of the most recommended measures to evaluate the nutritional status of cirrhotic patients with HE are triceps skinfold (TSF) and mid-arm muscle circumference (MAMC)[55]. MAMC is calculated through TSF and mid-arm circumference (AC). In a study of 212 cirrhotic inpatients monitored for 2 years, the authors suggested that MAMC and TSF could be included in the Child-Pugh classification in order to improve the predictive accuracy of this score, although the prognostic power of TSF was lower than that of MAMC[11]. Another study of 300 subjects showed that MAMC values were expressively more affected in male than in female cirrhotic patients, while loss of fat deposits based on TSF was more significant in females[48]. In a study evaluating 143 patients before liver transplantation, the authors considered MAMC to be the best reliable anthropometric tool[61]. In a study of 102 patients submitted to orthotopic liver transplantation (OLT), AC and TSF were used to categorize patients according to the values before OLT; those below the 25th percentile had an increased incidence of hepatic tests result abnormalities, suggesting a higher incidence of complications in this group[62].

A method in which MAMC and BMI were combined with SGA information as a new way to evaluate the nutritional status of cirrhotic patients was previously validated[63]. The proposed algorithm showed prognostic value in this population and we encourage its use as a screening method for patients in their hospital admission. However, there is no information whether this algorithm could be useful during the patient follow-up, or whether variations in weight/BMI could impair the accuracy found by the authors. Thus, a simple and precise technique for assessing malnutrition in cirrhotic individuals is not available yet[64].

Another anthropometric measure that may be used for diagnosing muscle wasting in cirrhotic patients is the adductor pollicis muscle thickness (APMT). APMT is a simple tool, with little influence from such body composition changes as edema, ascites and overweight. It has been applied to evaluate the nutritional status in normal populations as well as surgical, renal failure and cancer patients[65-68]. However, we have not found studies specially aimed to evaluate cirrhotic patients.

Specific data of anthropometric measurements in patients with HE were presented in a study of 300 cirrhotic patients (200 men and 100 women). The authors confirmed that the prevalence of overt HE during hospitalization was significantly higher in patients with muscle depletion assessed by MAMC and TSF, as well as in those with decreased muscle strength, showing the relevance of anthropometric measures and muscle strength in HE[39]. Of note, the diagnosis of muscle wasting and fat store depletion was based on MAMC and TSF < 5th percentile, respectively, in relation to normal values.

In a comprehensive review about nutrition in HE, the authors suggested that parameters not affected by ascites or edema include MAMC, AC, and TSF. They also proposed that the diagnosis of malnutrition in advanced liver disease could be done when MAMC and/or TSF values were lower than the 5th percentile in individuals aged 18-74 years, using the 10th percentile for those aged more[64].

Indeed, these measurements were the most widely used for a long time, so there is more evidence that they can be used in cirrhotic patients. Even without such a well-known scientific basis, we suggest that corrected arm muscle area (CAMA) and APMT should also be documented in these patients as supplementary data. Both could be useful to identify muscle wasting, which is extremely relevant for cirrhotic patients with HE. CAMA is calculated through AC and TSF values. As the others measurements proposed by Bémeur *et al*[64], CAMA and APMT are also easy to perform, given that they do not require much cooperation from patients. These advantages are important in cases with somnolence and/or confusion. Additionally, we recommend that the diagnosis of malnutrition in cirrhotic patients with HE should not be based only on anthropometry, unless it is the only method available.

Since anthropometric classifications were not based on cirrhotic patients, it is difficult to establish cutoff values according to the aforementioned studies because most of these values were obtained from standard measures for healthy subjects in different populations. Likewise, other limitations must be considered: The inter-observer disagreement in some procedures, the variations in skin compressibility and hydration, and the fact that in many cases the overweight can preclude the diagnosis of cirrhosis-related muscle loss. Anthropometric measurements should be confirmed by a skilled professional in order to increase their reliability[58]. Therefore, whether evaluations of cirrhotic patients with HE are based only on anthropometry, these issues can be a significant drawback. Given the severe disease found in this population, other methods should be used to support anthropometric findings whenever possible to provide greater accuracy for the data obtained.

Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) measures are safe and relatively accurate for estimating fat mass and fat-free mass. The water component of the body is appraised according to the capacity of the body to conduct an

electrical current[69]. Thus, the basic principle of BIA is that electrical conduction is faster through water and slower through fat tissue due to the resistance imposed by fat deposits, thus estimating the percentages of fat and fat-free tissues. The procedure takes less than 2 min to measure body electrical conductivity and resistance (impedance). This resistance value is then applied to the determination of total body water.

BIA has been applied in evaluations of cirrhotic patients for many years[70-77]. Among the results obtained, phase angle (PA) and body cell mass (BCM) deserve particular attention.

PA values are decreased in advanced cirrhosis. Furthermore, a prior study confirmed that PA was associated with muscle strength and muscle mass in cirrhotic patients. Moreover, a phase angle value less than or equal to 5.4 degrees was predictor of reduced survival[72]. Of note, Peres *et al*[75] found that the median PA was 4.17° (3.19°-7.42°) in a HE subpopulation, expressively worse compared to cirrhotic individuals in which HE was not present (5.04°).

BCM is a lean tissue compartment that is diminished in protein-calorie malnutrition. It estimates the body cellular elements, and has been considered one of the best nutritional references for appraising metabolic pathways like protein turnover and energy expenditure[58]. Thus, low BCM is often observed in advanced liver disease.

Analyzing 150 potential candidates for OLT equally divided into two categories (validation group and study group), Selberg *et al*[78] showed that hypermetabolism and low BCM proportion (< 35% of body weight) were related to short survival. Of note, when the authors compared the subjects who died with those who survived, HE and edema severity were expressively worse in the former group, and CAMA measures were correlated with BCM[78]. Figueiredo *et al*[79] evaluated 69 cirrhotic patients using SGA, anthropometry, handgrip strength, lab tests and dual-energy X-ray absorptiometry. Most of the methods applied were weakly associated with BCM measurements, but the authors showed that handgrip strength and MAMC values obtained in this study were the most sensitive markers of depleted BCM. Combining the cutoff points of 30 kg obtained by handgrip strength test and 23 cm obtained by MAMC, the authors showed that these methods could be used together in order to identify BCM depletion accurately in most patients[79].

Müller *et al*[80] in a study with 123 cirrhotic patients found that post-transplantation mortality was higher in patients with low BCM. The authors concluded that hypermetabolism was not present in all patients, but could be a cause of malnutrition, thus contributing to worsen prognosis[80].

As BIA results can be altered by physical activity, dehydration, diuretic use, fluid retention, as well as liquid and food ingestion before the test, the usage of this method for assessing cirrhotic patients has been controversial[56,81-84]. According to a prior study, total body water was comparable in compensated cirrhosis and in non-cirrhotic subjects, but was higher in patients with ascites[52]. Other studies assessing the changes caused by ascites have shown that fluid overload can be a cause of imprecision in BIA results[82,85,86].

In contrast, another research group postulated that ascites exerts an irrelevant influence on BIA measures since chest and abdomen together represent only 11% or less of the body resistance, showing that BCM appraised by BIA was associated with the same measure evaluated by the total body potassium count, regardless the presence of ascites[87]. However, it must be noticed that 72% of the subjects used diuretics.

Ascites should be viewed as a marker of fluid overload and not as a simple liquid accumulation. Accordingly, the instillation of 2-3 L of fluid into the peritoneal cavity has minimal effect on total body resistivity in patients submitted to peritoneal dialysis, and assessments of fat mass and fat-free mass do not vary significantly whether these patients are dry or filled[88,89]. According to ascites pathophysiology, it is a clinical sign that the patient presents diffuse water retention and not only in the abdomen. The controversial point is how much ascites can change BIA results.

To estimate this effect in cirrhotic patients with HE, we evaluated 50 patients with HE that were submitted to BIA and DEXA exams to compare the percentage of fat mass obtained in each exam (non-published data). The median of their Child-Pugh classification scores was 8 (Child-Pugh A/B/C = 11/33/6), 32% of them had ascites and 38% had edema. The mean fat mass according to BIA and DEXA exams were 29.3% ± 7.7% and 29.8% ± 7.1%, respectively. Pearson's r was used to test the correlation and Bland-Altman analysis to evaluate the agreement between the fat percentages obtained by BIA and DEXA[90]. Pearson correlation indicated good agreement (P = 0.0000882 and r = 0.526). However, we found some discrepancies when the differences were observed on the scatter graph and through Bland-Altman analysis (Figure 1). The results obtained by DEXA seem to be similar to the values previously found in cirrhotic patients without fluid retention (29.6 ± 9.2)[60]. Even so, it is important to notice that DEXA exams can also be affected by fluid alterations, as addressed below in this review[54,91].

These limitations and controversial issues must be pondered when a cirrhotic patient with HE has been evaluated, because the precision of BIA results could be affected by all the body changes induced by the liver disease. Nevertheless, when there is not a single sign of fluid retention that could be clinically detected, BIA can be used for predicting total body water in cirrhotic patients, but precision is somewhat worse than in normal conditions[92]. Taking in account these limitations, when more accurate methods are not available and the patient has no signals of fluid overload, we consider that BIA results are still valuable in cirrhotic patients with HE because phase angle and BCM convey prognostic information[72]. Finally, multi-frequency BIA should be

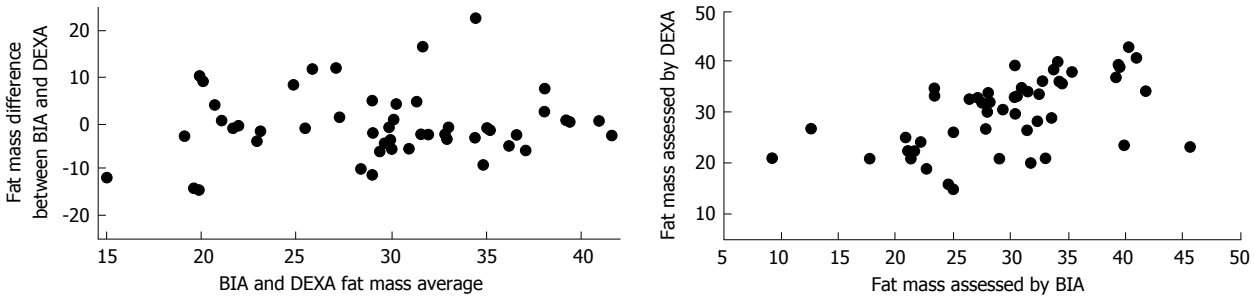

Figure 1 Bland-Altman analysis and scatter graph of fat mass percentages obtained from 50 cirrhotic patients with hepatic encephalopathy. Despite the correlation obtained between the two methods, the differences were also significant. DEXA: Dual-energy X-ray absorptiometry; BIA: Bioelectrical impedance analysis.

preferred in order to achieve more accurate results.

Handgrip strength

There is a growing interest in muscle activity evaluation for nutrition assessment. Walking tests and other tests requiring extensive collaboration could not be fully applied in patients stricken by severe illnesses like HE. Thus, handgrip strength (HS) can be a good option for patients with low-grade HE. Muscle strength is closely related to muscle activity, which is another valuable parameter for cirrhotic patients with HE, because HE tends to decrease physical activity.

A probable reason for decreased muscle activity in these patients is that hyperammonemia is related to fatigue, which is frequently reported in HE[19,93,94]. As previously mentioned in this review, in healthy adults the muscle role in ammonia clearance is minimal during rest, so the physical activity can be very important for ammonia metabolism. Accordingly, a previous study postulated that walking at least 5000 steps daily could be recommended for patients with compensated cirrhosis[95]. Although techniques of muscle activity evaluation are not considered to be methods of nutrition assessment, the information obtained would be of great value in patients with HE and more studies are needed in this setting.

HS has been suggested as a good indicator of nutritional status in liver disease patients by several guidelines[1,32,49]. The test is simple and a significant advantage is that the grip strength value is an independent predictor of cirrhosis decompensation[38,79,96,97]. As commented above, Merli *et al*[39] found that the prevalence of overt HE was higher in patients with decreased HS, suggesting a connection between strength and HE.

However, according to the ISHEN Consensus, skeletal muscle function is well associated with muscle mass only in men, and handgrip dynamometry would not be a reliable tool for appraising nutritional status in cirrhotic women. The degree of muscle loss caused by liver disease is different in each gender[32].

DEXA

The DEXA exam has received special attention because it is widely used to validate the results of body composition obtained by other methods, such as anthropometry and BIA. The procedure is based on measurement of body composition according to a model dividing the body elements in bone, fat, lean and bone-free lean masses, which can be distinct according to the energy photons passing through the body[98]. Additionally, radiation exposure is minimal with this technique[54,99]. The current guidelines recommend DEXA as a specific method for the diagnosis of malnutrition in liver diseases[1,32,49].

The DEXA exam allows assessment of fat-free mass and lean mass, which are technically different. Fat-free mass is composed by non-fat constituents of the body, including a small part of the adipose tissue. On the other hand, lean mass is composed by non-adipose constituents, which includes the lipids found in the nervous tissue and in cell membranes[100].

Riggio *et al*[101] evaluated 22 cirrhotic patients without ascites and 16 matched healthy controls using the DEXA exam. The authors found that the patterns of soft tissue loss in cirrhotic patients vary according to the gender. In women, the fat stores are more reduced, while lean tissue is maintained, as in early starvation. In men, the loss of lean tissue is more prominent, as seen under conditions of stress[101]. This finding could explain the poor correlation between muscle mass and muscle strength in cirrhotic women, making HS not so adequate for evaluating cirrhotic women according to the ISHEN Consensus[32]. Furthermore, it is in agreement with the finding of a prior study in which MAMC values were dramatically more affected in male than in female cirrhotic patients, while loss of fat deposits based on TSF was more significant in females, as formerly presented in this review[48].

Another study employed DEXA to evaluate 53 cirrhotic subjects, of whom only 30 seemed to be free of fluid retention. Results obtained by DEXA were compared with measurements from total body potassium, BIA and skinfold anthropometry. The authors found a good association between total body fat obtained from anthropometry and DEXA, regardless the existence of ascites. Even so, they found some discrepancy between DEXA and the other methods[102].

According to a prior review about the role of DEXA in the evaluation of cirrhotic patients, one of the main advantages of this exam is the high reproducibility of the measurements, with fat variations of less than 1% in

normal individuals. Accuracy errors are minor, amounting to 1.5% for quantifying lean mass. Additionally, the findings estimate the body compartments properly. Even so, a disadvantage is that fluid imbalances can change the X-ray passage, leading to an inappropriate appraisal of the lean mass[54].

DEXA allows obtainment of the ratio of the lean mass content divided by the square of the height, which is named fat-free mass index (FFMI). In a study of 108 cirrhotic liver transplant candidates, muscle wasting detected by FFMI was an independent predictor of HE[103]. Estimating the content of body segments (upper and lower limbs and trunk), DEXA can also be used to calculate the appendicular muscle mass index (AMMI), which is obtained by dividing the sum of appendicular muscle mass of the four members (free of fat and bone tissue) by the square of the height[100,104]. The AMMI provides a precise estimation of muscle mass because it does not use bone density, which varies with age, ethnicity, and response to drugs[100]. It also disregards the trunk mass, which is commonly affected by fluid retention in cirrhotic patients.

According to AMMI values, the diagnosis of sarcopenia can be made when individuals present less than 2 standard deviations from the healthy adults. The cutoff points were established as 7.26 kg/m^2 and 5.45 kg/m^2 for men and women, respectively[43,44,104]. In the presence of sarcopenia associated with fat mass percentages higher than 27% for men or higher than 38% for women, the diagnosis of sarcopenic obesity can be made[44,104,105]. AMMI is supposed to be very useful in studies about cirrhosis because it is not altered by fluid retention nor overweight status, focusing only on the fat-free mass[95,106].

Additionally, DEXA screening is also important for assessing bone mass before the incidence of fractures in patients with cirrhosis, because bone mass reduction is frequent among them. Thus, AMMI and body composition analysis can be obtained in the same exam performed to evaluate the bone mass.

Computed tomography scan

Recently, there has been a growing interest in employing a computed tomography (CT) scan to assess cirrhosis-related lean mass depletion. One of the reasons is that cirrhotic patients are commonly submitted to this exam to evaluate liver nodules or other alterations previously detected by ultrasonography screening exams. In addition, CT can also be used to evaluate portosystemic shunts in HE patients in order to plan hemodynamic corrections, and another advantage is that CT allows obtainment of information on abdominal muscles that are not commonly accessible. In theory, it could be possible to assess body composition retrospectively from images previously registered. For instance, in a recent CT-based study to assess nutritional status of cirrhotic patients who are candidates for OLT, the authors commented that the CT scan is often implemented in such patients to appraise the blood vessels distribution into the liver, to study the biliary tree and for hepatocellular carcinoma screening[106]. Furthermore, another aim of employing CT to evaluate body composition is that the transversal muscle thickness is associated with prognosis in these patients[107,108].

The measures are recorded by a single cross-sectional image at the level of the third lumbar vertebra or between the vertebrae L3 and L4[106,109]. Skeletal muscle tissue can be identified among other tissues by density limits: A cutoff of 35 Hounsfield units (HU) is used to distinct muscle and fat, whereas the maximum of 150 HU is used to separate muscle and bones, although the former limit can vary between the studies[91,106].

Thus, the exam allows calculating the third lumbar vertebra skeletal muscle index (L3 SMI), established as the muscle area contained in this axial plane divided by the square of the height. The cutoffs for sarcopenia in cirrhotic patients are 42 cm^2/m^2 and 50 cm^2/m^2 for women and men, respectively[110].

Hanai *et al*[109], in a retrospective study of 130 cirrhotic patients submitted to CT exams, found that sarcopenia was significantly associated with mortality. Of interest, the authors suggested that the use of branched-chain amino acids could improve survival of such patients, although the latter finding should be evaluated in prospective studies[109].

The possibility of measurements using previous exams make this method very attractive as a means of bringing more precision to the evaluations of body composition in cirrhotic patients. However, the use of ionizing radiation, the costs and the fact that the patient needs to be removed to the CT equipment are considerable limitations, especially when applied to patients with HE and/or CT is proposed to be repeated during the patient follow-up.

To facilitate choosing appropriately among the methods presented above according to the HE grade, Figure 2 summarizes some of the main points discussed about each method in relation to its advantages and limitations.

Other methods of nutritional assessment

In addition to the aforementioned methods for the assessment of body composition, other multi-compartmental techniques have been applied to cirrhotic patients, especially for scientific research.

Strauss *et al*[111] analyzed the usefulness of DEXA compared with a multi-compartmental model as a gold standard technique for evaluating body composition in 198 cirrhotic patients. The fat-free mass in this model was calculated adding bone content obtained from DEXA plus body water quantified by D_2O dilution and whole body protein quantified by *in vivo* neutron activation analysis (IVNAA). Consequently, fat mass was obtained subtracting fat-free mass from body weight. DEXA showed good accuracy and proved to be a suitable technique to evaluate fat-free mass and fat mass. The only restriction was that DEXA could not provide details on the water amount inside of the fat-free mass. It is

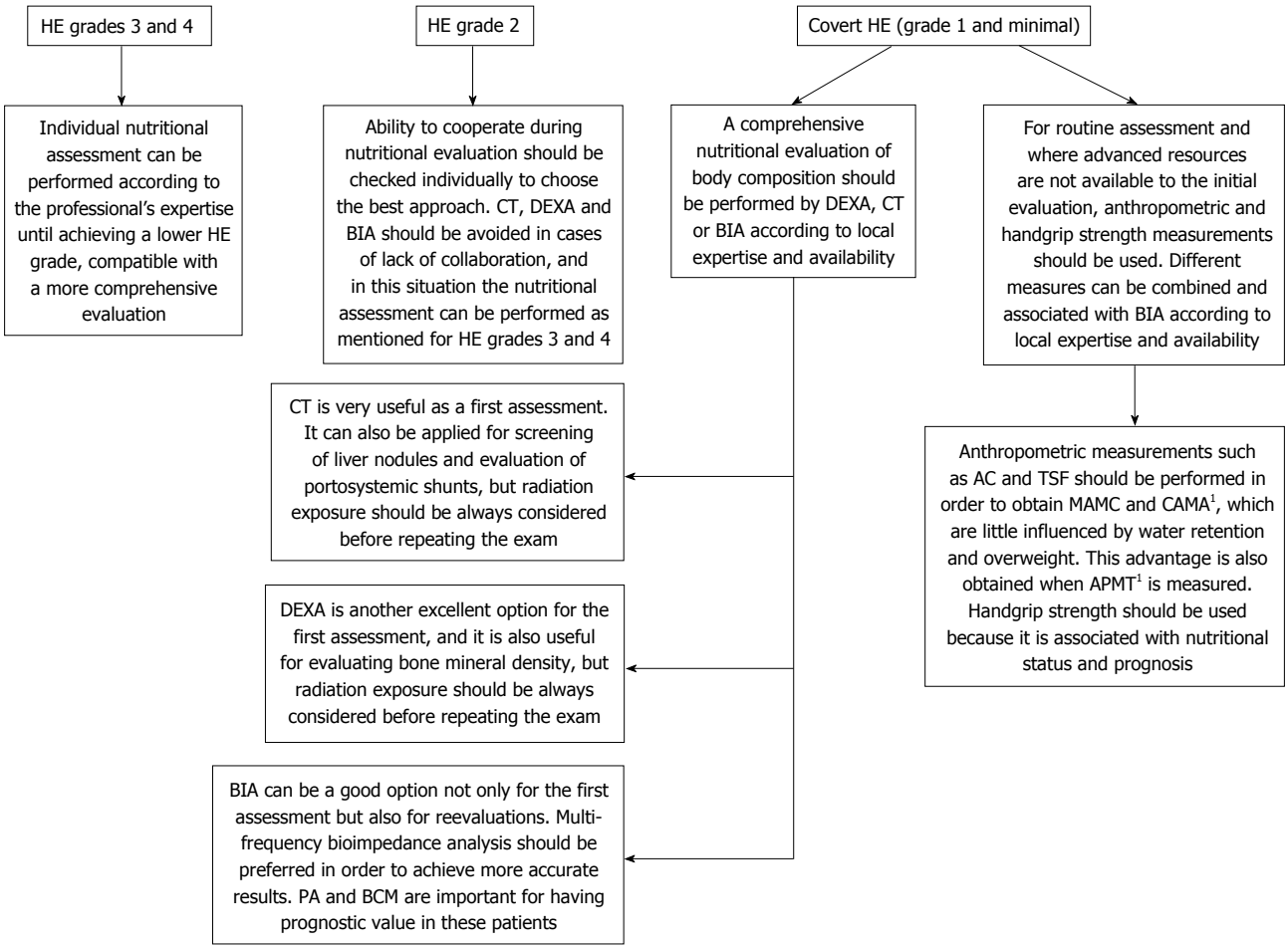

Figure 2 Proposal for choosing the best approaches during nutritional assessment of cirrhotic patients with hepatic encephalopathy. [1]APMT and CAMA are also associated with nutritional status in many conditions, but were not well evaluated in patients with cirrhosis and HE. AC: Arm circumference; APMT: Adductor pollicis muscle thickness; BCM: Body cell mass; BIA: Bioelectrical impedance analysis; CAMA: Corrected arm muscle area; CT: Computed tomography scan; DEXA: Dual-energy X-ray absorptiometry; HE: Hepatic encephalopathy; MAMC: Mid-arm muscle circumference; PA: Phase angle; TSF: Triceps skinfold.

noteworthy that these patients presented overhydrated fat-free mass in both sexes, especially in women[111].

Figueiredo *et al*[112], in a study of 79 cirrhotic patients and 17 controls, compared nutritional evaluation based on anthropometry, SGA, albumin and lymphocytes with a multi-compartmental model combining DEXA and methods of dilution spaces (bromide and deuterium). The multi-compartment model was composed by total bone mineral mass, total body fat, extracellular water and body cell mass. Accordingly, the sum of these four variables corresponded to the body weight. The authors found that the two-compartment assessment was not sufficiently accurate to diagnose malnutrition nor to estimate the severity of this complication in cirrhotic patients[112].

The guidelines from the European Society for Clinical Nutrition and Metabolism in liver disease published in 1997 and 2006 encourage the use of total body potassium count, IVNAA and isotope dilution for accurate quantification of changes in body composition in cirrhotic patients[49,113]. The ISHEN consensus also recommends the usage of magnetic resonance imaging. However, the guidelines warn that while these methods are not biased by hepatic impairment and fluid imbalance, they can involve radiation and can be difficult to repeat during the patient follow up[32,49,113]. Additionally, specific studies employing these other methods in HE patients are still lacking.

Finally, in spite of the usage of methods based on laboratory tests to estimate the degree of liver impairment of cirrhotic patients with HE, they are inadequate to assess their nutritional status. For instance, in such patients the creatinine height index is less accurate, because muscle wasting can reduce creatinine levels whereas renal impairment can increase them[114].

CONCLUSION

HE is associated with many pathophysiological changes induced by liver disease and influenced by nutrition, such as ammonia accumulation and muscle wasting. Likewise, nutritional status has a big impact on outcomes of HE patients. The alterations in body composition induced by liver cirrhosis and the limitations superimposed by HE hamper the obtainment of an accurate nutritional assessment of these patients by a simple test. Fluid

retention, metabolic alterations and depletion in muscle and fat deposits should be carefully investigated, whereas sufficient knowledge on the advantages and drawbacks of the main methods used in this population is essential to achieve consistent results by combining different techniques to assemble detailed data. Furthermore, for each method applied, it is important to recognize which measures are more trustworthy and which should be interpreted with caution due to possible bias. Prior studies in cirrhotic populations indicated that some anthropometric, BIA, DEXA and CT data convey prognostic information, whereas other measures remain controversial when obtained in patients with fluid overload. Most of these studies in cirrhotic patients did not mention the HE rate among the subjects included, so that specific data from patients with HE are scarce.

Knowledge on HE pathophysiology and evidence of the prognostic impact of muscle wasting on cirrhosis make muscle mass and muscle function some of the main points to evaluate in cirrhotic patients with HE. Ideally, a comprehensive assessment should gather information on food intake, anthropometric data not influenced by fluid overload, measurement of lean mass and tests of muscle function. The addition of complex techniques to measure body composition in HE patients increases the need for patient collaboration, the time spent, the demand for health professionals and the cost, but is useful to validate bedside measurements and should be encouraged. According to the local availability of each method and the patient condition, nutritional evaluation should be frequent during the patient follow-up, but the need to remove patients to the equipment room, the exposure to ionizing radiation and the costs can make the repetition of some of these methods less attractive. Therefore, some techniques can be combined to offer HE patients the safest and most cost-effective options, while maintaining the best accuracy in each nutritional evaluation.

REFERENCES

1 **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]

2 **Poordad FF**. Review article: the burden of hepatic encephalopathy. *Aliment Pharmacol Ther* 2007; **25** Suppl 1: 3-9 [PMID: 17295846 DOI: 10.1111/j.1746-6342.2006.03215.x]

3 **Bass NM**. Review article: the current pharmacological therapies for hepatic encephalopathy. *Aliment Pharmacol Ther* 2007; **25** Suppl 1: 23-31 [PMID: 17295849]

4 **Ortiz M**, Jacas C, Córdoba J. Minimal hepatic encephalopathy: diagnosis, clinical significance and recommendations. *J Hepatol* 2005; **42** Suppl: S45-S53 [PMID: 15777572 DOI: 10.1016/j.jhep.2004.11.028]

5 **Moriwaki H**, Shiraki M, Iwasa J, Terakura Y. Hepatic encephalopathy as a complication of liver cirrhosis: an Asian perspective. *J Gastroenterol Hepatol* 2010; **25**: 858-863 [PMID: 20546438 DOI: 10.1111/j.1440-1746.2010.06242.x]

6 **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]

7 **Stewart CA**, Malinchoc M, Kim WR, Kamath PS. Hepatic encephalopathy as a predictor of survival in patients with end-stage liver disease. *Liver Transpl* 2007; **13**: 1366-1371 [PMID: 17520742 DOI: 10.1002/lt.21129]

8 **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]

9 **Montagnese S**, De Rui M, Schiff S, Ceranto E, Valenti P, Angeli P, Cillo U, Zanus G, Gatta A, Amodio P, Merkel C. Prognostic benefit of the addition of a quantitative index of hepatic encephalopathy to the MELD score: the MELD-EEG. *Liver Int* 2015; **35**: 58-64 [PMID: 24517387 DOI: 10.1111/liv.12490]

10 **Yun BC**, Kim WR. Hyponatremia in hepatic encephalopathy: an accomplice or innocent bystander? *Am J Gastroenterol* 2009; **104**: 1390-1391 [PMID: 19455127 DOI: 10.1038/ajg.2009.287]

11 **Alberino F**, Gatta A, Amodio P, Merkel C, Di Pascoli L, Boffo G, Caregaro L. Nutrition and survival in patients with liver cirrhosis. *Nutrition* 2001; **17**: 445-450 [PMID: 11399401 DOI: 10.1016/S0899-9007(01)00521-4]

12 **Fialla AD**, Israelsen M, Hamberg O, Krag A, Gluud LL. Nutritional therapy in cirrhosis or alcoholic hepatitis: a systematic review and meta-analysis. *Liver Int* 2015; **35**: 2072-2078 [PMID: 25645300 DOI: 10.1111/liv.12798]

13 **Ney M**, Abraldes JG, Ma M, Belland D, Harvey A, Robbins S, Den Heyer V, Tandon P. Insufficient Protein Intake Is Associated With Increased Mortality in 630 Patients With Cirrhosis Awaiting Liver Transplantation. *Nutr Clin Pract* 2015; **30**: 530-536 [PMID: 25667232 DOI: 10.1177/0884533614567716]

14 **Ruiz-Margáin A**, Macías-Rodríguez RU, Duarte-Rojo A, Ríos-Torres SL, Espinosa-Cuevas Á, Torre A. Malnutrition assessed through phase angle and its relation to prognosis in patients with compensated liver cirrhosis: a prospective cohort study. *Dig Liver Dis* 2015; **47**: 309-314 [PMID: 25618555 DOI: 10.1016/j.dld.2014.12.015]

15 **Waghray A**, Waghray N, Kanna S, Mullen K. Optimal treatment of hepatic encephalopathy. *Minerva Gastroenterol Dietol* 2014; **60**: 55-70 [PMID: 24632768]

16 **Walker V**. Ammonia metabolism and hyperammonemic disorders. *Adv Clin Chem* 2014; **67**: 73-150 [PMID: 25735860 DOI: 10.1016/bs.acc.2014.09.002]

17 **Romero-Gómez M**, Montagnese S, Jalan R. Hepatic encephalopathy in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. *J Hepatol* 2015; **62**: 437-447 [PMID: 25218789 DOI: 10.1016/j.jhep.2014.09.005]

18 **Al Sibae MR**, McGuire BM. Current trends in the treatment of hepatic encephalopathy. *Ther Clin Risk Manag* 2009; **5**: 617-626 [PMID: 19707277 DOI: 10.2147/TCRM.S4443]

19 **Wilkinson DJ**, Smeeton NJ, Watt PW. Ammonia metabolism, the brain and fatigue; revisiting the link. *Prog Neurobiol* 2010; **91**: 200-219 [PMID: 20138956 DOI: 10.1016/j.pneurobio.2010.01.012]

20 **Cooper AJ**. The role of glutamine synthetase and glutamate dehydrogenase in cerebral ammonia homeostasis. *Neurochem Res* 2012; **37**: 2439-2455 [PMID: 22618691 DOI: 10.1007/s11064-012-0803-4]

21 **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]

22 **Wunsch E**, Koziarska D, Milkiewicz M, Naprawa G, Nowacki P, Hartleb M, Milkiewicz P. In patients with liver cirrhosis, proinflammatory interleukins correlate with health-related quality of life irrespective of minimal hepatic encephalopathy. *Eur J Gastroenterol Hepatol* 2013; **25**: 1402-1407 [PMID: 24025977 DOI: 10.1097/MEG.0b013e328365a447]

23 **Romeiro FG**, da Silva Yamashiro F, Américo MF, Corá LA, Silva GF, Miranda JR, Caramori CA. Erythromycin versus neomycin in the treatment of hepatic encephalopathy in cirrhosis: a randomized double-blind study. *BMC Gastroenterol* 2013; **13**: 13 [PMID:

23324408 DOI: 10.1186/1471-230X-13-13]
24 **Alempijević T**, Štulić M, Popovic D, Culafic D, Dragasevic S, Milosavljevic T. The role of fecal calprotectin in assessment of hepatic encephalopathy in patients with liver cirrhosis. *Acta Gastroenterol Belg* 2014; **77**: 302-305 [PMID: 25509200]
25 **Bajaj JS**, Betrapally NS, Hylemon PB, Heuman DM, Daita K, White MB, Unser A, Thacker LR, Sanyal AJ, Kang DJ, Sikaroodi M, Gillevet PM. Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. *Hepatology* 2015; **62**: 1260-1271 [PMID: 25820757 DOI: 10.1002/hep.27819]
26 **Nolan JP**. The role of intestinal endotoxin in liver injury: a long and evolving history. *Hepatology* 2010; **52**: 1829-1835 [PMID: 20890945 DOI: 10.1002/hep.23917]
27 **Liu Q**, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004; **39**: 1441-1449 [PMID: 15122774 DOI: 10.1002/hep.20194]
28 **Gupta A**, Dhiman RK, Kumari S, Rana S, Agarwal R, Duseja A, Chawla Y. Role of small intestinal bacterial overgrowth and delayed gastrointestinal transit time in cirrhotic patients with minimal hepatic encephalopathy. *J Hepatol* 2010; **53**: 849-855 [PMID: 20675008 DOI: 10.1016/j.jhep.2010.05.017]
29 **John S**, Thuluvath PJ. Hyponatremia in cirrhosis: pathophysiology and management. *World J Gastroenterol* 2015; **21**: 3197-3205 [PMID: 25805925 DOI: 10.3748/wjg.v21.i11.3197]
30 **Abu Hossain S**, Chaudhry FA, Zahedi K, Siddiqui F, Amlal H. Cellular and molecular basis of increased ammoniagenesis in potassium deprivation. *Am J Physiol Renal Physiol* 2011; **301**: F969-F978 [PMID: 21795646 DOI: 10.1152/ajprenal.00010.2011]
31 **Yang SS**, Lai YC, Chiang TR, Chen DF, Chen DS. Role of zinc in subclinical hepatic encephalopathy: comparison with somatosensory-evoked potentials. *J Gastroenterol Hepatol* 2004; **19**: 375-379 [PMID: 15012773 DOI: 10.1111/j.1440-1746.2003.03281.x]
32 **Amodio P**, Bemeur C, Butterworth R, Cordoba J, Kato A, Montagnese S, Uribe M, Vilstrup H, Morgan MY. The nutritional management of hepatic encephalopathy in patients with cirrhosis: International Society for Hepatic Encephalopathy and Nitrogen Metabolism Consensus. *Hepatology* 2013; **58**: 325-336 [PMID: 23471642 DOI: 10.1002/hep.26370]
33 **Romeiro FG**, Américo MF, Yamashiro FS, Caramori CA, Schelp AO, Santos AC, Silva GF. Acquired hepatocerebral degeneration and hepatic encephalopathy: correlations and variety of clinical presentations in overt and subclinical liver disease. *Arq Neuropsiquiatr* 2011; **69**: 496-501 [PMID: 21755129 DOI: 10.1590/S0004-282X2011000400017]
34 **Maffeo E**, Montuschi A, Stura G, Giordana MT. Chronic acquired hepatocerebral degeneration, pallidal T1 MRI hyperintensity and manganese in a series of cirrhotic patients. *Neurol Sci* 2014; **35**: 523-530 [PMID: 23712371 DOI: 10.1007/s10072-013-1458-x]
35 **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; **2**: CD001939 [PMID: 25715177 DOI: 10.1002/14651858.CD001939.pub2]
36 **Zhu GQ**, Shi KQ, Huang S, Wang LR, Lin YQ, Huang GQ, Chen YP, Braddock M, Zheng MH. Systematic review with network meta-analysis: the comparative effectiveness and safety of interventions in patients with overt hepatic encephalopathy. *Aliment Pharmacol Ther* 2015; **41**: 624-635 [PMID: 25684317 DOI: 10.1111/apt.13122]
37 **Kalaitzakis E**, Olsson R, Henfridsson P, Hugosson I, Bengtsson M, Jalan R, Björnsson E. Malnutrition and diabetes mellitus are related to hepatic encephalopathy in patients with liver cirrhosis. *Liver Int* 2007; **27**: 1194-1201 [PMID: 17919230 DOI: 10.1111/j.1478-3231.2007.01562.x]
38 **Huisman EJ**, Trip EJ, Siersema PD, van Hoek B, van Erpecum KJ. Protein energy malnutrition predicts complications in liver cirrhosis. *Eur J Gastroenterol Hepatol* 2011; **23**: 982-989 [PMID: 21971339 DOI: 10.1097/MEG.0b013e32834aa4bb]
39 **Merli M**, Giusto M, Lucidi C, Giannelli V, Pentassuglio I, Di Gregorio V, Lattanzi B, Riggio O. Muscle depletion increases the risk of overt and minimal hepatic encephalopathy: results of a prospective study. *Metab Brain Dis* 2013; **28**: 281-284 [PMID: 23224378 DOI: 10.1007/s11011-012-9365-z]
40 **Periyalwar P**, Dasarathy S. Malnutrition in cirrhosis: contribution and consequences of sarcopenia on metabolic and clinical responses. *Clin Liver Dis* 2012; **16**: 95-131 [PMID: 22321468 DOI: 10.1016/j.cld.2011.12.009]
41 **Berzigotti A**, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Morillas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Groszmann RJ. Obesity is an independent risk factor for clinical decompensation in patients with cirrhosis. *Hepatology* 2011; **54**: 555-561 [PMID: 21567436 DOI: 10.1002/hep.24418]
42 **Everhart JE**, Lok AS, Kim HY, Morgan TR, Lindsay KL, Chung RT, Bonkovsky HL, Ghany MG; HALT-C Trial Group. Weight-related effects on disease progression in the hepatitis C antiviral long-term treatment against cirrhosis trial. *Gastroenterology* 2009; **137**: 549-557 [PMID: 19445938 DOI: 10.1053/j.gastro.2009.05.007]
43 **Jeejeebhoy KN**. Malnutrition, fatigue, frailty, vulnerability, sarcopenia and cachexia: overlap of clinical features. *Curr Opin Clin Nutr Metab Care* 2012; **15**: 213-219 [PMID: 22450775 DOI: 10.1097/MCO.0b013e328352694f]
44 **Prado CM**, Wells JC, Smith SR, Stephan BC, Siervo M. Sarcopenic obesity: A Critical appraisal of the current evidence. *Clin Nutr* 2012; **31**: 583-601 [PMID: 22809635 DOI: 10.1016/j.clnu.2012.06.010]
45 **Dupont B**, Dao T, Joubert C, Dupont-Lucas C, Gloro R, Nguyen-Khac E, Beaujard E, Mathurin P, Vastel E, Musikas M, Ollivier I, Piquet MA. Randomised clinical trial: enteral nutrition does not improve the long-term outcome of alcoholic cirrhotic patients with jaundice. *Aliment Pharmacol Ther* 2012; **35**: 1166-1174 [PMID: 22452620 DOI: 10.1111/j.1365-2036.2012.05075.x]
46 **Campillo B**, Richardet JP, Scherman E, Bories PN. Evaluation of nutritional practice in hospitalized cirrhotic patients: results of a prospective study. *Nutrition* 2003; **19**: 515-521 [PMID: 12781851 DOI: 10.1016/S0899-9007(02)01071-7]
47 **Roongpisuthipong C**, Sobhonslidsuk A, Nantiruj K, Songchit-somboon S. Nutritional assessment in various stages of liver cirrhosis. *Nutrition* 2001; **17**: 761-765 [PMID: 11527674 DOI: 10.1016/S0899-9007(01)00626-8]
48 **Carvalho L**, Parise ER. Evaluation of nutritional status of nonhospitalized patients with liver cirrhosis. *Arq Gastroenterol* 2006; **43**: 269-274 [PMID: 17406753 DOI: 10.1590/S0004-28032006000400005]
49 **Plauth M**, Cabré E, Riggio O, Assis-Camilo M, Pirlich M, Kondrup J, Ferenci P, Holm E, Vom Dahl S, Müller MJ, Nolte W. ESPEN Guidelines on Enteral Nutrition: Liver disease. *Clin Nutr* 2006; **25**: 285-294 [PMID: 16707194 DOI: 10.1016/j.clnu.2006.01.018]
50 **Cheung K**, Lee SS, Raman M. Prevalence and mechanisms of malnutrition in patients with advanced liver disease, and nutrition management strategies. *Clin Gastroenterol Hepatol* 2012; **10**: 117-125 [PMID: 21893127 DOI: 10.1016/j.cgh.2011.08.016]
51 **Moctezuma-Velázquez C**, García-Juárez I, Soto-Solís R, Hernández-Cortés J, Torre A. Nutritional assessment and treatment of patients with liver cirrhosis. *Nutrition* 2013; **29**: 1279-1285 [PMID: 23867207 DOI: 10.1016/j.nut.2013.03.017]
52 **McCullough AJ**, Mullen KD, Kalhan SC. Measurements of total body and extracellular water in cirrhotic patients with and without ascites. *Hepatology* 1991; **14**: 1102-1111 [PMID: 1959861 DOI: 10.1002/hep.1840140626]
53 **Oldroyd B**, Bramley PN, Stewart SP, Simpson M, Truscott JG, Losowsky MS, Smith MA. A four-compartment model to determine body composition in liver cirrhosis. *Basic Life Sci* 1993; **60**: 221-224 [PMID: 8110115 DOI: 10.1007/978-1-4899-1268-8_51]
54 **Madden AM**, Morgan MY. The potential role of dual-energy X-ray absorptiometry in the assessment of body composition in cirrhotic patients. *Nutrition* 1997; **13**: 40-45 [PMID: 9058447 DOI: 10.1016/S0899-9007(97)90877-7]
55 **Chadalavada R**, Sappati Biyyani RS, Maxwell J, Mullen K. Nutrition in hepatic encephalopathy. *Nutr Clin Pract* 2010; **25**:

257-264 [PMID: 20581319 DOI: 10.1177/0884533610368712]
56 **Morgan MY**, Madden AM, Jennings G, Elia M, Fuller NJ. Two-component models are of limited value for the assessment of body composition in patients with cirrhosis. *Am J Clin Nutr* 2006; **84**: 1151-1162 [PMID: 17093169]
57 **Detsky AS**, McLaughlin JR, Baker JP, Johnston N, Whittaker S, Mendelson RA, Jeejeebhoy KN. What is subjective global assessment of nutritional status? *JPEN J Parenter Enteral Nutr* 1987; **11**: 8-13 [PMID: 3820522 DOI: 10.1177/014860718701100108]
58 **Matos C**, Porayko MK, Francisco-Ziller N, DiCecco S. Nutrition and chronic liver disease. *J Clin Gastroenterol* 2002; **35**: 391-397 [PMID: 12394227 DOI: 10.1097/00004836-200211000-00007]
59 **Campillo B**, Richardet JP, Bories PN. Validation of body mass index for the diagnosis of malnutrition in patients with liver cirrhosis. *Gastroenterol Clin Biol* 2006; **30**: 1137-1143 [PMID: 17075467]
60 **Fiore P**, Merli M, Andreoli A, De Lorenzo A, Masini A, Ciuffa L, Valeriano V, Balotta MT, Riggio O. A comparison of skinfold anthropometry and dual-energy X-ray absorptiometry for the evaluation of body fat in cirrhotic patients. *Clin Nutr* 1999; **18**: 349-351 [PMID: 10634919 DOI: 10.1016/S0261-5614(99)80014-4]
61 **Guex E**, Cheseaux M, Bertrand PC, Piquet MA, Bouvry S, Pilon N, Halkic N, Pascual M, Roulet M. [Prevalence of undernutrition in 143 patients before liver transplantation. The Lausanne experience between 1997 and 2005]. *Rev Med Suisse* 2008; **4**: 927-930 [PMID: 18578434]
62 **Harrison J**, McKiernan J, Neuberger JM. A prospective study on the effect of recipient nutritional status on outcome in liver transplantation. *Transpl Int* 1997; **10**: 369-374 [PMID: 9287402 DOI: 10.1111/j.1432-2277.1997.tb00931.x]
63 **Morgan MY**, Madden AM, Soulsby CT, Morris RW. Derivation and validation of a new global method for assessing nutritional status in patients with cirrhosis. *Hepatology* 2006; **44**: 823-835 [PMID: 17006918 DOI: 10.1002/hep.21358]
64 **Bémeur C**, Desjardins P, Butterworth RF. Role of nutrition in the management of hepatic encephalopathy in end-stage liver failure. *J Nutr Metab* 2010; **2010**: 489823 [PMID: 21234351 DOI: 10.1155/2010/489823]
65 **Lameu EB**, Gerude MF, Campos AC, Luiz RR. The thickness of the adductor pollicis muscle reflects the muscle compartment and may be used as a new anthropometric parameter for nutritional assessment. *Curr Opin Clin Nutr Metab Care* 2004; **7**: 293-301 [PMID: 15075921 DOI: 10.1097/00075197-200405000-00009]
66 **de Oliveira CM**, Kubrusly M, Mota RS, Choukroun G, Neto JB, da Silva CA. Adductor pollicis muscle thickness: a promising anthropometric parameter for patients with chronic renal failure. *J Ren Nutr* 2012; **22**: 307-316 [PMID: 22056150 DOI: 10.1053/j.jrn.2011.07.006]
67 **Bering T**, Maurício SF, Silva JB, Correia MI. Nutritional and metabolic status of breast cancer women. *Nutr Hosp* 2015; **31**: 751-758 [PMID: 25617559 DOI: 10.3305/nh.2015.31.2.8056]
68 **Gonzalez MC**, Pureza Duarte RR, Orlandi SP, Bielemann RM, Barbosa-Silva TG. Adductor pollicis muscle: A study about its use as a nutritional parameter in surgical patients. *Clin Nutr* 2015; **34**: 1025-1029 [PMID: 25467064 DOI: 10.1016/j.clnu.2014.11.006]
69 **Thomas BJ**, Cornish BH, Ward LC. Bioelectrical impedance analysis for measurement of body fluid volumes: a review. *J Clin Eng* 1992; **17**: 505-510 [PMID: 10124463 DOI: 10.1097/00004669-199211000-00016]
70 **Pirlich M**, Schütz T, Spachos T, Ertl S, Weiss ML, Lochs H, Plauth M. Bioelectrical impedance analysis is a useful bedside technique to assess malnutrition in cirrhotic patients with and without ascites. *Hepatology* 2000; **32**: 1208-1215 [PMID: 11093726 DOI: 10.1053/jhep.2000.20524]
71 **Cabré E**, Gassull MA. Nutritional aspects of liver disease and transplantation. *Curr Opin Clin Nutr Metab Care* 2001; **4**: 581-589 [PMID: 11706297 DOI: 10.1097/00075197-200111000-00020]
72 **Selberg O**, Selberg D. Norms and correlates of bioimpedance phase angle in healthy human subjects, hospitalized patients, and patients with liver cirrhosis. *Eur J Appl Physiol* 2002; **86**: 509-516 [PMID: 11944099 DOI: 10.1007/s00421-001-0570-4]
73 **Norman K**, Kirchner H, Lochs H, Pirlich M. Malnutrition affects quality of life in gastroenterology patients. *World J Gastroenterol* 2006; **12**: 3380-3385 [PMID: 16733855 DOI: 10.3748/wjg.v12.i21.3385]
74 **Fernandes SA**, Bassani L, Nunes FF, Aydos ME, Alves AV, Marroni CA. Nutritional assessment in patients with cirrhosis. *Arq Gastroenterol* 2012; **49**: 19-27 [PMID: 22481682 DOI: 10.1590/S0004-28032012000100005]
75 **Peres WA**, Lento DF, Baluz K, Ramalho A. Phase angle as a nutritional evaluation tool in all stages of chronic liver disease. *Nutr Hosp* 2012; **27**: 2072-2078 [PMID: 23588459 DOI: 10.3305/nh.2012.27.6.6015]
76 **Kalafateli M**, Triantos C, Tsochatzis E, Michalaki M, Koutroumpakis E, Thomopoulos K, Kyriazopoulou V, Jelastopulu E, Burroughs A, Lambropoulou-Karatza C, Nikolopoulou V. Adipokines levels are associated with the severity of liver disease in patients with alcoholic cirrhosis. *World J Gastroenterol* 2015; **21**: 3020-3029 [PMID: 25780301 DOI: 10.3748/wjg.v21.i10.3020]
77 **Liboredo JC**, Vilela EG, Ferrari Mde L, Lima AS, Correia MI. Nutrition status and intestinal permeability in patients eligible for liver transplantation. *JPEN J Parenter Enteral Nutr* 2015; **39**: 163-170 [PMID: 24255087 DOI: 10.1177/0148607113513465]
78 **Selberg O**, Böttcher J, Tusch G, Pichlmayr R, Henkel E, Müller MJ. Identification of high- and low-risk patients before liver transplantation: a prospective cohort study of nutritional and metabolic parameters in 150 patients. *Hepatology* 1997; **25**: 652-657 [PMID: 9049214 DOI: 10.1002/hep.510250327]
79 **Figueiredo FA**, Dickson ER, Pasha TM, Porayko MK, Therneau TM, Malinchoc M, DiCecco SR, Francisco-Ziller NM, Kasparova P, Charlton MR. Utility of standard nutritional parameters in detecting body cell mass depletion in patients with end-stage liver disease. *Liver Transpl* 2000; **6**: 575-581 [PMID: 10980056 DOI: 10.1053/jlts.2000.9736]
80 **Müller MJ**, Lautz HU, Plogmann B, Bürger M, Körber J, Schmidt FW. Energy expenditure and substrate oxidation in patients with cirrhosis: the impact of cause, clinical staging and nutritional state. *Hepatology* 1992; **15**: 782-794 [PMID: 1568718 DOI: 10.1002/hep.1840150507]
81 **Madden AM**, Morgan MY. A comparison of skinfold anthropometry and bioelectrical impedance analysis for measuring percentage body fat in patients with cirrhosis. *J Hepatol* 1994; **21**: 878-883 [PMID: 7890906 DOI: 10.1016/S0168-8278(94)80253-X]
82 **Wu TJ**, Huang JJ, Lin CY. Effects of fluid retention on the measurement of body composition using bioelectric impedance. *J Formos Med Assoc* 1994; **93**: 939-943 [PMID: 7633198]
83 **Panella C**, Guglielmi FW, Mastronuzzi T, Francavilla A. Whole-body and segmental bioelectrical parameters in chronic liver disease: effect of gender and disease stages. *Hepatology* 1995; **21**: 352-358 [PMID: 7843705 DOI: 10.1002/hep.1840210214]
84 **Lehnert ME**, Clarke DD, Gibbons JG, Ward LC, Golding SM, Shepherd RW, Cornish BH, Crawford DH. Estimation of body water compartments in cirrhosis by multiple-frequency bioelectrical-impedance analysis. *Nutrition* 2001; **17**: 31-34 [PMID: 11165885 DOI: 10.1016/S0899-9007(00)00473-1]
85 **Guglielmi FW**, Contento F, Laddaga L, Panella C, Francavilla A. Bioelectric impedance analysis: experience with male patients with cirrhosis. *Hepatology* 1991; **13**: 892-895 [PMID: 2029993 DOI: 10.1002/hep.1840130515]
86 **Zillikens MC**, van den Berg JW, Wilson JH, Swart GR. Whole-body and segmental bioelectrical-impedance analysis in patients with cirrhosis of the liver: changes after treatment of ascites. *Am J Clin Nutr* 1992; **55**: 621-625 [PMID: 1550033]
87 **Sörös P**, Böttcher J, Weissenborn K, Selberg O, Müller MJ. Malnutrition and hypermetabolism are not risk factors for the presence of hepatic encephalopathy: a cross-sectional study. *J Gastroenterol Hepatol* 2008; **23**: 606-610 [PMID: 18005013 DOI: 10.1111/j.1440-1746.2007.05222.x]
88 **Böhm D**, Odaischi M, Beyerlein C, Overbeck W. Total body water: changes during dialysis estimated by bioimpedance analysis. *Infusionstherapie* 1990; **17** Suppl 3: 75-78 [PMID: 2401575]
89 **Kurtin PS**, Shapiro AC, Tomita H, Raizman D. Volume status and

body composition of chronic dialysis patients: utility of bioelectric impedance plethysmography. *Am J Nephrol* 1990; **10**: 363-367 [PMID: 2080787 DOI: 10.1159/000168151]

90 **Bland JM**, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**: 307-310 [PMID: 2868172 DOI: 10.1016/S0140-6736(86)90837-8]

91 **Montano-Loza AJ**. Clinical relevance of sarcopenia in patients with cirrhosis. *World J Gastroenterol* 2014; **20**: 8061-8071 [PMID: 25009378 DOI: 10.3748/wjg.v20.i25.8061]

92 **Zillikens MC**, van den Berg JW, Wilson JH, Rietveld T, Swart GR. The validity of bioelectrical impedance analysis in estimating total body water in patients with cirrhosis. *J Hepatol* 1992; **16**: 59-65 [PMID: 1484169 DOI: 10.1016/S0168-8278(05)80095-9]

93 **Malaguarnera M**, Vacante M, Giordano M, Pennisi G, Bella R, Rampello L, Malaguarnera M, Li Volti G, Galvano F. Oral acetyl-L-carnitine therapy reduces fatigue in overt hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. *Am J Clin Nutr* 2011; **93**: 799-808 [PMID: 21310833 DOI: 10.3945/ajcn.110.007393]

94 **Banister EW**, Cameron BJ. Exercise-induced hyperammonemia: peripheral and central effects. *Int J Sports Med* 1990; **11** Suppl 2: S129-S142 [PMID: 2193891 DOI: 10.1055/s-2007-1024864]

95 **Hayashi F**, Matsumoto Y, Momoki C, Yuikawa M, Okada G, Hamakawa E, Kawamura E, Hagihara A, Toyama M, Fujii H, Kobayashi S, Iwai S, Morikawa H, Enomoto M, Tamori A, Kawada N, Habu D. Physical inactivity and insufficient dietary intake are associated with the frequency of sarcopenia in patients with compensated viral liver cirrhosis. *Hepatol Res* 2013; **43**: 1264-1275 [PMID: 23489325 DOI: 10.1111/hepr.12085]

96 **Alvares-da-Silva MR**, Reverbel da Silveira T. Comparison between handgrip strength, subjective global assessment, and prognostic nutritional index in assessing malnutrition and predicting clinical outcome in cirrhotic outpatients. *Nutrition* 2005; **21**: 113-117 [PMID: 15723736 DOI: 10.1016/j.nut.2004.02.002]

97 **Peng S**, Plank LD, McCall JL, Gillanders LK, McIlroy K, Gane EJ. Body composition, muscle function, and energy expenditure in patients with liver cirrhosis: a comprehensive study. *Am J Clin Nutr* 2007; **85**: 1257-1266 [PMID: 17490961]

98 **Kohrt WM**. Body composition by DXA: tried and true? *Med Sci Sports Exerc* 1995; **27**: 1349-1353 [PMID: 8531604]

99 **Kim J**, Wang Z, Heymsfield SB, Baumgartner RN, Gallagher D. Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *Am J Clin Nutr* 2002; **76**: 378-383 [PMID: 12145010]

100 **Bosy-Westphal A**, Müller MJ. Identification of skeletal muscle mass depletion across age and BMI groups in health and disease--there is need for a unified definition. *Int J Obes* (Lond) 2015; **39**: 379-386 [PMID: 25174451 DOI: 10.1038/ijo.2014.161]

101 **Riggio O**, Andreoli A, Diana F, Fiore P, Meddi P, Lionetti R, Montagnese F, Merli M, Capocaccia L, De Lorenzo A. Whole body and regional body composition analysis by dual-energy X-ray absorptiometry in cirrhotic patients. *Eur J Clin Nutr* 1997; **51**: 810-814 [PMID: 9426355]

102 **Bramley P**, Oldroyd B, Stewart S, Simpson M, Truscott J, Losowsky M, Smith M. Body composition analysis in liver cirrhosis. The measurement of body fat by dual energy X-ray absorptiometry in comparison to skinfold anthropometry, bioelectrical impedance and total body potassium. *Basic Life Sci* 1993; **60**: 211-214 [PMID: 8110112 DOI: 10.1007/978-1-4899-1268-8_48]

103 **Kalaitzakis E**, Josefsson A, Castedal M, Henfridsson P, Bengtsson M, Andersson B, Björnsson E. Hepatic encephalopathy is related to anemia and fat-free mass depletion in liver transplant candidates with cirrhosis. *Scand J Gastroenterol* 2013; **48**: 577-584 [PMID: 23452072 DOI: 10.3109/00365521.2013.777468]

104 **Baumgartner RN**, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD. Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* 1998; **147**: 755-763 [PMID: 9554417 DOI: 10.1093/oxfordjournals.aje.a009520]

105 **Baumgartner RN**. Body composition in healthy aging. *Ann N Y Acad Sci* 2000; **904**: 437-448 [PMID: 10865787 DOI: 10.1111/j.1749-6632.2000.tb06498.x]

106 **Giusto M**, Lattanzi B, Albanese C, Galtieri A, Farcomeni A, Giannelli V, Lucidi C, Di Martino M, Catalano C, Merli M. Sarcopenia in liver cirrhosis: the role of computed tomography scan for the assessment of muscle mass compared with dual-energy X-ray absorptiometry and anthropometry. *Eur J Gastroenterol Hepatol* 2015; **27**: 328-334 [PMID: 25569567 DOI: 10.1097/MEG.0000000000000274]

107 **Montano-Loza AJ**, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, Sawyer MB. Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2012; **10**: 166-173, 173.e1 [PMID: 21893129 DOI: 10.1016/j.cgh.2011.08.028]

108 **Durand F**, Buyse S, Francoz C, Laouénan C, Bruno O, Belghiti J, Moreau R, Vilgrain V, Valla D. Prognostic value of muscle atrophy in cirrhosis using psoas muscle thickness on computed tomography. *J Hepatol* 2014; **60**: 1151-1157 [PMID: 24607622 DOI: 10.1016/j.jhep.2014.02.026]

109 **Hanai T**, Shiraki M, Nishimura K, Ohnishi S, Imai K, Suetsugu A, Takai K, Shimizu M, Moriwaki H. Sarcopenia impairs prognosis of patients with liver cirrhosis. *Nutrition* 2015; **31**: 193-199 [PMID: 25441595 DOI: 10.1016/j.nut.2014.07.005]

110 **Montano-Loza AJ**, Meza-Junco J, Prado CMM, Tandon P, Bain VG, Ma M, Beaumont C, Esfandiari N, Sawyer MB, Baracos VE. New cutoff values for sarcopenia for predicting 6-months mortality in cirrhotic patients. *J Hepatol* 2013; **58**: S95 [DOI: 10.1016/S0168-8278(13)60223-8]

111 **Strauss BJ**, Gibson PR, Stroud DB, Borovnicar DJ, Xiong DW, Keogh J. Total body dual X-ray absorptiometry is a good measure of both fat mass and fat-free mass in liver cirrhosis compared to "gold-standard" techniques. Melbourne Liver Group. *Ann N Y Acad Sci* 2000; **904**: 55-62 [PMID: 10865710 DOI: 10.1111/j.1749-6632.2000.tb06421.x]

112 **Figueiredo FA**, Perez RM, Freitas MM, Kondo M. Comparison of three methods of nutritional assessment in liver cirrhosis: subjective global assessment, traditional nutritional parameters, and body composition analysis. *J Gastroenterol* 2006; **41**: 476-482 [PMID: 16799890 DOI: 10.1007/s00535-006-1794-1]

113 **Plauth M**, Merli M, Kondrup J, Weimann A, Ferenci P, Müller MJ. ESPEN guidelines for nutrition in liver disease and transplantation. *Clin Nutr* 1997; **16**: 43-55 [PMID: 16844569 DOI: 10.1016/S0261-5614(97)80022-2]

114 **Francoz C**, Prié D, Abdelrazek W, Moreau R, Mandot A, Belghiti J, Valla D, Durand F. Inaccuracies of creatinine and creatinine-based equations in candidates for liver transplantation with low creatinine: impact on the model for end-stage liver disease score. *Liver Transpl* 2010; **16**: 1169-1177 [PMID: 20879015 DOI: 10.1002/lt.22128]

P- Reviewer: Montagnese S, Sargsyants N
S- Editor: Kong JX **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.i30.2955

World J Hepatol 2015 December 28; 7(30): 2955-2961
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis B Virus

Hepatitis B virus infection in immigrant populations

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

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

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

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

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

Author contributions: All the authors contributed to conception and design, acquisition of data, analysis and interpretation of data, and final approval of the version to be published.

Conflict-of-interest statement: All the authors of the manuscript declare that they have no commercial, personal, political, intellectual or religious interests in connection with this paper.

Correspondence to: Nicola Coppola, MD, PhD, Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, Via L. Armanni 5, 80131 Naples, Italy. nicola.coppola@unina2.it
Telephone: +39-81-5666719
Fax: +39-81-5666013

Received: April 22, 2015
Peer-review started: April 23, 2015
First decision: August 31, 2015
Revised: November 4, 2015
Accepted: December 4, 2015
Article in press: December 8, 2015
Published online: December 28, 2015

Abstract

Hepatitis B virus (HBV) is the most common cause of hepatitis worldwide, with nearly 350 million people chronically infected and 600000 deaths per year due to acute liver failure occurring during acute hepatitis or, more frequently, in HBV-related liver cirrhosis or hepatocellular carcinoma. Ongoing immigration from countries with a high HBV endemicity to those with a low HBV endemicity warrants particular attention to prevent the spread of HBV infection to the native population. This review article analyzes the epidemiology and virological and clinical characteristics of HBV infection in immigrant populations and in their host countries, and suggests prophylactic measures to prevent the spread of this infection. Among the immigrants from different geographical areas, those from South East Asia and sub-Saharan Africa show the highest prevalences of hepatitis B surface antigen (HBsAg) carriers, in accordance with the high endemicity of the countries of origin. The molecular characteristics of HBV infection in immigrants reflect those of the geographical areas of origin: HBV genotype A and D predominate in immigrants from Eastern Europe, B and C in those from Asia and genotype E in those from Africa. The literature data on the clinical course and treatment of HBsAg-positive immigrants are scanty. The management of HBV infection in immigrant populations is difficult and requires expert personnel and dedicated structures for their assistance. The social services, voluntary operators and cultural mediators are essential to achieve optimized psychological and clinical intervention.

Key words: Hepatitis B virus infection; Chronic hepatitis B; Immigration; Immigrants; Developing countries

Core tip: Extensive immigration from countries with a high hepatitis B virus (HBV) endemicity to those with a low HBV diffusion warrants particular attention to prevent the spread of HBV infection to the native population. This review article analyzes: (1) the prevalence of subjects with hepatitis B in screened immigrants; (2) the distribution of the HBV genotypes; (3) the cost effectiveness of screening immigrants for hepatitis B; and (4) the clinical and therapeutic approach.

Coppola N, Alessio L, Pisaturo M, Macera M, Sagnelli C, Zampino R, Sagnelli E. Hepatitis B virus infection in immigrant populations. *World J Hepatol* 2015; 7(30): 2955-2961 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i30/2955.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i30.2955

INTRODUCTION

Due to numerous socio-economic and political crises that have occurred in Africa, Eastern Europe, Asia and South and Central America in recent decades, Western countries have become lands of immigration for citizens of these sub-continents. Immigrants are frequently poor, out of work, carry on the cultural and religious traditions of their country of origin and do not speak the language of the host country. One of the most important forces driving young and middle-aged adults to emigrate to a western country in search of work is the low income in their countries of origin. Consequently, the typical immigrant is a healthy young male who leaves his country in the hope of new opportunities to improve his living conditions[1]. This phenomenon is called "healthy migrant effect", a kind of self-selection whereby only those subjects in good physical condition who are young and with enough initiative and psychological stability emigrate. For these subjects their good health is the only certainty to invest in their own future and in that of their family. Although in good clinical conditions, these subjects may carry asymptomatic chronic infections, such as those related to hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV), all widespread in low-income countries. In addition, political reasons often favor the flow of immigrants, such as dictatorships, persecution, war and genocide, all of which induce numerous families to seek freedom outside their country.

The immigrant populations frequently move from geographical areas with intermediate or high HBV, HCV and HIV endemicity to countries where these infections have a low endemicity level. The high or intermediate endemicity in low-income countries is most frequently a consequence of scanty knowledge of the routes of transmission of these infections, due to a low level of schooling and difficulty obtaining information from the media[2]. In addition, tribal rituals may favor the parenteral spread of these infections in some countries. Concluding on this point, immigration from developing countries may influence the epidemiology of these infections in western countries, which can be controlled by improving the immigrants standard of life and by taking prophylactic measures in the host countries[3].

This review article analyzes the epidemiology of HBV infection in immigrant populations and in their host countries and suggests prophylactic measures to prevent the spread of this infection in western countries.

WORLDWIDE EPIDEMIOLOGY OF HBV INFECTION

HBV is the most common cause of hepatitis worldwide, with nearly 350 million people chronically infected[4] and 600000 deaths per year due to acute liver failure occurring during acute hepatitis or, more frequently, in HBV-related liver cirrhosis or hepatocellular carcinoma[5-7]. HBV is mainly transmitted at birth from an infected mother to the newborn baby, by parenteral route mainly in adults (intravenous drug use, surgery, dialysis, tattooing and piercing) or by unsafe sexual intercourse. The spread of chronic HBV infection differs widely from one geographical area to another, and the prevalences of hepatitis B surface antigen (HBsAg) chronic carriers classify the level of endemicity as low (< 2%), intermediate (2%-7%) and high (> 8%) in different countries[7]. HBV endemicity is high in Asia, sub-Saharan Africa and Alaska, but enclaves with high rates of HBsAg chronic carriers have been discovered in Southern and Eastern Europe and South and Central America. The prevalence of the HBV chronic infection varies in European countries from 0.1% to 8.0%. In Italy, the HBsAg seroprevalence is around 1% and the incidence of acute hepatitis B registered in 2013 is one case per 100000 inhabitants, the new cases almost exclusively involving non-vaccinated adults infected by sexual route[8]. In the United States and Australia the prevalence of HBsAg chronic carriers is lower than 0.5%.

In geographical areas with a high prevalence of HBsAg chronic carriers, HBV infection is frequently transmitted at birth or during early childhood[4], while in areas with a low prevalence it is typically acquired during adulthood by percutaneous or sexual transmission[9].

HBV INFECTION IN IMMIGRANT POPULATIONS

This review was prepared in accordance with the PRISMA guidelines. PubMed searches considered papers published between March 1974 and January 2015, and combined "Country" with the free-text search terms "hepatitis B, HBsAg" and "epidemiologic studies, prevalence, and seroprevalence" and "immigrants, foreigners and refugees".

The studies included in this review reported original

Table 1 Studies and immigrants screened, according to the geographical area of origin

Region	No. of studies	No. of immigrants investigated
Eastern Europe	8	4163402
Africa	13	959046
Latin America	5	130003914
Asia	17	8468256

data on the HBsAg seroprevalence in immigrants. The data on the immigrants' status was classified in the following categories: Immigrants, refugees, asylum seekers. We excluded studies on acute hepatitis B in immigrants.

The data on the study design, decade of study, immigrant status, immigrants' region of origin, mean or median age, gender distribution, co-morbidities, method of participant identification for the study, and serological testing method used were also extracted. The method of participant selection was categorized as occurring in reception centers at the time of arrival, in the context of a clinic or hospital visit, screening or others situations (*i.e.* screening studies in general host populations that included a subset of immigrants or studies which invited certain immigrant groups to be screened).

A total of 273 citations were identified in the electronic search. After screening the titles and abstracts, 245 were excluded because they were considered irrelevant and 5 were duplicated. A total of 23 articles were assessed with the predefined eligibility criteria and included in this review.

In these studies we evaluated: (1) the prevalence of subjects with hepatitis B in screened immigrants; (2) the distribution of the HBV genotypes; (3) the cost effectiveness of screening immigrants for hepatitis B; and (4) the clinical and therapeutic approach.

Prevalence of HBV infection in immigrants

The 23 selected articles involved 27948420 immigrants from several geographical regions (Table 1). All studies but two were prospective and evaluated the demographic, epidemiological and clinical data from immigrant screening programs performed in countries with a low HBV endemicity.

Among immigrants from different geographical areas, those from South East Asia (0%-27.3%) and sub-Saharan Africa (0%-15%) showed the highest prevalences of HBsAg carriers, in accordance with the high endemicity of the countries of origin (Table 2).

McCarthy *et al*[10] observed 15421 immigrants at 41 GeoSentinel clinics and found that 17% of the cases were HBV chronic.

Studies performed in Western Europe: The HBsAg seroprevalence in sub-Saharan immigrants ranged from 7.4% to 13.9% in four Italian studies[11-14] and was 8% and 15% in two studies from Spain[15,16], but another Italian study showed a prevalence of 3.7%[17]. Italy is a land of immigration also from Eastern Europe, and immigrants from this subcontinent living in Italy showed HBsAg-positivity rates ranging from 6.94% to 36.7%[11,14,17]. High prevalences were also observed in Albanian refugees in two Greek studies, 11.7% and 15.3%, respectively[18,19]. In Germany the Turkish community accounts for about 20% of the whole population, with an HBsAg prevalence of 5%[20]. In studies carried out in England and Holland the Chinese immigrants showed a percentage of HBV chronic carriers of 8.5% and 8.7%, respectively[21,22].

Studies performed in North America: Several American studies screened subjects from Asia (China, Pakistan, Afghanistan, Vietnam, Cambodia, Laos)[23-29]. The study by Mitchell *et al*[25] is an impressive retrospective investigation that tested for the HBsAg prevalence the immigrants to the United States from 1974 to 2008 (Table 2). Nearly 27 million immigrants from 225 countries were examined. The largest geographical subgroup came from Latin American countries (13 million cases) and the smallest from African countries (940000 cases). The percentage of HBsAg-positive immigrants was 1.6% in those from Latin America, 11.1% in those from South Africa, 5% in those from the Mediterranean countries, 2.9% from Europe, 4% from South East Asia and 11% from The Pacific countries.

Studies performed in Australia: At present, immigrant populations come to Australia prevalently from countries of South-East Asia. Caruana *et al*[30] screened 95 Laotian and 236 Cambodian immigrants, of whom 9.5% of the Laotians and 8% of the Cambodians were HBV chronic carriers. Most of the HBsAg-positive subjects were unaware of their HBV status. Further studies are needed to extend the knowledge of the impact of immigration on the HBV endemicity in Australia.

HBV Genotypes in immigrants

The molecular characteristics of HBV infection in immigrants reflect those of their geographical areas of origin. HBV genotyping in immigrants was investigated only in small studies (Table 3). Mixson-Hayden *et al*[23] studied immigrants from Asia and Somalia and detected HBV genotypes B and C in 90% of subjects with chronic hepatitis B. Rivas *et al*[15] studied 34 HBV viremic patients from Equatorial Guinea and other sub-Saharan countries and found HBV genotype A in 22, genotype E in 10, and genotype D in 2. El-Hamad *et al*[17] investigated 45 immigrants and found that HBV genotype D predominated in those from Eastern Europe, HBV genotypes B and C in those from Asia, genotype A in those from India and genotype E in those from West Africa. Scotto *et al*[11] evaluated 144 HBV-DNA-positive immigrants, prevalently from Africa, and identified 65 (45.1%) with HBV genotype E, 26 (18.1%) with D, 22 (15.3%) with B, 19 (13.2%) with C and 12 (8.3%) with A.

Table 2 Prevalence of hepatitis B virus infection in immigrant populations

Ref.	Country	Type of study	No. of patients[1]	Area of origin and percentage (%)	HBsAg positive (%)
Roussos *et al*[18]	Greece	Prospective	130	Eastern Europe: 86	15.3[3]
				Asia: 8.5	27.3
				Africa: 6.2	0
Caruana *et al*[30]	Australia	Prospective	329	South east Asia	Laos 9.5
				(Laos and Cambodia)	Cambodia 8
Toro *et al*[16]	Spain	Prospective	1303	Latin America: 46	0
				Sub-Saharan Africa: 23.7	15
				Eastern Europe: 9.4	0
				Northern Africa: 9.2	3.2
				Asia: 4.9	Unknown
Hislop *et al*[29]	Canada	Prospective	504	China: 100	6
Majori *et al*[13]	Italy	Prospective	182	Sub-Saharan Africa: 100	9.3
Museru *et al*[24]	United States	Retrospective	9570	Asia: 24	10.7[2]
				Africa: 71	
				Eastern Europe: 4	
Tafuri *et al*[12]	Italy	Prospective	529	Africa: 96.4	8.3
				Asia: 3.6	0
Milionis *et al*[19]	Greece	Prospective	504	Albania: 100	11.7
Lee *et al*[27]	United States	Prospective	567	Asia: 100	6
Levy *et al*[26]	United States	Prospective	684	South America: 96.4	0.3
			1502	Asia: 7.7	3.8
Mitchell *et al*[25]	United States	Retrospective	27900000	Latin America: 46.6	1.6
				Africa: 3.36	11.1
				Mediterranean countries: 6	5
				Europe: 14.3	2.9
				South East Asia: 6.6	4
				Pacific Countries: 23.7	11
Kallman *et al*[28]	United States	Prospective	322	Vietnam: 100	9.3
Veldhuijzen *et al*[22]	Holland	Prospective	1090	China: 100	8.5
Rivas et *al*[15]	Spain	Retrospective	1493	Sub-Saharan Africa: 100	8.4
McCarthy *et al*[10]	Canada, Europe,	Retrospective	15421	Africa: 41	12.5
	United States,			Asia: 35.8	11.7
	Australia, New			South America: 16.1	2
	Zeland			Eastern Europe: 4	0
Vedio *et al*[21]	United Kingdom	Prospective	229	China: 100	8.7
Zuure *et al*[38]	The Netherlands	Prospective	465	Egypt: 100	1.1
Richter *et al*[39]	The Netherlands	Prospective	959	Eastern Asia: 100	2.2
Burgazli *et al*[20]	Germany	Prospective	1287	Turkey: 100	5
Mixson-Hayden *et al*[23]	United States	Prospective	4890	Somalia: 14.5	5.5[4]
				Asia: 86.5	6.8[4]
El-Hamad *et al*[17]	Italy	Prospective	3728	North Africa: 12.4	2.8
				Eastern Europe: 44	6.9
				Sub-Saharan Africa: 21.4	3.7
				Asia: 16.8 (37 China)	3.4
				Central-South America: 5.4	3.3
Coppola *et al*[14]	Italy	Prospective	882	Northern Africa: 9	2.5
				Sub-Saharan Africa: 50.3	13.9
				Eastern Europe: 22	6.2
				India-Pakistan Area: 14.3	3.2

[1]Estimated cases; [2]It is not possible to differentiate for geographical area of origin; [3]Albania 22.4%; [4]HBV-DNA-positive. HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

Table 3 Prevalence of hepatitis B virus genotype in immigrant populations *n* (%)

Ref.	No. of HBV-DNA-positive patients (%)	HBV genotype A	HBV genotype B	HBV genotype C	HBV genotype D	HBV genotype E	HBV genotype F	HBV genotype G
Toro *et al*[16]	43 (81)	15 (34.9)	-	-	16 (37.2)	10 (23.2)	-	1 (2.3)
Scotto *et al*[11]	144 (75.4)	12 (8.3)[4]	22 (15.3)	19 (13.2)	26 (18.1)	65 (45.13)		
Rivas *et al*[15]	40 (52.6)[2]	22 (64.7)[3]	-	-	2 (5)	10 (25)	-	-
Zuure *et al*[38]	5 (100)	-	-	-	5 (100)	-	-	
Mixson-Hayden *et al*[23]	331 (12.1)[1]	9 (2.7)	164 (49.5)	137 (41.4)	-	-	-	1 (0.3)
El-Hamad *et al*[17]	45 (27)	8 (18)	5 (11)	5 (11)	27 (60)			

[1]Twenty specimens could not be genotyped; [2]Six specimens could not be genotyped; [3]Five subjects presented mixed genotypes (A/D); [4]Four subjects presented mixed genotypes (2 A/D; 2 A/F). HBV: Hepatitis B virus.

The flow of immigrants may modify the distribution of HBV genotype in host countries with a low endemicity, since the immigrants frequently come from countries with a high or intermediate HBV endemicity. An example of this has occurred in Italy, where HBV genotype D was responsible for acute hepatitis B in 95% of cases observed until two decades ago, whereas in recent years HBV genotypes non-D has been found to be responsible for 60% of the cases[31]. This phenomenon is of clinical value, since some studies reported that the natural history and the response to antiviral treatment of HBV infection may differ in relation to the HBV genotypes[32].

Clinical and therapeutic approach in relation to the legal status of the immigrants

The literature data on the clinical course and treatment of HBsAg-positive immigrants are scanty. Interesting information, however, comes from a study performed on a Chinese community in Rotterdam in the Netherlands[22] where 1090 Chinese immigrants were tested for HBsAg and 92 (8.5%) were found positive. Of these 92, the 35 (38%) with chronic hepatitis were referred to a specialist and 15 of them started antiviral treatment, whereas the 57 (62%) HBsAg asymptomatic carriers were referred to general practitioners for a long-term follow-up. The good clinical practice applied in this study is a good example to imitate in host countries. Instead, in most host countries the management of HBV infection in the immigrant population is a complex issue, due to bureaucratic difficulties and to the numerous language, economic, social, religious and cultural barriers the immigrants finding a foreign country.

Risk for host countries and cost effectiveness of screening immigrants for hepatitis B

Extensive immigration from countries with a high HBV endemicity to those with a low HBV endemicity requires vigilance to prevent the risk of spreading HBV infection to the native population. In fact, in most western countries, apart from the vaccination of subjects with a greater risk of acquiring HBV infection, universal vaccination now regards only newborn babies and, consequently, the majority of middle-aged adults and the elderly lack immunological protection against HBV. Extensive screening programs should be implemented to identify the HBsAg-positive immigrants and acquaint them with the correct information on HBV, its routes of transmission and prevention. HBV vaccination should be offered free of charge to the household members of HBsAg carriers.

Veldhuijzen *et al*[22] in Holland used an interesting screening strategy. In a 3-mo campaign targeting the Chinese community in Rotterdam with free HBV-testing at an outdoor location, 49% of 1090 immigrants had positive serology for a past or current HBV infection. The Chinese community organizations gave their support to the campaign, which started at the time of celebrations for the Chinese New Year. Ninety-two HBsAg-positive subjects were invited by telephone in their native language for clinical consultation and counseling.

In an Italian prospective study, 926 illegal or refugee immigrants were observed for a clinical consultation at a first-level clinical center with the help of cultural mediators[14]. During the clinical consultation, screening for HBV, HCV and HIV infections offered to all immigrants was accepted by 95% of them. The 81 subjects found to be HBsAg-positive were referred to a 3rd level clinic of infectious diseases to complete the diagnostic procedures and be considered for a clinical or therapeutic follow-up.

A particular screening strategy, based on a peer-to-peer communication, was applied in nine Italian prisons, where more than one third of the prisoners were immigrants. Briefly, a former prisoner acquainted with the correct information on HBV, HCV and HIV infections and correlated diseases and treatments acted as a peer educator for the inmates in the nine prisons. The sampling, performed on a voluntary basis, improved the percentages of screened prisoners from 25%-30% of the previous year to 65%. Active HBV infection was diagnosed in 15.2% of the immigrant inmates[33].

Of 1970 immigrant pregnant women recently screened in an Italian study, 143 (7.3%) were found to be HBsAg-positive and screening for HBsAg was offered also to their family members. All immigrants found to be HBsAg-positive in this study were referred to a unit of infectious diseases to complete the diagnostic procedures and be considered for a clinical and therapeutic follow-up (unpublished data).

CONCLUSION

People fleeing from cruel wars and/or extreme need are often destitute, but the current social and economic crises in some western countries do not favor their integration in the host country[34]. Correct management of the healthcare problems of immigrants requires expert personnel, funds and dedicated structures for their assistance. The experience of personnel on HBV infection is essential, also considering the suggestion of literature; in fact, recently in a survey on the knowledge on HBV infection in physicians in various stages of their training in Santa Clara, California, United States, Chao *et al*[35] demonstrated that both medical school and residency training had no adequately prepared physicians on the management of HBV infection.

Moreover, the assistance of the social services, voluntary operators and cultural mediators is essential to obtain an optimized psychological and clinical approach[36,37]. The first-level clinical centers that usually have the initial clinical contact with immigrants should have proven experience in the clinical, psychological and legal management of vulnerable groups, since HBV infection is only part of a global social and clinical problem. The role of the cultural mediators during clinical consultations is of great importance, since they can reassure the immigrants in their own language and be of help to a skilled physician in explaining to them

the importance of screening, diagnostic tests, clinical follow-up and treatment. Only if trust is established, will the immigrants listen and follow the suggestions put forward. The cultural mediators can also act as tutors to help the immigrants deal with the bureaucratic requirements to be carried out.

Information to immigrants is a key point. As regards HBV infection, they should improve their knowledge on the infection and related diseases, risk factors of transmission, methods for prevention including vaccination programs and available treatments. This information could be more easily delivered in illustrative brochures and informative cartoons prepared in different languages and with educational videos uploaded on the social networks (facebook, twitter, *etc.*). It is of great importance to stress the practical measures to prevent the acquisition and transmission of HBV and other infectious agents. The importance of condom use in preventing HBV transmission during sexual intercourse should always be stressed.

Healthcare operators offering the immigrants HBV screening free of charge, and hopefully for HCV and HIV infection, should act in accordance with the local laws of privacy. Adhesion to the screening and a signed informed consent, written in the immigrant's native language, should be obtained on a voluntary basis. Patients should be asked about their geographical origin, time of immigration, level of education, religion, family history, cohabitation conditions, sexual habits, history of previous surgery, dental care, tattooing, piercing, drug addiction, blood transfusion and tribal rituals, and for females previous abortions. These data should be recorded in a pre-coded questionnaire.

A skilled physician should discuss the result of the screening with the help of a cultural mediator, and the HBsAg-positive subjects should be referred to a 3rd level liver unit to complete the diagnostic course, clinical evaluation, monitoring and treatment if necessary. Each HBsAg-positive subject should be assisted by a cultural mediator at the 3rd level clinical center throughout the monitoring or treatment period. Vaccination against HBV should be considered for each HBsAg-negative subject exposed to HBV infection.

In conclusion, the integration of immigrants in the host country where they will start a new life should be a major objective of governments and those working in dedicated associations. Good quality medical care and improved quality of life are the first steps to ensuring integration.

REFERENCES

1 **Hatton TJ**, Williamson JG. Demographic and Economic Pressure on Emigration out of Africa. Scand. *J of Economics* 2003; **105**: 465-486 [DOI: 10.1111/1467-9442.t01-2-00008]

2 **Sidibé M**. Putting education first. Available from: URL: http://www.huffingtonpost.com/michel-sidib/aids-education_b_1915767.html

3 **Rossi C**, Shrier I, Marshall L, Cnossen S, Schwartzman K, Klein MB, Schwarzer G, Greenaway C. Seroprevalence of chronic hepatitis B virus infection and prior immunity in immigrants and refugees: a systematic review and meta-analysis. *PLoS One* 2012; **7**: e44611 [PMID: 22957088 DOI: 10.1371/journal.pone.0044611]

4 **World Health Organization**. Hepatitis B. World Health Organization Fact Sheet No. 204. [Accessed 2005 Jun 5]. Available from: http://who.int/mediacentre/factsheets/fs204/en

5 **Sagnelli E**, Stroffolini T, Mele A, Imparato M, Almasio PL. Chronic hepatitis B in Italy: new features of an old disease--approaching the universal prevalence of hepatitis B e antigen-negative cases and the eradication of hepatitis D infection. *Clin Infect Dis* 2008; **46**: 110-113 [PMID: 18171224 DOI: 10.1086/524074]

6 **Sagnelli E**, Stroffolini T, Mele A, Imparato M, Sagnelli C, Coppola N, Almasio PL. Impact of comorbidities on the severity of chronic hepatitis B at presentation. *World J Gastroenterol* 2012; **18**: 1616-1621 [PMID: 22529690 DOI: 10.3748/wjg.v18.i14.1616]

7 **Sagnelli E**, Sagnelli C, Pisaturo M, Macera M, Coppola N. Epidemiology of acute and chronic hepatitis B and delta over the last 5 decades in Italy. *World J Gastroenterol* 2014; **20**: 7635-7643 [PMID: 24976701 DOI: 10.3748/wjg.v20.i24.7635]

8 **Sagnelli E**, Stroffolini T, Mele A, Almasio P, Coppola N, Ferrigno L, Scolastico C, Onofrio M, Imparato M, Filippini P. The importance of HCV on the burden of chronic liver disease in Italy: a multicenter prevalence study of 9,997 cases. *J Med Virol* 2005; **75**: 522-527 [PMID: 15714480 DOI: 10.1002/jmv.20313]

9 **Lavanchy D**. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004; **11**: 97-107 [PMID: 14996343 DOI: 10.1046/j.1365-2893.2003.00487.x]

10 **McCarthy AE**, Weld LH, Barnett ED, So H, Coyle C, Greenaway C, Stauffer W, Leder K, Lopez-Velez R, Gautret P, Castelli F, Jenks N, Walker PF, Loutan L, Cetron M. Spectrum of illness in international migrants seen at GeoSentinel clinics in 1997-2009, part 2: migrants resettled internationally and evaluated for specific health concerns. *Clin Infect Dis* 2013; **56**: 925-933 [PMID: 23223602 DOI: 10.1093/cid/cis1016]

11 **Scotto G**, Martinelli D, Di Tullio R, Fazio V. Epidemiological and Clinical Features of Hepatitis B Virus Genotypes among Immigrants in Southern Italy. *Hepat Res Treat* 2010; **2010**: 878356 [PMID: 21188202 DOI: 10.1155/2010/878356]

12 **Tafuri S**, Prato R, Martinelli D, Melpignano L, De Palma M, Quarto M, Germinario C. Prevalence of Hepatitis B, C, HIV and syphilis markers among refugees in Bari, Italy. *BMC Infect Dis* 2010; **10**: 213 [PMID: 20646306 DOI: 10.1186/1471-2334-10-213]

13 **Majori S**, Baldo V, Tommasi I, Malizia M, Floreani A, Monteiro G, Ferrari A, Accordini A, Guzzo P, Baldovin T. Hepatitis A, B, and C infection in a community of sub-Saharan immigrants living in Verona (Italy). *J Travel Med* 2008; **15**: 323-327 [PMID: 19006505 DOI: 10.1111/j.1708-8305.2008.00230.x]

14 **Coppola N**, Alessio L, Gualdieri L, Pisaturo M, Sagnelli C, Caprio N, Maffei R, Starace M, Angelillo IF, Pasquale G, Sagnelli E. Hepatitis B virus, hepatitis C virus and human immunodeficiency virus infection in undocumented migrants and refugees in southern Italy, January 2012 to June 2013. *Euro Surveill* 2015; **20**: [PMID: 26530499 DOI: 10.2807/1560-7917.ES.2015.20.35.30009]

15 **Rivas P**, Herrero MD, Poveda E, Madejón A, Treviño A, Gutiérrez M, Ladrón de Guevara C, Lago M, de Mendoza C, Soriano V, Puente S. Hepatitis B, C, and D and HIV infections among immigrants from Equatorial Guinea living in Spain. *Am J Trop Med Hyg* 2013; **88**: 789-794 [PMID: 23339201 DOI: 10.4269/ajtmh.12-0319]

16 **Toro C**, Jiménez V, Rodríguez C, Del Romero J, Rodés B, Holguín A, Alvarez P, García-Campello M, Gómez-Hernando C, Guelar A, Sheldon J, de Mendoza C, Simón A, Soriano V. Molecular and epidemiological characteristics of blood-borne virus infections among recent immigrants in Spain. *J Med Virol* 2006; **78**: 1599-1608 [PMID: 17063510 DOI: 10.1002/jmv.20744]

17 **El-Hamad I**, Pezzoli MC, Chiari E, Scarcella C, Vassallo F, Puoti M, Ciccaglione A, Ciccozzi M, Scalzini A, Castelli F. Point-of-care screening, prevalence, and risk factors for hepatitis B infection among 3,728 mainly undocumented migrants from non-EU countries in northern Italy. *J Travel Med* 2015; **22**: 78-86 [PMID:

25424439 DOI: 10.1111/jtm.12176]
18 **Roussos A**, Goritsas C, Pappas T, Spanaki M, Papadaki P, Ferti A. Prevalence of hepatitis B and C markers among refugees in Athens. *World J Gastroenterol* 2003; **9**: 993-995 [PMID: 12717844]
19 **Milionis C**. Serological markers of Hepatitis B and C among juvenile immigrants from Albania settled in Greece. *Eur J Gen Pract* 2010; **16**: 236-240 [PMID: 20954813 DOI: 10.3109/13814788.2010.525631]
20 **Burgazli KM**, Mericliler M, Sen C, Tuncay M, Gökce Y, Nayir B, Klör HU, Sinterhauf K, Karatay E, Bilgin Y. The prevalence of hepatitis B virus (HBV) among Turkish immigrants in Germany. *Eur Rev Med Pharmacol Sci* 2014; **18**: 869-874 [PMID: 24706312]
21 **Vedio AB**, Ellam H, Rayner F, Stone B, Kudesia G, McKendrick MW, Green ST. Hepatitis B: report of prevalence and access to healthcare among Chinese residents in Sheffield UK. *J Infect Public Health* 2013; **6**: 448-455 [PMID: 23999342 DOI: 10.1016/j.jiph.2013.05.004]
22 **Veldhuijzen IK**, Wolter R, Rijckborst V, Mostert M, Voeten HA, Cheung Y, Boucher CA, Reijnders JG, de Zwart O, Janssen HL. Identification and treatment of chronic hepatitis B in Chinese migrants: results of a project offering on-site testing in Rotterdam, The Netherlands. *J Hepatol* 2012; **57**: 1171-1176 [PMID: 22885717 DOI: 10.1016/j.jhep.2012.07.036]
23 **Mixson-Hayden T**, Lee D, Ganova-Raeva L, Drobeniuc J, Stauffer WM, Teshale E, Kamili S. Hepatitis B virus and hepatitis C virus infections in United States-bound refugees from Asia and Africa. *Am J Trop Med Hyg* 2014; **90**: 1014-1020 [PMID: 24732462 DOI: 10.4269/ajtmh.14-0068]
24 **Museru OI**, Vargas M, Kinyua M, Alexander KT, Franco-Paredes C, Oladele A. Hepatitis B virus infection among refugees resettled in the U.S.: high prevalence and challenges in access to health care. *J Immigr Minor Health* 2010; **12**: 823-827 [PMID: 20204515 DOI: 10.1007/s10903-010-9335-7]
25 **Mitchell T**, Armstrong GL, Hu DJ, Wasley A, Painter JA. The increasing burden of imported chronic hepatitis B--United States, 1974-2008. *PLoS One* 2011; **6**: e27717 [PMID: 22163270 DOI: 10.1371/journal.pone.0027717]
26 **Levy V**, Yuan J, Ruiz J, Morrow S, Reardon J, Facer M, Molitor F, Allen B, Ajufo BG, Bell-Sanford G, McFarland W, Raymond HF, Kellogg T, Page K. Hepatitis B sero-prevalence and risk behaviors among immigrant men in a population-based household survey in low-income neighborhoods of northern California. *J Immigr Minor Health* 2010; **12**: 828-833 [PMID: 19319680 DOI: 10.1007/s10903-009-9239-6]
27 **Lee J**, Lok AS, Chen J. Hepatitis B prevalence among Asian Americans in Michigan: an assessment to guide future education and intervention strategies. *J Community Health* 2010; **35**: 534-542 [PMID: 20300810 DOI: 10.1007/s10900-010-9237-6]
28 **Kallman JB**, Tran S, Arsalla A, Haddad D, Stepanova M, Fang Y, Wrobel VJ, Srishord M, Younossi ZM. Vietnamese community screening for hepatitis B virus and hepatitis C virus. *J Viral Hepat* 2011; **18**: 70-76 [PMID: 20196807 DOI: 10.1111/j.1365-2893.2010.01278.x]
29 **Hislop TG**, Teh C, Low A, Li L, Tu SP, Yasui Y, Taylor VM. Hepatitis B knowledge, testing and vaccination levels in Chinese immigrants to British Columbia, Canada. *Can J Public Health* 2007; **98**: 125-129 [PMID: 17441536]
30 **Caruana SR**, Kelly HA, De Silva SL, Chea L, Nuon S, Saykao P, Bak N, Biggs BA. Knowledge about hepatitis and previous exposure to hepatitis viruses in immigrants and refugees from the Mekong Region. *Aust N Z J Public Health* 2005; **29**: 64-68 [PMID: 15782875 DOI: 10.1111/j.1467-842X.2005.tb00751.x]
31 **Zuccaro O**, Romanò L, Mele A, Mariano A, Clementi M, Tosti ME, Taliani G, Galli C, Zanetti AR, Spada E. Clinical, epidemiological and virological features of acute hepatitis B in Italy. *Infection* 2015; **43**: 431-441 [PMID: 25697541]
32 **Mayerat C**, Mantegani A, Frei PC. Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? *J Viral Hepat* 1999; **6**: 299-304 [PMID: 10607244 DOI: 10.1007/s15010-015-0747-0]
33 **Sagnelli E**, Starnini G, Sagnelli C, Monarca R, Zumbo G, Pontali E, Gabbuti A, Carbonara S, Iardino R, Armignacco O, Babudieri S. Blood born viral infections, sexually transmitted diseases and latent tuberculosis in italian prisons: a preliminary report of a large multicenter study. *Eur Rev Med Pharmacol Sci* 2012; **16**: 2142-2146 [PMID: 23280032]
34 **Robert G**, Martínez JM, García AM, Benavides FG, Ronda E. From the boom to the crisis: changes in employment conditions of immigrants in Spain and their effects on mental health. *Eur J Public Health* 2014; **24**: 404-409 [PMID: 24632339 DOI: 10.1093/eurpub/cku020]
35 **Chao SD**, Wang BM, Chang ET, Ma L, So SK. Medical training fails to prepare providers to care for patients with chronic hepatitis B infection. *World J Gastroenterol* 2015; **21**: 6914-6923 [PMID: 26078568 DOI: 10.3748/wjg.v21.i22.6914]
36 **Tarricone I**, Atti AR, Salvatori F, Braca M, Ferrari S, Malmusi D, Berardi D. Psychotic symptoms and general health in a socially disadvantaged migrant community in Bologna. *Int J Soc Psychiatry* 2009; **55**: 203-213 [PMID: 19383664 DOI: 10.1177/0020764008093445]
37 **Tarricone I**, Atti AR, Braca M, Pompei G, Morri M, Poggi F, Melega S, Stivanello E, Tonti L, Nolet M, Berardi D. Migrants referring to the Bologna Transcultural Psychiatric Team: reasons for drop-out. *Int J Soc Psychiatry* 2011; **57**: 627-630 [PMID: 20851829 DOI: 10.1177/0020764010382368]
38 **Zuure FR**, Bouman J, Martens M, Vanhommerig JW, Urbanus AT, Davidovich U, van Houdt R, Speksnijder AG, Weegink CJ, van den Hoek A, Prins M. Screening for hepatitis B and C in first-generation Egyptian migrants living in the Netherlands. *Liver Int* 2013; **33**: 727-738 [PMID: 23448397 DOI: 10.1111/liv.12131]
39 **Richter C**, Ter Beest G, Gisolf EH, VAN Bentum P, Waegemaekers C, Swanink C, Roovers E. Screening for chronic hepatitis B and C in migrants from Afghanistan, Iran, Iraq, the former Soviet Republics, and Vietnam in the Arnhem region, The Netherlands. *Epidemiol Infect* 2014; **142**: 2140-2146 [PMID: 24398373 DOI: 10.1017/S0950268813003415]

P- Reviewer: Ma L, Wang L, Zamani F **S- Editor**: Kong JX
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.i30.2962

World J Hepatol 2015 December 28; 7(30): 2962-2967
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

2015 Advances in Nonalcoholic Fatty Liver Disease

Management of non-alcoholic fatty liver disease in 2015

Neel Malhotra, Melanie D Beaton

Neel Malhotra, Melanie D Beaton, Department of Medicine, Division of Gastroenterology, Western University, London, ON N6A 3A5, Canada

Melanie D Beaton, London Health Sciences Centre, University Hospital, London, ON N6A 5A5, Canada

Author contributions: Malhotra N and Beaton MD analyzed the literature and wrote the manuscript.

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

Correspondence to: Dr. Melanie D Beaton, London Health Sciences Centre, University Hospital, Room A10-223, 339 Windermere Road, London, ON N6A 5A5, Canada. melanie.beaton@lhsc.on.ca
Telephone: +1-519-6633344
Fax: +1-519-6633220

Received: June 25, 2015
Peer-review started: June 26, 2015
First decision: September 8, 2015
Revised: September 25, 2015
Accepted: December 17, 2015
Article in press: December 18, 2015
Published online: December 28, 2015

Abstract

There is no single pharmacologic therapy that has been approved to treat nonalcoholic fatty liver disease in the general population. The backbone of therapy currently includes intensive lifestyle modification with established targets for diet and weight loss. The use of unsweetened, unfiltered coffee along with limiting high fructose corn syrup have emerged as beneficial dietary recommendations. The use of empiric oral hypoglycemic agents and vitamin E, however, has not been widely accepted. Developing bariatric surgical techniques are promising, but additional studies with long-term follow up are needed before it can be widely recommended. Finally, liver transplantation is an increasingly frequent consideration once complications of end-stage disease have developed. The future treatment of those with nonalcoholic fatty liver disease will likely involve a personalized approach. The importance of the gut microbiome in mediating hepatocyte inflammation and intestinal permeability is emerging and may offer avenues for novel treatment. The study of anti-fibrotic agents such as pentoxifylline and FXR agonists hold promise and new pathways, such as hepatocyte cannabinoid receptor antagonists are being studied. With the incidence of obesity and the metabolic syndrome increasing throughout the developed world, the future will continue to focus on finding novel agents and new applications of existing therapies to help prevent and to mediate the progression of nonalcoholic fatty liver disease.

Key words: Lobular inflammation; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Liver fibrosis

Core tip: Lifestyle modification with diet and exercise remain the mainstay of therapy for nonalcoholic fatty liver disease. Loss of at least 7%-10% of body weight with limiting high fructose corn syrup and high-saturated/high glycemic index foods should be combined with regular, vigorous physical activity. The future of treatment will continue to evolve and likely include the role of anti fibrotic agents, surgical management and transplantation when indicated.

Malhotra N, Beaton MD. Management of non-alcoholic fatty liver disease in 2015. *World J Hepatol* 2015; 7(30): 2962-2967 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i30/2962.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i30.2962

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a form of chronic hepatitis that affects a wide range of individuals. It is estimated that up to 95 million adults in the United States have been affected and that the prevalence will continue to increase[1]. The most common documented comorbidities that have been associated with NAFLD include obesity, impaired insulin sensitivity and dyslipidemia. There is growing evidence that there is also a connection to a number of other disorders including obstructive sleep apnea, hypothyroidism, hypopituitarism, hypogonadism and polycystic ovarian syndrome[2].

NAFLD exists as a spectrum and is best characterized histologically. Important features include steatosis, inflammation, hepatocellular ballooning and fibrosis. NAFLD can be classified as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). In NAFL hepatocellular injury is absent, whereas NASH involves the presence of inflammation with hepatocyte damage, with or without fibrosis[2]. Once present, these specific changes mediate the risk of future disease progression.

The importance of treatment for NAFLD comes from the knowledge of its complications. It is well established that patients with NASH can progress to develop cirrhosis and its complications[2] including hepatocellular carcinoma (HCC)[2]. However, the most common cause of death in patients with NAFLD is cardiovascular disease. Today, the medical community continues to look for evidence-based therapy to improve liver histology and reduce associated risks. These include lifestyle management with a goal of sustained weight loss, treating associated features of the metabolic syndrome to reduce cardiovascular risk, as well as liver specific pharmacologic therapy and in some cases transplantation. As we look beyond 2015, we are hopeful that studies currently evaluating the role of the microbiome, novel insulin sensitizers and anti-fibrotic agents will provide effective therapies for this condition. We aim to discuss the current management of NAFLD and future directions of treatment.

LIFESTYLE MODIFICATION: EXERCISE

Based on the 2012 published practice guidelines, lifestyle intervention has shown to improve hepatic aminotransferases along with steatosis[2]. It broadly encompasses both dietary changes and regular exercise. One of the objective end points for many studies has included the NAFLD activity score (NAS), which was validated by the NASH Clinical Research Network[3]. The components are based on histologic examination and include hepatic steatosis, lobular inflammation and hepatocyte ballooning. A randomized study by Promrat *et al*[3] showed that there was a significant improvement in the NAS score in those treated with lifestyle modification. It required subjects to achieve 7%-10% reduction in body weight by limiting caloric intake and completing moderate intensity activities for 200 min/wk. They also included regular support with behavior modification and dieticians for meal planning. Unfortunately, this study failed to identify a significant difference in fibrosis on biopsy[3]. Although specific targets exist for weight loss, sustained results over the long-term are the backbone for continued success. Even alone, there has been improvement seen with exercise without dietary modification[2].

We now know that the intensity of physical activity can effect the histologic improvements demonstrated in patients. Those with vigorous activity had decreased odds of NASH and lower glucose and insulin values[4]. It can be quantified as 75 min/wk with activities that include running on a treadmill or using a step machine. Doubling the time was shown to provide additional benefits of reduced NASH activity[4].

LIFESTYLE MODIFICATION: DIET

Dietary composition has been scrutinized as a possible modifiable factor in altering the course of NAFLD development and progression. As the North American diet continues to evolve, so does the need for finding easy meal options to keep up with a fast paced lifestyle. That often leads to consumption of high caloric, processed foods and carbonated beverages. Magnetic resonance spectroscopy (MRS) has been helpful to quantify the accumulation of fat in the liver. The cut off in NAFLD is based on intrahepatic triglyceride (IHTG) content and set at greater than 5.6%[5]. Fructose, trans-fatty acids and saturated fat may contribute to increased IHTG, while poly and mono-unsaturated fats may play a protective role[5]. The result is an imbalance of free fatty output from the liver. Saturated fats have been especially shown to contribute toward the development of cardiovascular disease, type 2 diabetes mellitus and the metabolic syndrome[6]. Although they have also been shown to increase the IHTG, studies of high calorie diets may have other potential cofounders to explain the results[7]. Trans-fatty acids are unsaturated and produced due to hydrogenation. They have at least one double bond in the trans configuration and are found in many processed food items. Their role in humans and NAFLD has not been well described at this time[5]. Polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids have been shown to regulate expression of proteins involved with fatty acid oxidation[8]. As a result, this may lead to an overall improvement in the IHTG content in the liver[5].

The monosaccharide fructose and its disaccharide form, sucrose are primary dietary sweeteners that are highly lipogenic sugars[9]. This has lead to examine

fructose and its role in NAFLD. Intake of sucrose and high fructose corn syrup has increased 1000% over the past 40 years and can account for up to 10% of caloric food intake[10]. Overall, fructose is a component of insulin resistance and a contributor to visceral adiposity and plasma triglycerides[9]. The downstream effect is fat deposition in the liver in excess amounts. Steatosis is the eventual result of the liver's inability to keep up with synthesis, utilization and expenditure of fat[9]. A cross sectional analysis by Vos *et al*[9] showed that daily consumption of fructose was associated with significantly more fibrosis (OR = 2.6, P = 0.004) and significantly lower plasma high density lipoprotein (HDL) (P < 0.001). More studies will be needed to further determine how fructose contributes to NAFLD development and progression to NASH and how this can be mediated[9]. Overall, a balanced diet consisting of lower glycemic index fruits and vegetables, low saturated fats along with whole grains has been shown to reduce liver fat on MRS[1].

The effect of coffee has been previously studied in other causes of liver disease, including HCC. A recent meta-analysis by Bravi *et al*[11] revealed a significant decrease in relative risk for HCC with increasing coffee consumption. Similarly in NAFLD, a study by Molloy *et al*[12] looked at caffeine and coffee intake. There appeared to be an inverse relationship between regular unsweetened, unfiltered caffeinated coffee consumption and hepatic fibrosis[12]. This effect could not be extrapolated to other types of caffeine. Unfortunately, there have been no prospective trials identifying a specific amount required for achieve maximal benefit. Although the exact ingredient responsible for these benefits is unknown, it has been postulated that it due to the reduced amount of cafestol and kahweol in unfiltered coffee. These are diterpene molecules that are thought to have anti-inflammatory properties, but also potentially increase serum cholesterol. By filtering coffee, it may prevent an elevation in low-density lipoprotein cholesterol and serve as a reasonable adjunct to therapy in NAFLD patients[13].

PHARMACOLOGIC THERAPY

Given the related pathophysiology of NAFLD and associated metabolic comorbidities, it has been hoped that the pharmacologic treatment of these conditions could lead to an improvement in liver histology. This has lead to numerous clinical trials, although none have demonstrated significant benefit to be approved as liver-specific therapy[3].

Poly unsaturated fatty acids have been shown to reduce insulin resistance, lipid production and systemic inflammation. The n-3 form of PUFAs represent the family of omega 3 fatty acids. The physiologic forms exist as eicosapentanoic acid (EPA) along with docosahexanoic acid and are significantly decreased in NASH[14]. Therefore, supplementation with the synthetic ethyl-EPA (EPA-E) that is found in many fish oil preparations was studied by Sanyal *et al*[14]. Twelve months of therapy was compared to placebo by Sanyal *et al*[14]. During the trial, up to 25% of patients dropped out, mainly due to side effects including nausea, diarrhea and abdominal discomfort[14]. Using histologic improvement as a primary endpoint, there was unfortunately no significant effect on steatosis or fibrosis stage when comparing placebo with lower (1800 mg/d) or higher (2700 mg/d) doses of of EPA-E[14].

The evaluation of insulin sensitizing agents in NAFLD has mainly focused on the role metformin and the family of thiazolidinedione. While pioglitazone has shown improvement in steatosis and inflammation in non diabetics, there are safety concerns including bladder cancer risk, weight gain, fractures and heart failure[2]. The PIVENS study was a multicenter, randomized, placebo-controlled trial published in 2010. In nondiabetic and non-cirrhotic adults with biopsy proven NASH there was a decrease in fibrosis after 96 wk (P = 0.04, NNT = 6.9), but it did not meet the pre-specified statistical endpoint[15]. The lack of significance might be attributed to the fact that 28% of the initial biopsy specimens lacked hepatocellular ballooning in the pioglitazone group compared to 17% in the placebo group[15]. As result, it would be harder to meet the targets set in the primary outcome, including improvement in hepatocellular ballooning and fibrosis score.

Metformin is widely used as a first line agent to control blood sugar and its role in NAFLD has been studied. In a recent systematic review and meta-analysis there was an improvement in aminotransferases, but no significant histologic response was identified[16]. In contrast, a small randomized controlled trial published by Bugianesi *et al*[17] showed a decrease in liver fat, necroinflammation and fibrosis in nondiabetic adults treated with metformin. Further to this, metformin's effect on overall mortality was examined in a cohort study of DM patients who developed NASH related cirrhosis, with a 57% reduced risk of death and no evidence of lactic acidosis in those taking metformin[18]. Although current guidelines do not recommend metformin as a specific treatment for liver disease in patients with NASH, it may be a useful adjunct in management of associated DM[2].

Vitamin E is a well-known antioxidant that prevents the propagation of free radicals. This was also examined in the PIVENS trial with a dose of 800 IU/d over 96 wk[15]. Overall, there was a significant improvement in the primary endpoint of hepatocellular ballooning and steatosis (P = 0.001, NNT = 4.2)[15]. This occurred without significant improvement in fibrosis score[15]. Vitamin E is not free from adverse effects, with associations including risk of hemorrhagic stroke, prostate cancer and all-cause mortality. As such, physicians must be mindful in using it, and when patients are placed on vitamin E to treat NASH, its efficacy should be assessed after 6 to 12 mo of therapy[15]. The current recommendations advocate for use of Vitamin E in nondiabetic patients with biopsy proven NASH[2].

Angiotensin-converting enzyme inhibitors (ACE-I)

and angiotensin receptor blockers (ARBs) are important renin-angiotensin-aldosterone system (RAAS) modulators used in treating hypertension and proteinuria. There is also evidence that these agents help with overall insulin sensitivity in DM patients[19]. A meta-analysis by Al-Mallah *et al*[20] established that there was a 20% reduction in the incidence of new onset DM with the use of ACE-I and ARBs. The mechanism behind this may be through vasodilation and improved blood flow to the pancreas, promoting insulin secretion and delivery to tissues[19]. Studies for the role of the RAAS in NAFLD are ongoing. Animal models have shown a down regulation in pro-inflammatory and pro-fibrotic cytokines, leading to prevention of lobular inflammation along with hepatic fibrosis[21]. In humans, studies have largely looked at the role for ARBs only. A study by Georgescu *et al*[22] determined that Telmisartan improved transaminase levels and insulin resistance more than Valsartan. Only Telmisartan, however, showed a significant decrease in NAS activity score and fibrosis[22].

LIVER TRANSPLANTATION

End stage disease leads to consideration of liver transplantation in applicable patients. A meta-analysis looking at this issue found that NASH patients had equivalent survival compare to those who were transplanted for other causes[23]. Although it can be successful, transplantation in this patient population also carries with it significant risk. When compared to other etiologies for liver transplantation, death in subjects with underlying NASH was more likely due to cardiovascular events (OR = 1.65) and sepsis (OR = 1.71)[23]. This should emphasize the importance of modifying underlying risk factors. With the increase in the obesity epidemic in the developed world, NAFLD is projected to become the leading cause of liver transplantation in the near future[24]. Given the limited resources for transplantation, the need for effective therapies to prevent end-stage liver disease is all the more important.

FUTURE MANAGEMENT

An area of significant interest and study in the treatment of NAFLD is bariatric surgery. Interventions include gastric bypass, gastric banding and sleeve gastrectomy[2]. Early on, weight loss goals could be achieved with use of bariatric surgery as an adjuvant treatment to lifestyle modification. The reduced gastric remnant works to decrease hunger and stimulate satiety[25]. In addition to loss of central adiposity in subcutaneous tissues, it could also lead to a decrease fat deposition in the liver. Prospective data is mixed and indicates an improvement in steatosis and hepatocyte ballooning in NAFL[2]. Reversal of NASH with fibrosis, however, is less clear and appears more resistant[25]. As a result, current guidelines have not indicated that bariatric surgery should be used a specific treatment of NASH at this time.

An evolving area of interest in NAFLD pathogenesis and management involves the gut microbiome. Its role is complex and at present, incompletely understood. Resident microbiota consist of millions of microbial genes that influence metabolism, physiology and gene regulation[26]. They aid in synthesis of vitamins, digestion of fibers and prevention of pathogen colonization[27]. As such, the role in intestinal disorders such as C difficile infection, inflammatory bowel disease and irritable bowel syndrome is increasingly recognized[26]. It is thought that the microbiome's role in NAFLD may be mediated *via* the development of endotoxins involved in obesity and insulin resistance. The major fermentation products of microbiota are short chain fatty acids (SCFAs)[27]. There is also an association with increased production of SCFAs in overweight and obese subjects when compared to lean individuals[28]. The pathophysiology is thought to be due to an increased ability to harvest energy through the glucagon-like peptide 2 receptor[27]. The resident species of bacteria in the gut that are involved in these complex interactions is also important to consider. A study in obese patients by Ley *et al*[29] showed that the proportion of specific microbacteria (Bacteroidetes) was lower in obese compared to lean patients. This proportion subsequently increased with weight loss[29].

During digestion, bacterial metabolites are presented to the liver through the portal vein[27]. This allows exposure of gut-derived factors from the small intestine to potentially cause a downstream inflammatory response in liver tissue[1]. Interestingly, SCFAs may also play a protective role with down-regulating insulin signaling in adipose tissue through G-protein couple receptor 43[27]. The future of the gut microbiome is exciting and may one day lead to a personalized approach to manage NAFLD. This is a promising development for possible future pharmacologic applications in NAFLD.

There are a wide range of additional pharmacologic agents currently under study. Endocannabinoid CB1 receptors expressed on hepatocytes and myofibroblasts are contributors to hepatic fat storage[1]. Peripheral receptor antagonists could prove to be a beneficial strategy in this patient population. Additional newer oral hypoglycemic agents GLP-1 receptor analogues (Exenatide™) and DPP-4 inhibitors (Sitagliptin™) may play an indirect role through glycemic control. In patients with NAFLD and DM, Exenatide™ has been shown to improve body weight and decrease transaminases[1]. In addition, Sitagliptin™ decreases liver triglyceride content[1]. Pentoxifylline has been shown to decrease free-radical induced oxidative stress and inhibit lipid oxidation[1]. A meta-analysis of randomized double-blind controlled trials by Zeng *et al*[30] examined the application of pentoxifylline in NAFLD. Not only were there a decrease in aminotransferases, but also significant improvement in steatosis, lobular inflammation and fibrosis[30], suggesting this may become a potential treatment option[31].

Obeticholic acid is a bile acid derivative that may have a future role in non-cirrhotic NASH patients. Bile acids bind to Farnesoid X nuclear receptors to promote

insulin sensitivity and decrease hepatic gluconeogenesis along with circulating triglycerides[32]. A recent multi-center randomized controlled trial by Neuschwander-Tetri *et al*[33] showed a significant improvement in NAS score and fibrosis when compared to placebo (P = 0.0002). These derivatives also inhibit conversion of cholesterol to bile acids, which resulted in an increase in total cholesterol/low density lipoprotein along with decrease in HDL[33]. The clinical significance of this needs further exploration before recommendations can be made for its use.

Wolfberry, or Lycii fructus, is a well-known drug supplement in traditional Chinese medicine. Another common supplement now in North America is epigallocatechin-3-gallate (pure green tea extract). The premise behind their potential beneficial effect relies on reduction of oxidative stress and inflammation within hepatocytes. The endpoint would be less downstream damage within the liver. Xiao *et al*[34] noted that rat models showed an improvement in fat accumulation, fibrosis and eventual histology. The key molecules include many pro-inflammatory cytokines including interleukin molecules. Both of these supplements also mediate inflammation by regulating nuclear factor kappa B, which many chemokine signaling pathways depend on. The application in human models is ongoing.

CONCLUSION

There is currently no single pharmacologic or surgical therapy that has been shown to be universally effective in all patients with NAFLD. Histologic regression presently hinges on lifestyle modification through diet and exercise with the goal of improvement in weight as well as the detection and management of associated metabolic disorders. As such, caloric restriction along with regular exercise should be considered in all patients. The use of unfiltered, unsweetened coffee, reduction of ingestion of fructose and saturated fats are reasonable specific dietary recommendations for patients. Use of vitamin E should be restricted to non-diabetic patients with biopsy proven NASH and the use of oral hypoglycemic agents (*i.e.,* Pioglitazone) in nondiabetics should be considered on an individual patient basis. In patients with DM metformin is a reasonable first line agent. Statins have been proven to be safe in this population and given the increased cardiovascular mortality in NAFLD, should be used as indicated for management of hyperlipidemia.

Although not yet widely recommended, the use of surgical techniques including bariatric surgery may aid in weight loss and contribute to improved insulin resistance and hepatic fat deposition in carefully selected patients. Transplantation is an option for end stage disease in some individuals. Underlying cardiovascular disease is still a concern for post transplant mortality and recurrence of NAFLD is common if underlying behaviors and risk factors are not addressed. Promising targets for future management include bile acid transport, the gut microbiome, pro-inflammatory and liver fibrosis pathways. We have come a long way, but still have to advocate for aggressive management of risk factors to prevent progression of NAFLD and potential impact on healthcare resources.

REFERENCES

1 **Federico A**, Zulli C, de Sio I, Del Prete A, Dallio M, Masarone M, Loguercio C. Focus on emerging drugs for the treatment of patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 16841-16857 [PMID: 25492998 DOI: 10.3748/wjg.v20.i45.16841]

2 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

3 **Promrat K**, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava JL, Wing RR. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 121-129 [PMID: 19827166 DOI: 10.1002/hep.23276]

4 **Kistler KD**, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol* 2011; **106**: 460-468; quiz 469 [PMID: 21206486 DOI: 10.1038/ajg.2010.488]

5 **Sullivan S**. Implications of diet on nonalcoholic fatty liver disease. *Curr Opin Gastroenterol* 2010; **26**: 160-164 [PMID: 20010099 DOI: 10.1097/MOG.0b013e3283358a58]

6 **Funaki M**. Saturated fatty acids and insulin resistance. *J Med Invest* 2009; **56**: 88-92 [PMID: 19763019 DOI: 10.2152/jmi.56.88]

7 **Kechagias S**, Ernersson A, Dahlqvist O, Lundberg P, Lindström T, Nystrom FH. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. *Gut* 2008; **57**: 649-654 [PMID: 18276725 DOI: 10.1136/gut.2007.131797]

8 **Clarke SD**. Nonalcoholic steatosis and steatohepatitis. I. Molecular mechanism for polyunsaturated fatty acid regulation of gene transcription. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G865-G869 [PMID: 11557505]

9 **Vos MB**, Lavine JE. Dietary fructose in nonalcoholic fatty liver disease. *Hepatology* 2013; **57**: 2525-2531 [PMID: 23390127 DOI: 10.1002/hep.26299]

10 **Abdelmalek MF**, Suzuki A, Guy C, Unalp-Arida A, Colvin R, Johnson RJ, Diehl AM. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 1961-1971 [PMID: 20301112 DOI: 10.1002/hep.23535]

11 **Bravi F**, Bosetti C, Tavani A, Gallus S, La Vecchia C. Coffee reduces risk for hepatocellular carcinoma: an updated meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 1413-1421.e1 [PMID: 23660416 DOI: 10.1016/j.cgh.2013.04.039]

12 **Molloy JW**, Calcagno CJ, Williams CD, Jones FJ, Torres DM, Harrison SA. Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. *Hepatology* 2012; **55**: 429-436 [PMID: 21987293 DOI: 10.1002/hep.24731]

13 **Torres DM**, Harrison SA. Is it time to write a prescription for coffee? Coffee and liver disease. *Gastroenterology* 2013; **144**: 670-672 [PMID: 23453671 DOI: 10.1053/j.gastro.2013.02.015]

14 **Sanyal AJ**, Abdelmalek MF, Suzuki A, Cummings OW, Chojkier M. No significant effects of ethyl-eicosapentanoic acid on histologic features of nonalcoholic steatohepatitis in a phase 2 trial. *Gastroenterology* 2014; **147**: 377-384.e1 [PMID: 24818764 DOI: 10.1053/j.gastro.2014.04.046]

15 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

16 **Li Y**, Liu L, Wang B, Wang J, Chen D. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Biomed Rep* 2013; **1**: 57-64 [PMID: 24648894 DOI: 10.3892/br.2018.18]

17 **Bugianesi E**, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, David E, Rizzetto M, Marchesini G. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2005; **100**: 1082-1090 [PMID: 15842582 DOI: 10.1111/j.1572-0241.2005.41583.x]

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

19 **Paschos P**, Tziomalos K. Nonalcoholic fatty liver disease and the renin-angiotensin system: Implications for treatment. *World J Hepatol* 2012; **4**: 327-331 [PMID: 23355909 DOI: 10.4254/wjh.v4.i12.327]

20 **Al-Mallah M**, Khawaja O, Sinno M, Alzohaili O, Samra AB. Do angiotensin converting enzyme inhibitors or angiotensin receptor blockers prevent diabetes mellitus? A meta-analysis. *Cardiol J* 2010; **17**: 448-456 [PMID: 20865674]

21 **Warner FJ**, Lubel JS, McCaughan GW, Angus PW. Liver fibrosis: a balance of ACEs? *Clin Sci* (Lond) 2007; **113**: 109-118 [PMID: 17600527 DOI: 10.1042/CS20070026]

22 **Georgescu EF**, Ionescu R, Niculescu M, Mogoanta L, Vancica L. Angiotensin-receptor blockers as therapy for mild-to-moderate hypertension-associated non-alcoholic steatohepatitis. *World J Gastroenterol* 2009; **15**: 942-954 [PMID: 19248193 DOI: 10.3748/wjg.15.942]

23 **Wang X**, Li J, Riaz DR, Shi G, Liu C, Dai Y. Outcomes of liver transplantation for nonalcoholic steatohepatitis: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2014; **12**: 394-402.e1 [PMID: 24076414 DOI: 10.1016/j.cgh.2013.09.023]

24 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

25 **Caiazzo R**, Lassailly G, Leteurtre E, Baud G, Verkindt H, Raverdy V, Buob D, Pigeyre M, Mathurin P, Pattou F. Roux-en-Y gastric bypass versus adjustable gastric banding to reduce nonalcoholic fatty liver disease: a 5-year controlled longitudinal study. *Ann Surg* 2014; **260**: 893-898; discussion 898-899 [PMID: 25379859 DOI: 10.1097/SLA.0000000000000945]

26 **Kinross JM**, von Roon AC, Holmes E, Darzi A, Nicholson JK. The human gut microbiome: implications for future health care. *Curr Gastroenterol Rep* 2008; **10**: 396-403 [PMID: 18627653 DOI: 10.1186/gm228]

27 **Zhu L**, Baker RD, Baker SS. Gut microbiome and nonalcoholic fatty liver diseases. *Pediatr Res* 2015; **77**: 245-251 [PMID: 25310763 DOI: 10.1038/pr.2014.157]

28 **Schwiertz A**, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* (Silver Spring) 2010; **18**: 190-195 [PMID: 19498350 DOI: 10.1038/oby.2009.167]

29 **Ley RE**, Turnbaugah PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022-1023 [PMID: 17183309]

30 **Zeng T**, Zhang CL, Zhao XL, Xie KQ. Pentoxifylline for the treatment of nonalcoholic fatty liver disease: a meta-analysis of randomized double-blind, placebo-controlled studies. *Eur J Gastroenterol Hepatol* 2014; **26**: 646-653 [PMID: 24743504 DOI: 10.1097/MEG.0000000000000068]

31 **Du J**, Ma YY, Yu CH, Li YM. Effects of pentoxifylline on non-alcoholic fatty liver disease: a meta-analysis. *World J Gastroenterol* 2014; **20**: 569-577 [PMID: 24574727 DOI: 10.3748/wjg.v20.i2.569]

32 **Porez G**, Prawitt J, Gross B, Staels B. Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. *J Lipid Res* 2012; **53**: 1723-1737 [PMID: 22550135 DOI: 10.1194/jlr.R024794]

33 **Neuschwander-Tetri BA**, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 956-965 [PMID: 25468160 DOI: 10.1016/S0140-6736(14)61933-4]

34 **Xiao J**, Fai So K, Liong EC, Tipoe GL. Recent advances in the herbal treatment of non-alcoholic Fatty liver disease. *J Tradit Complement Med* 2013; **3**: 88-94 [PMID: 24716162 DOI: 10.4103/2225-4110.110411]

P- Reviewer: Cheng ML, Nair DG **S- Editor**: Gong ZM
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.i30.2968

World J Hepatol 2015 December 28; 7(30): 2968-2979
ISSN 1948-5182 (online)

REVIEW

Extragastric manifestations of *Helicobacter pylori* infection: Possible role of bacterium in liver and pancreas diseases

Elizabeth MA Rabelo-Gonçalves, Bruna M Roesler, José MR Zeitune

Elizabeth MA Rabelo-Gonçalves, Bruna M Roesler, Laboratory of Gastroenterology and Hepatology, Center of Diagnosis of Digestive Diseases, State University of Campinas, Campinas, São Paulo 13083-878, Brazil

José MR Zeitune, Department of Internal Medicine, Faculty of Medical Sciences, State University of Campinas, Campinas, São Paulo13083-887, Brazil

Author contributions: Rabelo-Gonçalves EMA contributed to the study idea, study design, literature search, manuscript writing and final revision of the article; Roesler BM contributed to the literature search, manuscript writing and final revision of the article; Zeitune JMR contributed to study design, manuscript writing and the final version of the article.

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

Correspondence to: Elizabeth MA Rabelo-Gonçalves, BSc, MSc, Laboratory of Gastroenterology and Hepatology, Center of Diagnosis of Digestive Diseases, State University of Campinas, Rua Carlos Chagas 420, Campinas, São Paulo 13083-878, Brazil. elizabeth.goncalves@gc.unicamp.br
Telephone: +55-19-35218557
Fax: +55-19-35218566

Received: April 27, 2015
Peer-review started: May 4, 2015
First decision: July 6, 2015
Revised: November 26, 2015
Accepted: December 13, 2015
Article in press: December 15, 2015
Published online: December 28, 2015

Abstract

Helicobacter pylori (*H. pylori*) is an ancient microorganism that has co-evolved with humans for over 60000 years. This bacterium typically colonizes the human stomach and it is currently recognized as the most common infectious pathogen of the gastroduodenal tract. Although its chronic infection is associated with gastritis, peptic ulcer, dysplasia, neoplasia, MALT lymphoma and gastric adenocarcinoma, it has been suggested the possible association of *H. pylori* infection with several extragastric effects including hepatobiliary and pancreatic diseases. Since a microorganism resembling *H. pylori* was detected in samples from patients with hepatobiliary disorders, several reports have been discussed the possible role of bacteria in hepatic diseases as hepatocellular carcinoma, cirrhosis and hepatic encephalopathy, nonalcoholic fatty liver disease and fibrosis. Additionally, studies have reported the possible association between *H. pylori* infection and pancreatic diseases, especially because it has been suggested that this infection could change the pancreatic physiology. Some of them have related a possible association between the microorganism and pancreatic cancer. *H. pylori* infection has also been suggested to play a role in the acute and chronic pancreatitis pathogenesis, autoimmune pancreatitis, diabetes mellitus and metabolic syndrome. Considering that association of *H. pylori* to liver and pancreas diseases needs further clarification, our work offers a review about the results of some investigations related to the potential pathogenicity of *H. pylori* in these extragastric diseases.

Key words: Hepatocellular carcinoma; Cirrhosis; Hepatic encephalopathy; *Helicobacter pylori*; Nonalcoholic fatty liver disease; Fibrosis; Pancreatitis; Pancreatic cancer; Diabetes mellitus; Metabolic syndrome

Core tip: *Helicobacter pylori* (*H. pylori*) has been

associated with several extragastric manifestations, including liver and pancreas diseases. Evidence for its role in the pathogenesis of chronic liver diseases and liver carcinoma is supported by several clinical and experimental studies. Furthermore, epidemiologic and serology-based works have reported a possible association between the microorganism and pancreatic cancer. *H. pylori* infection has also been linked to the acute and chronic pancreatitis pathogenesis and it could be related to the development of autoimmune pancreatitis, diabetes mellitus and metabolic syndrome. This review summarizes recent findings on the possible role of *H. pylori* infection in the etiology of liver and pancreas disorders.

Rabelo-Gonçalves EMA, Roesler BM, Zeitune JMR. Extragastric manifestations of *Helicobacter pylori* infection: Possible role of bacterium in liver and pancreas diseases. *World J Hepatol* 2015; 7(30): 2968-2979 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i30/2968.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i30.2968

INTRODUCTION

Helicobacter pylori (*H. pylori*) is an ancient organism that has co-evolved with humans for over 60000 years[1]. This bacterium typically colonizes the human stomach and it is currently recognized as the most common infectious pathogen of the gastroduodenal tract[2]. Most infected individuals do not develop disease, leading to the hypothesis that some *H. pylori* strains are harmless or even beneficial[3]. However, its chronic infection is associated with increased risk for several disease outcomes including gastritis, peptic ulcer, dysplasia, neoplasia, mucosa associated lymphoid tissue lymphoma and invasive gastric adenocarcinoma[4].

In recent years, it has been suggested the possible role of *H. pylori* infection with several extragastric effects including neurodegenerative, metabolic and cardiovascular conditions, as well as hepatobiliary, pancreatic and colorectal diseases[5-7]. Moreover, studies indicate that this bacterium may be related to the development of skin diseases such as urticaria as well as rheumatic disorders[8,9].

Since *Helicobacter* spp. have been isolated from the liver samples of a variety of mammals, it was suggested that bacteria may be involved in the pathogenesis of chronic liver diseases (CLD) and liver carcinoma[10-12]. Regarding to human studies, a microorganism resembling *H. pylori* was firstly detected in resected gallbladder mucosa of patient with gallstone[13]. Nilsson *et al*[14] reported the detection of *H. pylori* using molecular biology techniques such as polymerase chain reaction (PCR), hybridization and partial DNA sequencing in liver samples from patients with primary sclerosing cholangitis and primary biliary cirrhosis (PBC).

Subsequent studies have verified the possible association of *H. pylori* infection in the development of other liver diseases, particularly hepatocellular carcinoma (HCC), in different geographic areas. Some authors have reported a very high prevalence of antibodies to *H. pylori* and the prevalence of bacteria in patients with cirrhosis, compared with controls[15-18]. Although *Helicobacter* spp. DNA was successfully detected in liver samples of individuals with primary liver carcinoma[19-23], the remaining question was whether these findings corresponded to the true liver colonization by bacteria or whether *H. pylori* DNA could result from the retrograde transfer of this DNA from the duodenum to liver. Nevertheless, the isolation of *H. pylori* in culture medium using liver samples was crucial for supporting the true bacterial colonization[24,25].

Another extragastric digestive organ indicated as a possible target for *H. pylori* is the pancreas and some mechanisms by which *H. pylori* infection may influence pancreatic physiology have been object of several studies. It has been suggested that some aggressive factors produced by this microorganism such as ammonia and lypopolysaccharides (LPSs), as well as the production of inflammatory cytokines, could induce the pancreas damage. These conditions associated with the activation of leukocytes could be responsible for the clinical outcome of the pancreas diseases[26].

Considering these aspects, several epidemiologic and serology-based studies have reported a possible association between the microorganism and pancreatic cancer[27-30] and, in addition, epidemiological studies have examined if there could be any association between peptic ulcers development and risk of pancreatic cancer. Results from cohort studies with large number of pancreatic cancer cases and detailed information on type of peptic ulcers (*i.e.*, gastric *vs* duodenal) observed positive associations with gastric ulcers, but not duodenal ulcers[31,32]. *H. pylori* infection has also been suggested to play a role in the acute and chronic pancreatitis (CP) pathogenesis[33-35] and it has been reported that this infection could be related to autoimmune pancreatitis (AIP) mainly through induction of autoimmunity, by molecular mimicry, and apoptosis[36]. Finally, a possible role of *H. pylori* in diabetes mellitus (DM)[37,38] and metabolic syndrome have been investigated[39,40].

However, the interpretation of so different results obtained from the mentioned studies makes the interpretation of their relevance be inconclusive. *H. pylori* more virulent strains, such as the ones that harbor the CagA pathogenicity island, together with the host characteristics, could be important for the clinical expression of pancreatic diseases, and other systemic disorders. In some cases, *H. pylori* might be a minor factor contributing to disease development owing to the persistent inflammatory state of the gastric mucosa[41]. Considering these findings and that, the role of *H. pylori* infection in the liver and pancreatic diseases remains controversial, in the review we attempt to discuss about the results of some investigations related to the potential pathogenicity of *H. pylori* in these extra gastric diseases.

ROLE OF *H. PYLORI* IN LIVER DISEASES

HCC

The HCC is the main primary malignant tumor of the liver and the fifth most common type of cancer. It is estimated that more than 50000 cases are diagnosed annually and that the disease is the third leading cause of cancer-related death in the world population[42-47]. The cases of death due to HCC vary 250000-1 million individuals per year and its prevalence varies according to geographic location, gender, age and ethnicity[48].

The development of HCC is attributed to several factors such as alcoholism, exposure to mycotoxins (aflatoxins), hereditary hemochromatosis, PBC, deficiency of α-1-anti-trypsin, Wilson's disease and microcystin[21,49-51]. Chronic viral infections are considered the main risk factor of HCC in 75%-80% of cases[47], wherein hepatitis B virus (HBV) infection is responsible by 50%-55% of cases[52] and hepatitis C virus (HCV) infection occurs in 25%-30% of patients[53]. However, the detection of *H. pylori* in patients with HCC may suggest the role of bacteria in the pathogenesis of this disease.

Several studies have tried to correlate the *H. pylori* infection in the development of HCC. Avenaud *et al*[20] detected *H. pylori* in 100% (8/8) of the liver tissue employed in their study. In 2001, Swedish researchers identified *Helicobacter* spp. in patients with HCC and cholangiocarcinoma[54]. Evaluating patients with chronic hepatitis, cirrhosis and HCC, Dore *et al*[21] used the serology and PCR to verify the presence of *H. pylori* infection. The results showed that 54% of patients were positive for the bacteria, and the prevalence of infection was higher in patients with HCC (73%) compared with patients with cirrhosis (58%) and chronic hepatitis (39%). *Helicobacter* DNA was detected in 17% of liver cirrhosis patients and 55% of individuals with HCC.

Verhoef *et al*[55] revealed the presence of *Helicobacter* DNA in 45% of liver samples from patients with HCC in contrast to 10% positive samples in the control group. Sequence analysis indicated that fragments had similarity with DNA of *H. pylori*. In this study the authors also noted the similarity among three samples of gastric biopsies of patients with HCC who were positive for the liver culture, suggesting that gastric colonization with *H. pylori* strains may be associated with the induction of HCC.

In 2004, Pellicano *et al*[56] confirmed the presence of *Helicobacter* spp. DNA in 17 (85%) of 20 liver samples from patients with HCC compared with 33% of positive *Helicobacter* cases in the control samples. Recently, more studies emphasize the detection of bacteria in hepatic tissue from patients with HCC, even suggesting its possible role in the progression of CLD due to higher prevalence of *H. pylori* in more advanced stages of liver disease as cirrhosis and HCC[25,57].

In addition, several researchers have described the association between *H. pylori* and HBV or HCV in the development of HCC. In fact, the prevalence of anti-*H. pylori* antibodies in patients infected with HBV is significantly higher when compared to subjects without viral infection[17,18,58-63]. In an attempt to verify the role of *H. pylori* in the progression of CLD in patients infected with HCV, investigators have reported that *Helicobacter* spp. DNA was detected in 4.2% of controls and 3.5% of individuals with noncirrhotic chronic hepatitis, compared with 61%-68% in cirrhotic liver and 90% in HCC tumoral tissue[22]. These results reinforced that prevalence of *H. pylori* infection may be associated with later stages of CLD and suggested that disease increased with the severity of the cancer. Then, it may be possible that *H. pylori* co-infection with HBV or HCV results in the progression from cirrhosis to cancer, reinforcing the synergistic cooperation between *H. pylori* and hepatitis virus in the development of HCC[47].

The mechanism by which *H. pylori* colonizes the human liver is not fully understood. Some researchers hypothesized that *H. pylori* DNA detection in liver tissue can result from bacterial translocation from the stomach into the blood through the portal system, especially in the advanced stages of chronic liver disease, when occurs the portal hypertension[47,64]. Furthermore, bacteria can reach the liver *via* circulating phagocytes and macrophages or retrograde transfer from the duodenum[24]. However, reports that involved *H. pylori* culture from the liver samples of patients with HCC support a true hepatic colonization by bacteria, discarding the possibility of retrograde contamination[25,65]. Additionally, no other bacteria in the digestive tract are associated with human liver carcinogenesis[66,67].

The experimental murine models of *H. hepaticus* infection show that bacteria are able to induce chronic active hepatitis and HCC in various strains of animals[11,68]. Moreover, enteric *Helicobacter* species are capable of producing toxins which can cause hepatocellular injury *in vivo*[69]. Furthermore, *Helicobacter* spp. may induce the production of proinflammatory chemokines and cytokines which contributes to the development of liver cancer by DNA damage, growth stimulation, increase of survival, angiogenesis and invasion into host tissue[70].

In a report employing human hepatocytes culture infected with *H. pylori*, Ito *et al*[71] have shown that bacteria are able to adhere and penetrate into these cells. The authors suggest that the process of bacterial internalization can be an *H. pylori* strategy to avoid the host immune reaction and remain in the liver, resulting in morphological and physiological changes in the hepatocytes. Analyzing the *in vitro* proliferation, adhesion and invasion responses of the hepatic tumor cell lines to LPS authors demonstrated that these characteristics were increased in response to LPS, which may be related to increased gene expression of interleukin-8 (IL-8) and transforming growth factor-beta 1 (TGF-β1). Considering that *H. pylori* has LPS, they inferred that bacteria may be ignored by host immune system and directly promote adhesion and invasion of hepatoma cells mediated by LPS[72].

In addition to the host immune system evasion

mechanisms, *H. pylori* virulence factors detected in liver samples from patients with HCC, such as *vac*A and *cagA* genes, are supposed to be involved in liver carcinogenesis[21,63,73]. These findings were recently supported by Esmat *et al*[74] who found that the positivity of *cagA* gene was directly proportional to the severity of liver disease. They studied patients infected with HCV in the presence and absence of cirrhosis and HCC. The *cag*A positivity occurred in 75% of patients with cirrhosis and HCC, 52.9% of cirrhosis subjects without HCC and 32% of individuals with chronic hepatitis. The authors have also shown significant differences when compared METAVIR system among groups which confirmed *H. pylori* association with later stages of fibrosis.

Cirrhosis and hepatic encephalopathy

Cirrhosis is a major health problem with high incidence and prevalence worldwide[75]. Considering that patients with cirrhosis are more likely to develop gastrointestinal mucosal lesions, with increased risk for peptic ulcer disease (PUD)[76], it was suggested that *H. pylori* infection has an important role in the pathogenesis of PUD in cirrhotic patients and may be related to the development of hepatic encephalopathy (HE) and hyperammonemia[77]. In fact, a recent review has demonstrated that eradication of *H. pylori* infection in patients with cirrhosis may have a positive effect on the control of hyperammonemia and HE[78].

In a meta-analysis, researchers have described that prevalence of *H. pylori* infection in individuals with cirrhosis has increased significantly worldwide, especially in Europe and America, due to viral cirrhosis and PBC[79]. However, Pellicano *et al*[80] related that some papers which concluded that there was no association between the prevalence of bacteria and PBC[81,82] were not reported in this meta-analysis. Another report demonstrated that there is a significant association between *H. pylori* infection and portal hypertensive gastropathy (PHG) in cirrhotic patients which is also related to the severity of PHG, suggesting that eradication of *H. pylori* must be considered in cirrhotic patients with PHG[75].

HE is a frequent complication of liver cirrhosis and manifests itself as a wide variety of neuropsychiatric symptoms[83]. It is admitted that ammonia is the most relevant substance in the pathogenesis of HE. Then, reduction of ammonia production in the gastrointestinal tract is a current treatment strategy for HE[78]. Considering that *H. pylori* urease hydrolyses urea present in the gastric juice into ammonia and carbon dioxide, and the amount of ammonia produced in the gastric mucosa could increase blood ammonia levels in cirrhotic patients, the possible participation of *H. pylori* infection in the pathogenesis of HE has been studied.

The current data associating *H. pylori* infection to the pathophysiology of HE are inconclusive. Initially, some reports have demonstrated that *H. pylori* contributes to hyperammonemia in cirrhosis, and the eradication of bacteria may reduce the blood ammonia[84-86]. After that, other studies have not found a significant difference in the ammonia levels between cirrhotic patients with and without *H. pylori* infection[87,88]. In a review, Zullo *et al*[89] confirmed that gastric ammonia production by *H. pylori* urease appears to be inadequate to clinically affect ammonia levels in the majority if cirrhotic patients.

In a recent review, it was demonstrated that positivity of *H. pylori* infection was higher in HE patients compared to non-HE individuals, particularly in the older subjects. However, there are no strong evidences for an effect of bacteria on increasing blood ammonia level, nor there is strong evidence to support the hypothesis that *H. pylori* eradication can reduce blood ammonia level and improve HE symptoms[90].

However, authors have described that HE is not fully reversible and *H. pylori* might contribute to persistent cognitive impairment even after resolution of symptoms[91]. Furthermore, it is believed that inflammatory cytokines produced during *H. pylori* infection may cross the blood-brain barrier and contribute to the pathogenesis of cognitive dysfunction associated with cirrhosis[92].

Nonalcoholic fatty liver disease

The nonalcoholic fatty liver disease (NAFLD) is currently considered the most common liver disease in Western countries, affecting up to 25%-30% of subjects[93-95]. NAFLD includes a broad spectrum of liver disorders, which range from nonalcoholic fatty liver to nonalcoholic steatohepatitis (NASH) which may progress to cirrhosis and HCC without significant alcohol consumption[96-98].

Authors have shown the possible participation of *H. pylori* infection in the pathogenesis of insulin resistance (IR)[39]. Considering that IR is implicated in the etiology of NAFLD, the evaluation of *H. pylori* infection as a risk factor for IR may help clarify its effect on NAFLD[99].

In 2008, researchers have detected *H. pylori* 16S rDNA in hepatic tissue collected from a 44-year-old woman with NASH[100]. After that, in a study employing liver samples from patients with CLD, *H. pylori* DNA was amplified in 45.5% (5/11) of samples obtained from subjects with NAFLD[57].

The case report described by Abenavoli *et al*[101] related the improvement in IR and fatty liver indices after *H. pylori* eradication therapy, reinforcing the possible association among *H. pylori* infection, IR and NAFLD. In the same year, another study revealed that prevalence of anti-*H. pylori* IgG titers, together with lower circulating adiponectin and higher tumor necrosis factor-α levels, was higher in individuals with NAFLD compared with control group[102]. Moreover, using logistic regression analysis model, researchers mentioned that both *H. pylori* infection and Homeostasis Model of Assessment-Insulin Resistance which is the marker of the metabolic syndrome, were considered independent variables to predict NAFLD. In addition, no correlation was found between *H. pylori* infection and progression to NASH. Then, it was suggested that presence of *H. pylori* may be involved to early-stage NAFLD.

However, Sumida *et al*[103] performed a recent study

which was the first one to show that NASH is more prevalent in *H. pylori*-positive patients than in non-infected individuals. Histopathologic analysis revealed that bacteria was associated with hepatocyte ballooning; however, there was no association of *H. pylori* with steatosis or liver fibrosis. Authors still mentioned that although the exact pathogenic mechanisms involved in hepatocyte ballooning as well as its role in NAFLD remain unclear, it is considered as a key histologic feature of NASH.

Considering that the prevalence of NAFLD is increasing worldwide and that *H. pylori* infection may present a role in its pathogenesis, further studies on this area are needed, in order to provide better understanding of the role of *H. pylori* infection in NAFLD. However, once this association is confirmed, it is possible that *H. pylori* eradication regimens might have therapeutic implications on NAFLD[39].

Fibrosis

As mentioned before, *Helicobacter* spp. infection is more prevalent in advanced stages of liver diseases, reinforcing the possible association of bacteria with the progression of chronic hepatitis to cirrhosis and HCC[49].

In order to elucidate these findings, researchers have induced experimental hepatic fibrosis with carbon tetrachloride (CCl4) administration in mice and rats orally challenged with *H. pylori*. Authors verified a significant increase in the fibrotic score in *H. pylori*-positive animals treated with CCl4 when compared with non-infected animals treated with CCl4. Furthermore, they observed that alpha-smooth muscle actin and TGF-β1 also enhanced in *H. pylori* infected animals[104].

After that, authors have suggested that increased liver fibrosis in *H. pylori* infection may occur through increased TGF-β1 induced pro-inflammatory signaling pathways in hepatic stellate cell line (HSC). They still mentioned that *H. pylori* infection may be involved in increased risk TGF-β1-mediated tumorigenesis by disturbing the balance between apoptosis and proliferation of hepatocytes[105]. Another group has also described an increase in activated kupffer cells and hydrogen peroxide levels in *H. pylori* infection which might result in activation of HSC alone or in combination with TGF-β1, amplifying hepatic inflammation *via* release of proinflammatory cytokines[106,107].

More recently, Esmat *et al*[74] studied liver samples from patients with HCV-related chronic hepatitis and cirrhosis in the presence or absence of HCC in order to verify the possible role of *H. pylori* infection in the disease progression. They showed that prevalence of *cag*A gene was directly proportional to severity of liver disease and was more positive in advanced stages of fibrosis (28.2%) compared to early stages (5.9%); it was still suggested by authors that *H. pylori* can produce toxins that may interfere with hepatic cells.

One aspect that deserves further analysis of hepatology experts is the analysis of a possible role of *H. pylori* infection in the liver fibrosis determined by schistosomiasis.

Therefore, these findings confirm that hepatocytes can be stimulated by *H. pylori* infection, resulting in collagen accumulation and, consequently, in hepatic fibrosis[108].

ROLE OF *H. PYLORI* IN PANCREAS DISEASES

Acute and CP

In the last decades, acute pancreatitis, defined as an acute inflammatory response from unregulated activation of pancreatic enzymes, has demonstrated an increase in its incidence[109,110]. This disorder, which can present a persistent hypovolemia, besides a decreased intravascular volume, can lead to extrapancreatic complications[111]. In 2007, a publication revealed an important frequency of patients with acute pancreatitis that also presented acute gastrointestinal mucosal damage[112] and it suggests that this bacterium infection could somewhat influence the acute pancreatitis progression.

Two important mechanisms-hypergastrinemia and duodenal acidification-together with the translocation of the microorganism or its toxins into the pancreas, have been cited as important mechanisms by which *H. pylori* infection could have an effect on the acute pancreatitis progression[26]. Warzecha *et al*[33] observed the effect of *H. pylori* infection of the gastric mucosa on the clinical evaluation of the disease in a model of ischaemia/reperfusion-induced in rats. Their results have suggested an evidence of damage effect of *H. pylori* infection of the stomach in patients diagnosed with acute pancreatitis. Lee *et al*[113] trying to evaluate the relationship between PUD and acute pancreatitis, studied 78 patients with acute pancreatitis, and 41 of them suffered from peptic ulcer disease, but only 31.7% of these 41 patients were infected by *H. pylori*. They concluded that PUD is associated with severe acute pancreatitis and the treatment for PUD should be considered for patients with that disease.

As regards to the possible association between CP and *H. pylori* infection, different hypothesis have been suggested. According to Manes *et al*[26], there are three possible roles of this infection and the evolution of CP: The influence of this microorganism infection in the pathogenesis and evolution in idiopathic forms of CP, the influence of this infection on the exocrine pancreatic secretion in individuals with CP, and the possibility of CP influences the gastrointestinal physiology and, consequently, the pathogen colonization.

Besides, important alterations in the gastric function found in CP could demonstrate the presence of *H. pylori* infection in the stomach rather than changes induced by the pancreatic disease. Study developed by Manes *et al*[35] reported that the prevalence of *H. pylori* infection in patients with CP is similar that of two other studied groups of individuals, one with patients with alcoholic liver cirrhosis and other with healthy subjects, but the

frequency and severity of *H. pylori* negative chronic gastritis in the antrum was significantly higher in individuals with CP than in the other groups. Similar results were found by Niemann *et al*[34]. In their study, the prevalence of *H. pylori* infection was investigated in individuals with CP, with and without duodenal ulcers, in comparison to a control group which only included patients with duodenal ulcer. The results suggested that the bacterium infection can contribute to the CP development, but no as the main cause for the disease development.

Pancreatic cancer

The potential role of *H. pylori* infection in pancreatic cancer (exocrine pancreatic carcinoma or pancreatic ductal adenocarcinoma) has also been suggested. This cancer is the fifth leading cause of cancer related death worldwide. Its high degree of death incidence is especially due to the diagnosis generally done in the advanced stage and to the poor responses to current treatments[114-117].

Lowenfels *et al*[118] considered that similarly pathologic consequences of gastric tissue due to *H. pylori* chronic infection could be observed in CP and consequently in pancreatic cancer.

A meta-analysis of six cohort studies and one case-control study found that the pooled relative risk estimate for pancreatic cancer among patients with CP is 13.3[119]. Duell *et al*[120] analyzed 5048 patients with pancreatic cancer in ten case-control studies and found only a small association between pancreatic cancer and antecedent CP, although this study presented some limitations. Despite of these findings, it has been considered that CP is a rare cause of pancreatic cancer[121].

Other possible mechanisms for the association between *H. pylori* and pancreatic cancer include changes in gastrin (increased secretion) and somatostatin (low number of antral somatostatin cells) resulting from *H. pylori* gastritis[28,122-124], increased DNA synthesis, increased formation of N-nitroso components (due to bacterial overgrowth), and chronic inflammation properly, aspect that can be responsible by itself for initiating the carcinogenesis process[125-127].

In addition, *H. pylori* chronic infection, as responsible for the production of proinflammatory cytokines and reactive oxygen species, as well as of other inflammatory mediators, may induce the tissue inflammation. In consequence, the increase on genomic DNA damage and cell proliferation (aspects that may lead to an inactivation of tumor-suppressor genes), are factors which may contribute the malignant transformation of pancreatic cells[128]. Considering it, Takayama *et al*[129] reported that activities of nuclear factor-κb, activator protein-1, and serum response element of human pancreatic cancer cells were shown to be increased by *H. pylori* infection, as well as serum levels of IL-8, suggesting that the development of pancreatic cancer could be similar to the gastric carcinogenesis. Consequently, environmental aspects such as dietary habits, smoking and alcohol consumption can contribute for the development of pancreatic cancer[130], as well as they contribute in gastric cancer.

Finally, other important meta-analysis concluded that *H. pylori* infection can be considered a significantly factor to the pancreatic cancer development, also considering that regional aspects can be important for this. Xiao *et al*[29] reported countries regional aspects reporting that the association between *H. pylori* infection and pancreatic cancer development is more evident in Europe and East Asia, and decreases in North America. They also suggested that *H. pylori* CagA positive strains are not possibly associated with pancreatic cancer development. Despite of it, meta-analysis conducted by Wang *et al*[30] concluded that *H. pylori* infection and CagA positive strains are associated with a decreased risk of pancreatic cancer in Eastern populations but have no significant associations in Western countries. Lindkvist *et al*[131], in a prospective study, also concluded that no association between this pathogen infection and the risk for pancreatic cancer was found in their nested case-control study within a population based cohort. Nevertheless, recent study with 56 cases of pancreatic cancer analyzed anti-Hp IgG (*H. pylori*-specific antibodies), Hp IgM (*H. pylori* antibodies) and CagA-Hp-IgG (*H. pylori* serotoxin-associated protein A antibody), comparing the results with a control group. The results obtained demonstrated that *H. pylori* infection rate in the patients group was significantly higher than that in the control group ($P < 0.01$). Besides, the positive rate of CagA-Hp in the observation group was 38.88%, and 21.53% in the control group ($P < 0.05$). The researchers concluded that *H. pylori* infection, especially with CagA positive strains, besides smoking history and the history of CP, is one of the risk factors for pancreatic cancer development[132].

AIP

AIP has been recognized as a form of CP, which is always associated with autoimmune manifestations[133]. It is defined as an inflammatory process of the pancreas characterized by hypergammaglobulinemia, enlargement of the organ, fibrotic changes with lymphocytic infiltrations and presence of autoantibodies, among other alterations, all of them contributing to the tissue destruction possibly by apoptosis[36].

The coexistence of AIP with other autoimmune diseases has been reported in the literature. Among them, they can be cited Sjögren's syndrome, PBC, autoimmune hepatitis, Hashimoto's thyroiditis and gastric ulcer, among others[134-136].

As regards to the mechanisms by which *H. pylori* infection could trigger AIP, the molecular mimicry between bacterial antigens and human ones has been reported as the most plausible hypothesis, based on epidemiological studies[137,138].

According to Kountouras *et al*[36], bacterial heat shock proteins (Hsps), particularly Hsp-60 or Hsp-70 of *H. pylori*, may represent major target antigens

responsible for molecular mimicry causing autoreactivity between this microorganism and the host's immune gastric tissue, being probably responsible for the humoral and/or cellular (T-cell) response against these proteins and consequently influencing the pathogenesis of autoimmune diseases such as AIP[139,140].

Guarneri *et al*[141] reported the existence of two molecules possibly involved in the molecular mimicry (CA-Ⅱ and α-HpCA), that are homologous to the HLA molecule DRB1*0405, reported as a risk factor for the development of AIP[142]. Considering it, and the importance of α-HpCA for gastric colonization, the host immune response against this molecule could turn against the autoantigen CA-Ⅱ, promoting the appearance of AIP in genetically predisposed individuals[142,143].

Besides, HLA-DR antigens are expressed on the pancreatic duct cells as well as on $CD4^+$ suggesting an autoimmune mechanism involved in inflammation[134,144], event that, in conjunction with epithelial cells apoptosis, can be upregulated by *H. pylori* infection and, consequently, be important to the development of AIP[140].

Besides, important researches have indicate that apoptosis is a mechanism of cell death in several important *H. pylori*-associated upper gastrointestinal damages and in extradigestive disorders. These studies hypothesized that *H. pylori* could change the expression of some genes including that ones encoding growth factors, transcription factors, and apoptosis proteins, among others, consequently contributing to the development both of gastrointestinal and extradigestive diseases. In addition, some virulence factors of *H. pylori*, such as urease, could contribute to cell apoptosis probably to activation of T cells[36]. Finally, in addition, microcirculatory changes promoted by *H. pylori* infection through platelet and platelet-leukocyte aggregation could promote the amplification of the pancreatic injury[142,145,146].

Obviously the relationship between *H. pylori* infection and AIP development has to better studied, but it can be considered that various autoimmune and apoptotic sequelae induced by this chronic and long-term infection appear to influence the pathophysiology of AIP.

DM and metabolic disorders

Type 1 or type 2 DM development have also been reported to a high prevalence of *H. pylori* positive individuals[147]. In a meta-analysis, Zhou *et al*[148] found a high prevalence of this microorganism infection in individuals with DM, particularly type 2. Jeon *et al*[149] reported similar results and concluded in their sampling that individuals who were seropositive for *H. pylori* were 2.7 times more at risk to develop DM than seronegative patients. Nevertheless, this association remains controversial[150].

Gunji *et al*[151] evaluated the association between IR and *H. pylori* infection and suggested that this infection could really contribute even in an independent way to promoting this condition. Besides, study developed by So *et al*[152] in China concluded that *H. pylori* infection could be an independent predictor for hyperglycemia and reduced insulin sensitivity and this fact has shown to be important for the high prevalence of type 2 DM in this country population.

Metabolic syndrome, one of the most prevalent global health problems that predisposes to type 2 DM and it is linked to IR, has also be proposed to be associated to *H. pylori* infection[153,154]. Polyzos *et al*[39], through a quantitative homeostatic model, studied this possible association, but data concerning this relationship remain contradictory. In anyway, the eradication of the infection appears to prevent negative metabolic effects in the pathogenesis of DM[155].

CONCLUSION

The higher prevalence of *H. pylori* infection in subjects with hepatitis, cirrhosis, HE, NAFLD and HCC may suggest its possible role in the pathogenesis of CLD. Moreover, many studies have found a synergistic association between this bacterium and hepatitis viruses, mainly HCV, suggesting that *H. pylori* may represent a co-risk factor in the progression of liver diseases, especially HCC. However, high quality prospective studies in non-cirrhotic HCC patients co-infected with HCV are needed to confirm these findings.

Another important fact to consider is that patients with severe CLD are more likely to develop bacterial infection. Then, *H. pylori* infection could be related to immunological and inflammatory changes in the liver. In this context, bacterium would not be considered a risk factor for HCC and the colonization of hepatic tissue could result from tumor process.

Complex interactions in the gastric mucosal changes determined by the pathogen infection could contribute to the pancreatic cancer development, especially by N-nitrosamine exposure of the host, added by dietary and smoking habits. Important meta-analyses have reported an increased risk for pancreatic cancer development in subjects contaminated by *H. pylori*. In addition, it has also been suggested that *H. pylori* causes AIP due to molecular mimicry between bacterium and enzymes that are highly expressed in the pancreatic ductal and pancreatic cells, in addiction of apoptosis process. Finally, *H. pylori* infection has been associated to both type 1 and type 2 DM development, as well as metabolic syndrome, interaction that has not been determined. Despite some of these studies present some limitations, including a small number of patients and only hypothesis which have not been elucidated yet, the role played by *H. pylori* in the pathogenesis of such conditions have a substantial impact of healthcare and obviously its infection and the relationship with all the conditions described above has to be subject for further investigation.

Considering the worldwide liver and pancreatic diseases burden, as well as the possible association between *H. pylori* infection, which is commonly chronic and reported to poor sanitary conditions, besides the

widespread use of some medicines that can mask the real condition of the infection, a complete elucidation of the role played by *H. pylori* in the pathogenesis of such conditions have certainly a substantial impact of healthcare.

REFERENCES

1 **Moodley Y**, Linz B, Bond RP, Nieuwoudt M, Soodyall H, Schlebusch CM, Bernhöft S, Hale J, Suerbaum S, Mugisha L, van der Merwe SW, Achtman M. Age of the association between Helicobacter pylori and man. *PLoS Pathog* 2012; **8**: e1002693 [PMID: 22589724 DOI: 10.1371/journal.ppat.1002693]

2 **Roubaud Baudron C**, Franceschi F, Salles N, Gasbarrini A. Extragastric diseases and Helicobacter pylori. *Helicobacter* 2013; **18** Suppl 1: 44-51 [PMID: 24011245 DOI: 10.1111/hel.12077]

3 **Mishra S**. Is Helicobacter pylori good or bad? *Eur J Clin Microbiol Infect Dis* 2013; **32**: 301-304 [PMID: 23132690 DOI: 10.1007/s10096-012-1773-9]

4 **Makola D**, Peura DA, Crowe SE. Helicobacter pylori infection and related gastrointestinal diseases. *J Clin Gastroenterol* 2007; **41**: 548-558 [PMID: 17577110]

5 **Franceschi F**, Gasbarrini A. Helicobacter pylori and extragastric diseases. *Best Pract Res Clin Gastroenterol* 2007; **21**: 325-334 [PMID: 17382280]

6 **Roubaud-Baudron C**, Krolak-Salmon P, Quadrio I, Mégraud F, Salles N. Impact of chronic Helicobacter pylori infection on Alzheimer's disease: preliminary results. *Neurobiol Aging* 2012; **33**: 1009.e11-1009.e19 [PMID: 22133280 DOI: 10.1016/j.neurobiolaging.2011.10.021]

7 **Venerito M**, Selgrad M, Malfertheiner P. Helicobacter pylori: gastric cancer and extragastric malignancies - clinical aspects. *Helicobacter* 2013; **18** Suppl 1: 39-43 [PMID: 24011244 DOI: 10.1111/hel.12078]

8 **Tebbe B**, Geilen CC, Schulzke JD, Bojarski C, Radenhausen M, Orfanos CE. Helicobacter pylori infection and chronic urticaria. *J Am Acad Dermatol* 1996; **34**: 685-686 [PMID: 8601663]

9 **Zentilin P**, Seriolo B, Dulbecco P, Caratto E, Iiritano E, Fasciolo D, Bilardi C, Mansi C, Testa E, Savarino V. Eradication of Helicobacter pylori may reduce disease severity in rheumatoid arthritis. *Aliment Pharmacol Ther* 2002; **16**: 1291-1299 [PMID: 12144579]

10 **Fox JG**, Yan LL, Dewhirst FE, Paster BJ, Shames B, Murphy JC, Hayward A, Belcher JC, Mendes EN. Helicobacter bilis sp. nov., a novel Helicobacter species isolated from bile, livers, and intestines of aged, inbred mice. *J Clin Microbiol* 1995; **33**: 445-454 [PMID: 7536217]

11 **Fox JG**, Yan L, Shames B, Campbell J, Murphy JC, Li X. Persistent hepatitis and enterocolitis in germfree mice infected with Helicobacter hepaticus. *Infect Immun* 1996; **64**: 3673-3681 [PMID: 8751916]

12 **Franklin CL**, Beckwith CS, Livingston RS, Riley LK, Gibson SV, Besch-Williford CL, Hook RR. Isolation of a novel Helicobacter species, Helicobacter cholecystus sp. nov., from the gallbladders of Syrian hamsters with cholangiofibrosis and centrilobular pancreatitis. *J Clin Microbiol* 1996; **34**: 2952-2958 [PMID: 8940429]

13 **Kawaguchi M**, Saito T, Ohno H, Midorikawa S, Sanji T, Handa Y, Morita S, Yoshida H, Tsurui M, Misaka R, Hirota T, Saito M, Minami K. Bacteria closely resembling Helicobacter pylori detected immunohistologically and genetically in resected gallbladder mucosa. *J Gastroenterol* 1996; **31**: 294-298 [PMID: 8680555]

14 **Nilsson HO**, Taneera J, Castedal M, Glatz E, Olsson R, Wadström T. Identification of Helicobacter pylori and other Helicobacter species by PCR, hybridization, and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. *J Clin Microbiol* 2000; **38**: 1072-1076 [PMID: 10698999]

15 **Schmulson MJ**, De León G, Kershenovich A, Vargas-Vorackova F, Kershenobich D. Helicobacter pylori infection among patients with alcoholic and nonalcoholic cirrhosis. *Helicobacter* 1997; **2**: 149-151 [PMID: 9432344]

16 **Siringo S**, Vaira D, Menegatti M, Piscaglia F, Sofia S, Gaetani M, Miglioli M, Corinaldesi R, Bolondi L. High prevalence of Helicobacter pylori in liver cirrhosis: relationship with clinical and endoscopic features and the risk of peptic ulcer. *Dig Dis Sci* 1997; **42**: 2024-2030 [PMID: 9365129]

17 **Fan XG**, Zou YY, Wu AH, Li TG, Hu GL, Zhang Z. Seroprevalence of Helicobacter pylori infection in patients with hepatitis B. *Br J Biomed Sci* 1998; **55**: 176-178 [PMID: 10367401]

18 **Pellicano R**, Leone N, Berrutti M, Cutufia MA, Fiorentino M, Rizzetto M, Ponzetto A. Helicobacter pylori seroprevalence in hepatitis C virus positive patients with cirrhosis. *J Hepatol* 2000; **33**: 648-650 [PMID: 11059871]

19 **Ponzetto A**, Cutufia MA, D'Errico A, Angelino R, Pellicano R, Leone N. Demonstration of Helicobacter spp by PCR in liver of patients with liver cirrhosis and hepatocellular carcinoma. *Gut* 1999; **45**: A88

20 **Avenaud P**, Marais A, Monteiro L, Le Bail B, Bioulac Sage P, Balabaud C, Mégraud F. Detection of Helicobacter species in the liver of patients with and without primary liver carcinoma. *Cancer* 2000; **89**: 1431-1439 [PMID: 11013355]

21 **Dore MP**, Realdi G, Mura D, Graham DY, Sepulveda AR. Helicobacter infection in patients with HCV-related chronic hepatitis, cirrhosis, and hepatocellular carcinoma. *Dig Dis Sci* 2002; **47**: 1638-1643 [PMID: 12141829]

22 **Rocha M**, Avenaud P, Ménard A, Le Bail B, Balabaud C, Bioulac-Sage P, de Magalhães Queiroz DM, Mégraud F. Association of Helicobacter species with hepatitis C cirrhosis with or without hepatocellular carcinoma. *Gut* 2005; **54**: 396-401 [PMID: 15710989]

23 **Rabelo-Gonçalves EM**, Sgardioli IC, Lopes-Cendes I, Escanhoela CA, Almeida JR, Zeitune JM. Improved detection of Helicobacter pylori DNA in formalin-fixed paraffin-embedded (FFPE) tissue of patients with hepatocellular carcinoma using laser capture microdissection (LCM). *Helicobacter* 2013; **18**: 244-245 [PMID: 23350684 DOI: 10.1111/hel.12040]

24 **de Magalhães Queiroz DM**, Santos A. Isolation of a Helicobacter strain from the human liver. *Gastroenterology* 2001; **121**: 1023-1024 [PMID: 11665691]

25 **Xuan SY**, Li N, Qiang X, Zhou RR, Shi YX, Jiang WJ. Helicobacter infection in hepatocellular carcinoma tissue. *World J Gastroenterol* 2006; **12**: 2335-2340 [PMID: 16688821]

26 **Manes G**, Balzano A, Vaira D. Helicobacter pylori and pancreatic disease. *JOP* 2003; **4**: 111-116 [PMID: 12743416]

27 **Raderer M**, Wrba F, Kornek G, Maca T, Koller DY, Weinlaender G, Hejna M, Scheithauer W. Association between Helicobacter pylori infection and pancreatic cancer. *Oncology* 1998; **55**: 16-19 [PMID: 9428370]

28 **Stolzenberg-Solomon RZ**, Blaser MJ, Limburg PJ, Perez-Perez G, Taylor PR, Virtamo J, Albanes D. Helicobacter pylori seropositivity as a risk factor for pancreatic cancer. *J Natl Cancer Inst* 2001; **93**: 937-941 [PMID: 11416115 DOI: 10.1093/jnci/93.12.937]

29 **Xiao M**, Wang Y, Gao Y. Association between Helicobacter pylori infection and pancreatic cancer development: a meta-analysis. *PLoS One* 2013; **8**: e75559 [PMID: 24086571 DOI: 10.1371/journal.pone.0075559]

30 **Wang Y**, Zhang FC, Wang YJ. Helicobacter pylori and pancreatic cancer risk: a meta- analysis based on 2,049 cases and 2,861 controls. *Asian Pac J Cancer Prev* 2014; **15**: 4449-4454 [PMID: 24969867]

31 **Luo J**, Nordenvall C, Nyrén O, Adami HO, Permert J, Ye W. The risk of pancreatic cancer in patients with gastric or duodenal ulcer disease. *Int J Cancer* 2007; **120**: 368-372 [PMID: 17044024 DOI: 10.1002/ijc.22123]

32 **Bao Y**, Spiegelman D, Li R, Giovannucci E, Fuchs CS, Michaud DS. History of peptic ulcer disease and pancreatic cancer risk in men. *Gastroenterology* 2010; **138**: 541-549 [PMID: 19818786 DOI: 10.1053/j.gastro.2009.09.059]

33 **Warzecha Z**, Dembiński A, Ceranowicz P, Dembiński M, Sendur R, Pawlik WW, Konturek SJ. Deleterious effect of Helicobacter pylori infection on the course of acute pancreatitis in

rats. *Pancreatology* 2002; **2**: 386-395 [PMID: 12138227 DOI: 10.1159/000065086]
34 **Niemann T**, Larsen S, Mouritsen EA, Thorsgaard N. Helicobacter pylori infection in patients with chronic pancreatitis and duodenal ulcer. *Scand J Gastroenterol* 1997; **32**: 1201-1203 [PMID: 9438316]
35 **Manes G**, Dominguez-Muñoz JE, Hackelsberger A, Leodolter A, Rössner A, Malfertheiner P. Prevalence of Helicobacter pylori infection and gastric mucosal abnormalities in chronic pancreatitis. *Am J Gastroenterol* 1998; **93**: 1097-1100 [PMID: 9672337]
36 **Kountouras J**, Zavos C, Chatzopoulos D. A concept on the role of Helicobacter pylori infection in autoimmune pancreatitis. *J Cell Mol Med* 2005; **9**: 196-207 [PMID: 15784177 DOI: 10.1111/j.1582-4934.2005.tb00349x]
37 **Marietti M**, Gasbarrini A, Saracco G, Pellicano R. Helicobacter pylori infection and diabetes mellitus: the 2013 state of art. *Panminerva Med* 2013; **55**: 277-281 [PMID: 24088801]
38 **Franceschi F**, Tortora A, Gasbarrini G, Gasbarrini A. Helicobacter pylori and extragastric diseases. *Helicobacter* 2014; **19** Suppl 1: 52-58 [PMID: 25167946 DOI: 10.1111/hel.12159]
39 **Polyzos SA**, Kountouras J, Zavos C, Deretzi G. The association between Helicobacter pylori infection and insulin resistance: a systematic review. *Helicobacter* 2011; **16**: 79-88 [PMID: 21435084 DOI: 10.1111/j.1523-5378.2011.00822.x]
40 **Franceschi F**, Annalisa T, Teresa DR, Giovanna D, Ianiro G, Franco S, Viviana G, Valentina T, Riccardo LL, Antonio G. Role of Helicobacter pylori infection on nutrition and metabolism. *World J Gastroenterol* 2014; **20**: 12809-12817 [PMID: 25278679 DOI: 10.3748/wjg.v20.i36.12809]
41 **Malfertheiner P**, Link A, Selgrad M. Helicobacter pylori: perspectives and time trends. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 628-638 [PMID: 25001975 DOI: 10.1038/nrgastro.2014.99]
42 **Lau WY**. Primary liver tumors. *Semin Surg Oncol* 2000; **19**: 135-144 [PMID: 11126378]
43 **Lau WY**. Management of hepatocellular carcinoma. *J R Coll Surg Edinb* 2002; **47**: 389-399 [PMID: 11874260]
44 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750]
45 **Lai EC**, Lau WY. The continuing challenge of hepatic cancer in Asia. *Surgeon* 2005; **3**: 210-215 [PMID: 16076007]
46 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078]
47 **Tu QV**, Okoli AS, Kovach Z, Mendz GL. Hepatocellular carcinoma: prevalence and molecular pathogenesis of Helicobacter spp. *Future Microbiol* 2009; **4**: 1283-1301 [PMID: 19995189 DOI: 10.2217/fmb.09.90]
48 **Hall AJ**, Wild CP. Liver cancer in low and middle income countries. *BMJ* 2003; **326**: 994-995 [PMID: 12742895]
49 **Pellicano R**, Ménard A, Rizzetto M, Mégraud F. Helicobacter species and liver diseases: association or causation? *Lancet Infect Dis* 2008; **8**: 254-260 [PMID: 18353266 DOI: 10.1016/S1473-3099(08)70066-5]
50 **Bosch FX**, Ribes J, Cléries R, Díaz M. Epidemiology of hepatocellular carcinoma. *Clin Liver Dis* 2005; **9**: 191-211, v [PMID: 15831268]
51 **Humpage AR**, Falconer IR. Mycrocystin-LR and liver tumor promotion: effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes. *Env Toxicol* 1999; **14**: 61-75
52 **Liu CJ**, Kao JH. Hepatitis B virus-related hepatocellular carcinoma: epidemiology and pathogenic role of viral factors. *J Chin Med Assoc* 2007; **70**: 141-145 [PMID: 17475593]
53 **Farinati F**, Cardin R, Bortolami M, Burra P, Russo FP, Rugge M, Guido M, Sergio A, Naccarato R. Hepatitis C virus: from oxygen free radicals to hepatocellular carcinoma. *J Viral Hepat* 2007; **14**: 821-829 [PMID: 18070284]
54 **Nilsson HO**, Mulchandani R, Tranberg KG, Stenram U, Wadström T. Helicobacter species identified in liver from patients with cholangiocarcinoma and hepatocellular carcinoma. *Gastroenterology* 2001; **120**: 323-324 [PMID: 11246512]
55 **Verhoef C**, Pot RG, de Man RA, Zondervan PE, Kuipers EJ, IJzermans JN, Kusters JG. Detection of identical Helicobacter DNA in the stomach and in the non-cirrhotic liver of patients with hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 2003; **15**: 1171-1174 [PMID: 14560149]
56 **Pellicano R**, Mazzaferro V, Grigioni WF, Cutufia MA, Fagoonee S, Silengo L, Rizzetto M, Ponzetto A. Helicobacter species sequences in liver samples from patients with and without hepatocellular carcinoma. *World J Gastroenterol* 2004; **10**: 598-601 [PMID: 14966925]
57 **Pirouz T**, Zounubi L, Keivani H, Rakhshani N, Hormazdi M. Detection of Helicobacter pylori in paraffin-embedded specimens from patients with chronic liver diseases, using the amplification method. *Dig Dis Sci* 2009; **54**: 1456-1459 [PMID: 18975076 DOI: 10.1007/s10620-008-0522-5]
58 **Ponzetto A**, Pellicano R, Leone N, Berrutti M, Turrini F, Rizzetto M. Helicobacter pylori seroprevalence in cirrhotic patients with hepatitis B virus infection. *Neth J Med* 2000; **56**: 206-210 [PMID: 10821975]
59 **Ponzetto A**, Pellicano R, Leone N, Cutufia MA, Turrini F, Grigioni WF, D'Errico A, Mortimer P, Rizzetto M, Silengo L. Helicobacter infection and cirrhosis in hepatitis C virus carriage: is it an innocent bystander or a troublemaker? *Med Hypotheses* 2000; **54**: 275-277 [PMID: 10790764]
60 **Spinzi G**, Pellicano R, Minoli G, Terreni N, Cutufia MA, Fagoonee S, Rizzetto M, Ponzetto A. Helicobacter pylori seroprevalence in hepatitis C virus positive patients with cirrhosis. The Como cross-sectional study. *Panminerva Med* 2001; **43**: 85-87 [PMID: 11449177]
61 **Leone N**, Pellicano R, Brunello F, Cutufia MA, Berrutti M, Fagoonee S, Rizzetto M, Ponzetto A. Helicobacter pylori seroprevalence in patients with cirrhosis of the liver and hepatocellular carcinoma. *Cancer Detect Prev* 2003; **27**: 494-497 [PMID: 14642558]
62 **Ponzetto A**, Pellicano R, Redaelli A, Rizzetto M, Roffi L. Helicobacter pylori infection in patients with Hepatitis C Virus positive chronic liver diseases. *New Microbiol* 2003; **26**: 321-328 [PMID: 14596342]
63 **Queiroz DM**, Rocha AM, Rocha GA, Cinque SM, Oliveira AG, Godoy A, Tanno H. Association between Helicobacter pylori infection and cirrhosis in patients with chronic hepatitis C virus. *Dig Dis Sci* 2006; **51**: 370-373 [PMID: 16534683]
64 **Tsuneyama K**, Harada K, Kono N, Hiramatsu K, Zen Y, Sudo Y, Gershwin ME, Ikemoto M, Arai H, Nakanuma Y. Scavenger cells with gram-positive bacterial lipoteichoic acid infiltrate around the damaged interlobular bile ducts of primary biliary cirrhosis. *J Hepatol* 2001; **35**: 156-163 [PMID: 11580136]
65 **Leelawat K**, Suksumek N, Leelawat S, Lek-Uthai U. Detection of VacA gene specific for Helicobactor pylori in hepatocellular carcinoma and cholangiocarcinoma specimens of Thai patients. *Southeast Asian J Trop Med Public Health* 2007; **38**: 881-885 [PMID: 18041306]
66 **Ito K**, Nakamura M, Toda G, Negishi M, Torii A, Ohno T. Potential role of Helicobacter pylori in hepatocarcinogenesis. *Int J Mol Med* 2004; **13**: 221-227 [PMID: 14719127]
67 **Abu Al-Soud W**, Stenram U, Ljungh A, Tranberg KG, Nilsson HO, Wadström T. DNA of Helicobacter spp. and common gut bacteria in primary liver carcinoma. *Dig Liver Dis* 2008; **40**: 126-131 [PMID: 18083084]
68 **Ihrig M**, Schrenzel MD, Fox JG. Differential susceptibility to hepatic inflammation and proliferation in AXB recombinant inbred mice chronically infected with Helicobacter hepaticus. *Am J Pathol* 1999; **155**: 571-582 [PMID: 10433949]
69 **Taylor NS**, Fox JG, Yan L. In-vitro hepatotoxic factor in Helicobacter hepaticus, H. pylori and other Helicobacter species. *J Med Microbiol* 1995; **42**: 48-52 [PMID: 7739025]
70 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545 [PMID: 11229684]
71 **Ito K**, Yamaoka Y, Ota H, El-Zimaity H, Graham DY. Adherence, internalization, and persistence of Helicobacter pylori in hepatocytes. *Dig Dis Sci* 2008; **53**: 2541-2549 [PMID: 18320323 DOI: 10.1007/s10620-007-0164-z]
72 **Liu X**, Liang J, Li G. Lipopolysaccharide promotes adhesion and

invasion of hepatoma cell lines HepG2 and HepG2.2.15. *Mol Biol Rep* 2010; **37**: 2235-2239 [PMID: 19680784 DOI: 10.1007/s11033-009-9710-4]
73 **Huang Y**, Fan XG, Zhou JH. [Immunohistochemistry of Helicobacter pylori in primary liver carcinoma tissues]. *Zhongnan Daxue Xuebao Yixueban* 2004; **29**: 15-17 [PMID: 16136995]
74 **Esmat G**, El-Bendary M, Zakarya S, Ela MA, Zalata K. Role of Helicobacter pylori in patients with HCV-related chronic hepatitis and cirrhosis with or without hepatocellular carcinoma: possible association with disease progression. *J Viral Hepat* 2012; **19**: 473-479 [PMID: 22676359 DOI: 10.1111/j.1365-2893.2011.01567.x]
75 **Sathar SA**, Kunnathuparambil SG, Sreesh S, Narayanan P, Vinayakumar KR. Helicobacter pylori infection in patients with liver cirrhosis: prevalence and association with portal hypertensive gastropathy. *Ann Gastroenterol* 2014; **27**: 48-52 [PMID: 24714519]
76 **Rabinovitz M**, Yoo YK, Schade RR, Dindzans VJ, Van Thiel DH, Gavaler JS. Prevalence of endoscopic findings in 510 consecutive individuals with cirrhosis evaluated prospectively. *Dig Dis Sci* 1990; **35**: 705-710 [PMID: 2344804]
77 **Abdel-Hady H**, Zaki A, Badra G, Lotfy M, Selmi C, Giorgini A, El-Sayed M, Badr R. Helicobacter pylori infection in hepatic encephalopathy: Relationship to plasma endotoxins and blood ammonia. *Hepatol Res* 2007; **37**: 1026-1033 [PMID: 17610507]
78 **Schulz C**, Schütte K, Malfertheiner P. Does H. pylori eradication therapy benefit patients with hepatic encephalopathy?: systematic review. *J Clin Gastroenterol* 2014; **48**: 491-499 [PMID: 24583758 DOI: 10.1097/MCG.0000000000000108]
79 **Feng H**, Zhou X, Zhang G. Association between cirrhosis and Helicobacter pylori infection: a meta-analysis. *Eur J Gastroenterol Hepatol* 2014; **26**: 1309-1319 [PMID: 25304251 DOI: 10.1097/MEG0000000000000220]
80 **Pellicano R**, Fagoonee S. Helicobacter pylori and cirrhosis: any missing data in the meta-analysis? *Eur J Gastroenterol Hepatol* 2015; **27**: 182-183 [PMID: 25533432 DOI: 10.097/MEG.0000000000000243]
81 **Floreani A**, Biagini MR, Zappalà F, Farinati F, Plebani M, Rugge M, Surrenti C, Naccarato R. Chronic atrophic gastritis and Helicobacter pylori infection in primary biliary cirrhosis: a cross-sectional study with matching. *Ital J Gastroenterol Hepatol* 1997; **29**: 13-17 [PMID: 9265572]
82 **Durazzo M**, Rosina F, Premoli A, Morello E, Fagoonee S, Innarella R, Solerio E, Pellicano R, Rizzetto M. Lack of association between seroprevalence of Helicobacter pylori infection and primary biliary cirrhosis. *World J Gastroenterol* 2004; **10**: 3179-3181 [PMID: 15457568]
83 **Amodio P**, Montagnese S, Gatta A, Morgan MY. Characteristics of minimal hepatic encephalopathy. *Metab Brain Dis* 2004; **19**: 253-267 [PMID: 15554421]
84 **Shimamoto C**, Hirata I, Katsu K. Breath and blood ammonia in liver cirrhosis. *Hepatogastroenterology* 2000; **47**: 443-445 [PMID: 10791209]
85 **Demirtürk L**, Yazgan Y, zci O, Ozel M, Toğrol E, Gültepe M, Gürbüz AK, Yildirim S. The effect of Helicobacter pylori eradication on gastric juice and blood ammonia concentrations and on visual evoked potentials in cirrhotics. *Helicobacter* 2001; **6**: 325-330 [PMID: 11843965]
86 **Nandakumar R**, Naik AS, Pandit B, Kamat R, Bhatia SJ. Effect of Helicobacter pylori eradication on serum ammonia levels in patients with chronic liver disease. *Indian J Gastroenterol* 2003; **22**: 221-223 [PMID: 15030034]
87 **Miquel J**, Barcena R, Boixeda D, Fernández J, SanRoman AL, Martín-de-Argila C, Ramosa F. Role of Helicobacter pylori infection and its eradication in patients with subclinical hepatic encephalopathy. *Eur J Gastroenterol Hepatol* 2001; **13**: 1067-1072 [PMID: 11564957]
88 **Huber M**, Rössle M, Siegerstetter V, Ochs A, Haag K, Kist M, Blum HE. Helicobacter pylori infection does not correlate with plasma ammonia concentration and hepatic encephalopathy in patients with cirrhosis. *Hepatogastroenterology* 2001; **48**: 541-544 [PMID: 11379349]
89 **Zullo A**, Hassan C, Morini S. Hepatic encephalopathy and Helicobacter pylori: a critical reappraisal. *J Clin Gastroenterol* 2003; **37**: 164-168 [PMID: 12869889]
90 **Hu BL**, Wang HY, Yang GY. Association of Helicobacter pylori infection with hepatic encephalopathy risk: a systematic review. *Clin Res Hepatol Gastroenterol* 2013; **37**: 619-625 [PMID: 23830279 DOI: 10.1016/jclinre.2013.05.004]
91 **Kountouras J**, Zavos C, Deretzi G. Helicobacter pylori might contribute to persistent cognitive impairment after resolution of overt hepatic encephalopathy. *Clin Gastroenterol Hepatol* 2011; **9**: 624; author reply 624-625 [PMID: 21334456 DOI: 10.1016/j.cgh.2011.02.011]
92 **Kountouras J**, Zavos C, Deretzi G, Vardaka E, Boziki M, Gavalas E, Kouklakis G, Katsinelos P, Venizelos I, Nikolaidou C, Polyzos SA, Giartza-Taxidou E. Helicobacter pylori induced cognitive dysfunction might be associated with falls and fractures in cirrhosis. *Hepatology* 2013; **57**: 1284 [PMID: 22745033 DOI: 10.1002/hep.25926]
93 **de Alwis NM**, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. *J Hepatol* 2008; **48** Suppl 1: S104-S112 [PMID: 18304679 DOI: 10.16/jjhep.2008.01.009]
94 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]
95 **Bhala N**, Jouness RI, Bugianesi E. Epidemiology and natural history of patients with NAFLD. *Curr Pharm Des* 2013; **19**: 5169-5176 [PMID: 23394091]
96 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]
97 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419 [PMID: 10348825]
98 **Rafiq N**, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009; **7**: 234-238 [PMID: 19049831 DOI: 10.1016/j.cgh.2008.11.005]
99 **Li M**, Shen Z, Li YM. Potential role of Helicobacter pylori infection in nonalcoholic fatty liver disease. *World J Gastroenterol* 2013; **19**: 7024-7031 [PMID: 24222944 DOI: 10.3748/wjg.v19.i41.7024]
100 **Cindoruk M**, Cirak MY, Unal S, Karakan T, Erkan G, Engin D, Dumlu S, Turet S. Identification of Helicobacter species by 16S rDNA PCR and sequence analysis in human liver samples from patients with various etiologies of benign liver diseases. *Eur J Gastroenterol Hepatol* 2008; **20**: 33-36 [PMID: 18090988 DOI: 10.197/MEG.0b013e3282efa4f2]
101 **Abenavoli L**, Milic N, Masarone M, Persico M. Association between non-alcoholic fatty liver disease, insulin resistance and Helicobacter pylori. *Med Hypotheses* 2013; **81**: 913-915 [PMID: 24011768 DOI: 10.1016/j.mehy.2013.08.011]
102 **Polyzos SA**, Kountouras J, Papatheodorou A, Patsiaoura K, Katsiki E, Zafeiriadou E, Zavos C, Anastasiadou K, Terpos E. Helicobacter pylori infection in patients with nonalcoholic fatty liver disease. *Metabolism* 2013; **62**: 121-126 [PMID: 22841522 DOI: 10.1016/jmetabol.2012.06.007]
103 **Sumida Y**, Kanemasa K, Imai S, Mori K, Tanaka S, Shimokobe H, Kitamura Y, Fukumoto K, Kakutani A, Ohno T, Taketani H, Seko Y, Ishiba H, Hara T, Okajima A, Yamaguchi K, Moriguchi M, Mitsuyoshi H, Yasui K, Minami M, Itoh Y. Helicobacter pylori infection might have a potential role in hepatocyte ballooning in nonalcoholic fatty liver disease. *J Gastroenterol* 2015; **50**: 996-1004 [PMID: 25622927]
104 **Goo MJ**, Ki MR, Lee HR, Yang HJ, Yuan DW, Hong IH, Park JK, Hong KS, Han JY, Hwang OK, Kim DH, Do SH, Cohn RD, Jeong KS. Helicobacter pylori promotes hepatic fibrosis in the animal model. *Lab Invest* 2009; **89**: 1291-1303 [PMID: 19736546 DOI: 10.138/labinvest.2009.90]

105 **Ki MR**, Goo MJ, Park JK, Hong IH, Ji AR, Han SY, You SY, Lee EM, Kim AY, Park SJ, Lee HJ, Kim SY, Jeong KS. Helicobacter pylori accelerates hepatic fibrosis by sensitizing transforming growth factor-β1-induced inflammatory signaling. *Lab Invest* 2010; **90**: 1507-1516 [PMID: 20531291 DOI: 10.138/labinvest.2010.109]

106 **García-Trevijano ER**, Iraburu MJ, Fontana L, Domínguez-Rosales JA, Auster A, Covarrubias-Pinedo A, Rojkind M. Transforming growth factor beta1 induces the expression of alpha1(I) procollagen mRNA by a hydrogen peroxide-C/EBPbeta-dependent mechanism in rat hepatic stellate cells. *Hepatology* 1999; **29**: 960-970 [PMID: 10051504]

107 **Wheeler MD**, Kono H, Yin M, Nakagami M, Uesugi T, Arteel GE, Gäbele E, Rusyn I, Yamashina S, Froh M, Adachi Y, Iimuro Y, Bradford BU, Smutney OM, Connor HD, Mason RP, Goyert SM, Peters JM, Gonzalez FJ, Samulski RJ, Thurman RG. The role of Kupffer cell oxidant production in early ethanol-induced liver disease. *Free Radic Biol Med* 2001; **31**: 1544-1549 [PMID: 11744328]

108 **Franceschi F**, Zuccalà G, Roccarina D, Gasbarrini A. Clinical effects of Helicobacter pylori outside the stomach. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 234-242 [PMID: 24345888 DOI: 10.1038/nrgastro.2013.243]

109 **Imrie CW**. Acute pancreatitis: overview. *Eur J Gastroenterol Hepatol* 1997; **9**: 103-105 [PMID: 9058618]

110 **Whitcomb DC**. Clinical practice. Acute pancreatitis. *N Engl J Med* 2006; **354**: 2142-2150 [PMID: 16707751 DOI: 10.1056/NEJMc054958]

111 **Frossard JL**, Steer ML, Pastor CM. Acute pancreatitis. *Lancet* 2008; **371**: 143-152 [PMID: 18191686 DOI: 10.1016/S0140-6736(08)60107-5]

112 **Chen TA**, Lo GH, Lin CK, Lai KH, Wong HY, Yu HC, Hsu PI, Chen HH, Tsai WL, Chen WC. Acute pancreatitis-associated acute gastrointestinal mucosal lesions: incidence, characteristics, and clinical significance. *J Clin Gastroenterol* 2007; **41**: 630-634 [PMID: 17577121 DOI: 10.1097/01.mcg.0000225638.37533.8c]

113 **Lee KM**, Paik CN, Chung WC, Yang JM. Association between acute pancreatitis and peptic ulcer disease. *World J Gastroenterol* 2011; **17**: 1058-1062 [PMID: 21448359 DOI: 10.3748/wjg.v17j8.1058]

114 **Hassan MM**, Bondy ML, Wolff RA, Abbruzzese JL, Vauthey JN, Pisters PW, Evans DB, Khan R, Chou TH, Lenzi R, Jiao L, Li D. Risk factors for pancreatic cancer: case-control study. *Am J Gastroenterol* 2007; **102**: 2696-2707 [PMID: 17764494 DOI: 10.1111/j.1572-0241.2007.01510.x]

115 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]

116 **Maisonneuve P**, Lowenfels AB. Epidemiology of pancreatic cancer: an update. *Dig Dis* 2010; **28**: 645-656 [PMID: 21088417 DOI: 10.1159/000320068]

117 **Kang R**, Tang D. Autophagy in pancreatic cancer pathogenesis and treatment. *Am J Cancer Res* 2012; **2**: 383-396 [PMID: 22860230]

118 **Lowenfels AB**, Maisonneuve P, Lankisch PG. Chronic pancreatitis and other risk factors for pancreatic cancer. *Gastroenterol Clin North Am* 1999; **28**: 673-685, x [PMID: 10503143]

119 **Raimondi S**, Lowenfels AB, Morselli-Labate AM, Maisonneuve P, Pezzilli R. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. *Best Pract Res Clin Gastroenterol* 2010; **24**: 349-358 [PMID: 20510834 DOI: 10.1016/j.bpg.2010.02.007]

120 **Duell EJ**, Lucenteforte E, Olson SH, Bracci PM, Li D, Risch HA, Silverman DT, Ji BT, Gallinger S, Holly EA, Fontham EH, Maisonneuve P, Bueno-de-Mesquita HB, Ghadirian P, Kurtz RC, Ludwig E, Yu H, Lowenfels AB, Seminara D, Petersen GM, La Vecchia C, Boffetta P. Pancreatitis and pancreatic cancer risk: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). *Ann Oncol* 2012; **23**: 2964-2970 [PMID: 22767586 DOI: 10.1093/annonc/mds140]

121 **Yadav D**, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 2013; **144**: 1252-1261 [PMID: 23622135 DOI: 10.1053/j.gastro.2013.01.068]

122 **Tham TC**, Chen L, Dennison N, Johnston CF, Collins JS, Ardill JE, Buchanan KD. Effect of Helicobacter pylori eradication on antral somatostatin cell density in humans. *Eur J Gastroenterol Hepatol* 1998; **10**: 289-291 [PMID: 9855043]

123 **Park SM**, Lee HR, Kim JG, Park JW, Jung G, Han SH, Cho JH, Kim MK. Effect of Helicobacter pylori infection on antral gastrin and somatostatin cells and on serum gastrin concentrations. *Korean J Intern Med* 1999; **14**: 15-20 [PMID: 10063309]

124 **Larsson LI**. Developmental biology of gastrin and somatostatin cells in the antropyloric mucosa of the stomach. *Microsc Res Tech* 2000; **48**: 272-281 [PMID: 10700044 DOI: 10.1002/(SICI)1097-0029]

125 **Risch HA**. Etiology of pancreatic cancer, with a hypothesis concerning the role of N-nitroso compounds and excess gastric acidity. *J Natl Cancer Inst* 2003; **95**: 948-960 [PMID: 12837831 DOI: 10.1093/jnci/95.13.948]

126 **Trikudanathan G**, Philip A, Dasanu CA, Baker WL. Association between Helicobacter pylori infection and pancreatic cancer. A cumulative meta-analysis. *JOP* 2011; **12**: 26-31 [PMID: 21206097]

127 **Dobrila-Dintinjana R**, Vanis N, Dintinjana M, Radić M. Etiology and oncogenesis of pancreatic carcinoma. *Coll Antropol* 2012; **36**: 1063-1067 [PMID: 23213974]

128 **Nilsson HO**, Stenram U, Ihse I, Wadstrom T. Helicobacter species ribosomal DNA in the pancreas, stomach and duodenum of pancreatic cancer patients. *World J Gastroenterol* 2006; **12**: 3038-3043 [PMID: 16718784 DOI: 10.3748/wjg.v12.i19.3038]

129 **Takayama S**, Takahashi H, Matsuo Y, Okada Y, Manabe T. Effects of Helicobacter pylori infection on human pancreatic cancer cell line. *Hepatogastroenterology* 2007; **54**: 2387-2391 [PMID: 18265671]

130 **Michaud DS**. Role of bacterial infections in pancreatic cancer. *Carcinogenesis* 2013; **34**: 2193-2197 [PMID: 23843038 DOI: 10.1093/carcin/bgt249]

131 **Lindkvist B**, Johansen D, Borgström A, Manjer J. A prospective study of Helicobacter pylori in relation to the risk for pancreatic cancer. *BMC Cancer* 2008; **8**: 321 [PMID: 18986545 DOI: 10.1186/1471-2407-8-321]

132 **Ai F**, Hua X, Liu Y, Lin J, Feng Z. Preliminary study of pancreatic cancer associated with Helicobacter pylori infection. *Cell Biochem Biophys* 2015; **71**: 397-400 [PMID: DOI: 10.1007/s12013-014-0211-2]

133 **Fan BG**, Andrén-Sandberg A. Autoimmune pancreatitis. *N Am J Med Sci* 2009; **1**: 148-151 [PMID: 22666688]

134 **Okazaki K**, Chiba T. Autoimmune related pancreatitis. *Gut* 2002; **51**: 1-4 [PMID: 12077078 DOI: 10.1136/gut51.1.1]

135 **Kim KP**, Kim MH, Song MH, Lee SS, Seo DW, Lee SK. Autoimmune chronic pancreatitis. *Am J Gastroenterol* 2004; **99**: 1605-1616 [PMID: 15307882 DOI: 10.1111/j.1572-0241.2004.30336x]

136 **Shinji A**, Sano K, Hamano H, Unno H, Fukushima M, Nakamura N, Akamatsu T, Kawa S, Kiyosawa K. Autoimmune pancreatitis is closely associated with gastric ulcer presenting with abundant IgG4-bearing plasma cell infiltration. *Gastrointest Endosc* 2004; **59**: 506-511 [PMID: 15044886 DOI: 10.1016/S0016-5107(03)02874-8]

137 **Persidis A**. Autoimmune disease drug discovery. *Nat Biotechnol* 1999; **17**: 1038 [PMID: 10504714 DOI: 10.1038/13748]

138 **Benvenga S**, Guarneri F, Vaccaro M, Santarpia L, Trimarchi F. Homologies between proteins of Borrelia burgdorferi and thyroid autoantigens. *Thyroid* 2004; **14**: 964-966 [PMID: 15671776 DOI: 10.1089/thy.2004.14.964]

139 **Spechler SJ.** Peptic ulcer disease and its complications. In: Sleisenger and Fordtran's Gastrointestinal and Liver Disease, 7th ed. Philadelphia, PA: Saunders, 2002: 747-781

140 **Kountouras J**, Zavos C, Chatzopoulos D. Autoimmune pancreatitis, Helicobacter pylori infection and apoptosis: a proposed relationship. *Pancreas* 2005; **30**: 192-193

141 **Guarneri F**, Guarneri C, Benvenga S. Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry? *J Cell Mol Med* 2005; **9**: 741-744 [PMID: 16202223 DOI: 10.1111/j.1582-4934.2005.tb00506x]

142 **Klöppel G**, Lüttges J, Löhr M, Zamboni G, Longnecker D. Autoimmune pancreatitis: pathological, clinical, and immunological features. *Pancreas* 2003; **27**: 14-19 [PMID: 12826900]

143 **Chirica LC**, Petersson C, Hurtig M, Jonsson BH, Borén T, Lindskog S. Expression and localization of alpha- and beta-carbonic anhydrase in Helicobacter pylori. *Biochim Biophys Acta* 2002; **1601**: 192-199 [PMID: 12445482]

144 **Marcus EA**, Moshfegh AP, Sachs G, Scott DR. The periplasmic alpha-carbonic anhydrase activity of Helicobacter pylori is essential for acid acclimation. *J Bacteriol* 2005; **187**: 729-738 [PMID: 15629943]

145 **Okazaki K**, Uchida K, Chiba T. Recent concept of autoimmune-related pancreatitis. *J Gastroenterol* 2001; **36**: 293-302 [PMID: 11388391 DOI: 10.1007/s005350170094]

146 **DiMagno EP**, Chari S. Acute pancreatitis. In: Sleisenger & Fordtran's *Gastrointestinal and Liver Disease*. 7th Ed. Philadelphia, PA: Saunders, 2002: 913-941

147 **Kountouras J**, Zavos C, Chatzopoulos D. Induction of apoptosis as a proposed pathophysiological link between glaucoma and Helicobacter pylori infection. *Med Hypotheses* 2004; **62**: 378-381 [PMID: 14975507]

148 **Zhou X**, Zhang C, Wu J, Zhang G. Association between Helicobacter pylori infection and diabetes mellitus: a meta-analysis of observational studies. *Diabetes Res Clin Pract* 2013; **99**: 200-208 [PMID: 23395214 DOI: 10.1016/j.diabres.2012.11.012]

149 **Jeon CY**, Haan MN, Cheng C, Clayton ER, Mayeda ER, Miller JW, Aiello AE. Helicobacter pylori infection is associated with an increased rate of diabetes. *Diabetes Care* 2012; **35**: 520-525 [PMID: 22279028 DOI: 10.2337/dc11-1043]

150 **Albaker WI**. Helicobacter pylori infection and its relationship to metabolic syndrome: is it a myth or fact? *Saudi J Gastroenterol* 2011; **17**: 165-169 [PMID: 21546717 DOI: 10.4103/1319-3767.80377]

151 **Gunji T**, Matsuhashi N, Sato H, Fujibayashi K, Okumura M, Sasabe N, Urabe A. Helicobacter pylori infection significantly increases insulin resistance in the asymptomatic Japanese population. *Helicobacter* 2009; **14**: 144-150 [PMID: 19751440 DOI: 10.1111/j.1523-5378.2009.00705.x]

152 **So WY**, Tong PC, Ko GT, Ma RC, Ozaki R, Kong AP, Yang X, Ho CS, Lam CC, Chan JC. Low plasma adiponectin level, white blood cell count and Helicobacter pylori titre independently predict abnormal pancreatic beta-cell function. *Diabetes Res Clin Pract* 2009; **86**: 89-95 [PMID: 19747747 DOI: 10.1016/j.diabres.2009.08.010]

153 **Shin DW**, Kwon HT, Kang JM, Park JH, Choi HC, Park MS, Park SM, Son KY, Cho B. Association between metabolic syndrome and Helicobacter pylori infection diagnosed by histologic status and serological status. *J Clin Gastroenterol* 2012; **46**: 840-845 [PMID: 23064216 DOI: 10.1097/MCG.0b013e3182522477]

154 **Banić M**, Franceschi F, Babić Z, Gasbarrini A. Extragastric manifestations of Helicobacter pylori infection. *Helicobacter* 2012; **17** Suppl 1: 49-55 [PMID: 22958156]

155 **Malfertheiner P**, Selgrad M, Bornschein J. Helicobacter pylori: clinical management. *Curr Opin Gastroenterol* 2012; **28**: 608-614 [PMID: 23010682 DOI: 10.1097/MOG.0b013e32835918a7]

P- Reviewer: Manguso F **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.i30.2980

World J Hepatol 2015 December 28; 7(30): 2980-2991
ISSN 1948-5182 (online)

REVIEW

Crosstalk between innate and adaptive immunity in hepatitis B virus infection

Li Wang, Kai Wang, Zhi-Qiang Zou

Li Wang, Zhi-Qiang Zou, Infectious Disease Hospital of Yantai, Yantai 264001, Shandong Province, China

Kai Wang, Hepatology Department, Qilu Hospital Affiliated to Shandong University, Jinan 250012, Shandong Province, China

Author contributions: Wang L, Wang K and Zou ZQ designed and performed literature search; Wang L wrote manuscript.

Supported by Grants of Yantai Science and Technology Plan Project, No. 2012116.

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

Correspondence to: Li Wang, PhD, Infectious Disease Hospital of Yantai, Huanshan Road 62, Zhifu District, Yantai 264001, Shandong Province, China. liliwang2200@163.com
Telephone: +86-535-6232253
Fax: +86-535-6628542

Received: September 23, 2015
Peer-review started: September 26, 2015
First decision: October 21, 2015
Revised: November 11, 2015
Accepted: December 8, 2015
Article in press: December 11, 2015
Published online: December 28, 2015

Abstract

Hepatitis B virus (HBV) infection is a major public health problem worldwide. HBV is not directly cytotoxic to infected hepatocytes; the clinical outcome of infection results from complicated interactions between the virus and the host immune system. In acute HBV infection, initiation of a broad, vigorous immune response is responsible for viral clearance and self-limited inflammatory liver disease. Effective and coordinated innate and adaptive immune responses are critical for viral clearance and the development of long-lasting immunity. Chronic hepatitis B patients fail to mount efficient innate and adaptive immune responses to the virus. In particular, HBV-specific cytotoxic T cells, which are crucial for HBV clearance, are hyporesponsiveness to HBV infection. Accumulating experimental evidence obtained from the development of animal and cell line models has highlighted the importance of innate immunity in the early control of HBV spread. The virus has evolved immune escape strategies, with higher HBV loads and HBV protein concentrations associated with increasing impairment of immune function. Therefore, treatment of HBV infection requires inhibition of HBV replication and protein expression to restore the suppressed host immunity. Complicated interactions exist not only between innate and adaptive responses, but also among innate immune cells and different components of adaptive responses. Improved insight into these complex interactions are important in designing new therapeutic strategies for the treatment HBV infection. In this review, we summarize the current knowledge regarding the cross-talk between the innate and adaptive immune responses and among different immunocytes in HBV infection.

Key words: Crosstalk; Hepatitis B virus; Innate immune; Adapative immune

Core tip: Hepatitis B virus (HBV) is poorly sensed by the innate immune system and can escape innate immune

recognition at the early stage of infection. HBV-specific T-cell responses are timely and efficiently induced in acute self-limited infections but are deeply exhausted in chronic hepatitis B. The tolerogenic effect of the liver environment and the persistent exposure of T cells to high antigen loads play a key role in the pathogenesis of T-cell inhibition in chronic HBV infection. Combination of reduction of HBV and virus antigen loads and restoration of the anti-viral T-cell function may represent a strategy to cure chronic HBV infections.

Wang L, Wang K, Zou ZQ. Crosstalk between innate and adaptive immunity in hepatitis B virus infection. *World J Hepatol* 2015; 7(30): 2980-2991 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i30/2980.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i30.2980

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem worldwide. Approximately 30% of the world's population show serological evidence of current or past HBV infection and 350 million people are chronically infected[1]. The outcome of HBV infection varies widely among infected patients from resolved acute infection, chronic hepatitis, and liver cirrhosis to hepatocellular carcinoma. Infections in approximately 5% of adults and 95% of neonates become persistent[2]. HBV itself is not directly cytotoxic to infected hepatocytes and the clinical outcome of infection results from complicated interactions between the virus and the host immune system[3-5]. The immune responses to HBV antigens, which are mediated through complex interactions between the innate immune and adaptive immune systems, are responsible both for viral clearance and disease pathogenesis. In acute HBV infection, a broad, vigorous immune response results in viral clearance associated with acute, self-limited inflammatory liver disease[6]. In contrast, chronic hepatitis B (CHB) patients fail to mount efficient innate and adaptive immune responses to the virus, with HBV-specific cytotoxic T cells (CTLs) in particular, being hyporesponsiveness to HBV infection[7,8]. The role of adaptive immune responses in the control of HBV infection is widely accepted, with HBV-specific T cell responses being essential for the termination of HBV infection. Furthermore, $CD4^+$ T cells serve as the chief regulators of the adaptive immune response to HBV[5]. The innate immune system is the first line of active host defense against viral infection, and once activated, is linked to a favorable clinical outcome and subsequent robust adaptive immune responses[8]. The induction of innate immune responses by HBV during the phase of early infection is a longstanding controversy. The development of animal and cell culture models has yielded great improvements in our understanding of the innate immune responses during HBV infection. Furthermore, the strategies employed by HBV to counteract the innate antiviral pathways are being gradually recognized. It is known that effective recognition of viral infection and successive activation of antiviral innate immune responses are vital for host antiviral defense and largely depend on multiple regulators, including Toll-like receptors (TLRs)[9,10] and cytokines[11]. Efficient control of virus infections requires the coordinated actions of both innate and adaptive immune responses. Mounting effective innate and adaptive immune responses is critical for viral clearance and the development of long-lasting immunity. Complicated interactions exist not only between the innate and adaptive systems, but also among innate immune cells and among different components of adaptive responses.

A better understanding of the interplay between innate and adaptive immune responses and between the host immune response and the virus is crucial for the development of new antiviral therapeutic strategies aimed at eradicating chronic infections.

In this review, we summarize the current knowledge regarding the interactions between the innate and adaptive immune systems and among different immunocytes during HBV infection.

TLRS

TLRs are a group of highly conserved molecules that sense pathogen-associated molecular patterns (PAMPs). So far in humans and mice, TLR1 to 13 have been identified, which are extensively expressed in various immune and non-immune cells. Stimulation by their ligands initiates the activation of complex intracellular signal transduction networks and innate and adaptive immune-related cells, including natural killer (NK) cells, NK-T cells, monocytes, dendritic cells (DCs), T cells, B cells, and Tregs, as well as the production of antiviral effector interferons (IFNs) and proinflammatory cytokines[12]. TLRs play important roles in innate immune responses[13] to viral infections, including HBV. TLRs can activate DCs, improve antigen presentation, and initiate T cell immune responses. *In vivo*, TLRs also directly modulate HBV-specific T and B cell responses, which are essential for the termination of HBV infection[14]. Therefore, TLR responses are cell type-specific.

TLRs and innate immunity

Innate immunity is important in controlling infection immediately after contact with the pathogen and to initiate efficient development of an adaptive immune response. TLRs play a key role in the activation of innate immune responses to infectious agents[13]. The TLR family consists of intracellular and cell surface subgroups. The intracellular subgroup (TLR3, TLR7, TLR8 and TLR9) is localized in endosomes and recognizes nucleic acids, such as viral DNA or RNA, while the cell surface subgroup (TLR1, TLR2, TLR4/MD-2, TLR5 and TLR6) recognizes extracellular bacterial and fungal cell wall components, as well as some viral proteins[13-17].

Binding of TLR agonists to their receptors initiates the activation of complex networks of intracellular signal transduction pathways that leads to the induction of type Ⅰ IFNs (IFNα/β), proinflammatory cytokines, and costimulatory molecules, which are involved in antiviral responses[18,19]. The importance of TLR receptor signaling in controlling HBV replication was confirmed by a study in which a single intravenous injection of ligands specific for TLR3, TLR4, TLR5, TLR7 and TLR9 provided efficient inhibition of HBV replication in a non-cytolytic and IFNα/β-dependent manner in HBV transgenic mice[20].

TLRs and DCs and peripheral blood mononuclear cells

TLRs are abundantly expressed on the surface of DCs, especially peripheral blood monocyte-derived DCs (moDCs). Plasmacytoid dendritic cells (pDCs) play a crucial role in triggering antiviral immunity through their ability to capture and process viral antigens and subsequently induce adaptive immune responses and the production of type Ⅰ IFNs. pDCs are the key sensors of viral infections through expression of both TLR7 and TLR9[21]. Myeloid DCs (mDCs) respond to TLR1, -2, -4 and -9 ligands resulting in upregulation of CD40 and activation of allogeneic T cells[22]. TLR9 detects intracellular viral double-stranded (ds)DNA, which leads to the activation of nuclear factor κB (NF-κB) *via* the myeloid differentiation primary response 88 (MyD88) pathway, resulting in the activation of immune responses against HBV. However, expression of TLR9, MyD88, IRAK1, TRAF6, and NF-κB in peripheral blood mononuclear cells (PBMCs) of CHB patients is significantly decreased in comparison with healthy controls[23,24], which may result in an attenuated responses that ultimately lead to long-lasting HBV infection[25]. Reduced TLR9 expression in pDCs of CHB patients is associated with impaired IFNα production[26]. TLR2 and TLR4 mediate the activation of the same signaling pathways downstream of MyD88, including NF-κB, MAPK, and PI-3k/Akt pathways to inhibit hepadnaviral replication. One study indicated that expressions of TLR2 and TLR4 were downregulated in PBMCs during HBV infection[27], while another study showed that expression of TLR2 and TLR-4 in moDCs was significantly increased with disease progression[28]. The role of TLR2 and TLR-4 in the pathogenesis of requires further evaluation.

TLRs and NK cells

NK cells possess receptors allowing them to sense and respond to viral and bacterial patterns, including TLRs. Upon TLR activation (mainly TLR3 and TLR7), NK cells produce IFNγ[29-31], which also contributes to deleterious inflammation if produced in excessive amounts[29]. NK cells in CHB patients have an impaired IFNγ response to TLR9 stimulation compared to healthy controls although no differences have been observed in responses to the other TLR ligands. This suggests that multiple mechanisms may be involved in NK activation[32] and although viral clearance is suppressed in chronic HBV infection, the potential to mediate tissue injury is maintained.

TLRs and non-parenchymal cells

Non-parenchymal cells (NPCs), like Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs), also participate in innate immune responses by producing various cytokines, including tumor necrosis factor-α (TNF-α) and IFNβ[28] in response to TLR signaling. Isogawa *et al*[20] demonstrated the involvement of NPCs rather than hepatocytes in antiviral activation induced by TLR ligands. HBV is recognized by the NPCs of the liver, mainly macrophages (KCs), although they are not infected. KCs respond to all TLR ligands by producing TNF-α or interleukin-6 (IL-6), to TLR3 and TLR4 ligands by producing IFNβ[22], to TLR1 and TLR8 ligands by upregulating major histocompatibility complex (MHC) class Ⅱ and costimulatory molecules, and to TLR1, -2, -4 and -6 ligands by inducing high levels of T cell proliferation and IFNλ production in the mixed lymphocyte reaction[22].

LSECs are liver-resident antigen-presenting cells that are capable of antigen cross-presentation and induction of $CD8^+$ T cell tolerance or immunity under different conditions[33,34]. Liu *et al*[35] demonstrated that pretreatment of LSECs with a TLR1/2 ligand or LPS (TLR4 ligand) relieved their suppressive functions to induce T cell immunity, while Wu *et al*[22] suggested that, on stimulation by TLR ligands, LSECs have similar responses to KCs. Another study that demonstrated that, among different TLR ligands, hepatic NPCs show significant production of IFNβ only in response to TLR3 stimulation[36]. However, in the presence of HBsAg, TLR-induced expression of IFNγ, interferon sensitive genes and proinflammatory cytokines in murine KCs and LSECs was efficiently suppressed, whereas the expression of anti-inflammatory cytokines was enhanced[37].

Although regarded as a type of antigen-presenting cell (APC), NPCs display a restricted TLR-mediated activation profile compared with "classical" APCs. Therefore, antiviral effects induced by TLR receptor activation should be carefully evaluated in therapeutic design to maintain the balance between viral control and liver injury. Furthermore, coordination of innate and adaptive immune responses may be highly important for the control of viral infection[19].

TLRs and adaptive immunity

Several studies have demonstrated that TLR2 is expressed by activated and memory $CD4^+$ and $CD8^+$ T cells and serves as a costimulatory molecule[38,39]. In some studies, TLR3 and TLR9 expression on human $CD8^+$ T cells was also demonstrated to promote IFNγ production upon stimulation[40,41]. However, one study showed that, although all TLRs were able to induce $CD8^+$ T cell activation *in vitro*, there were profound differences in their $CD8^+$ T cell activation capacity *in vivo*. TLR3 and TLR9 induced $CD8^+$ T cell activation, while, TLR2 and TLR4 were not only incapable of inducing $CD8^+$ T cell priming, but also inhibiting $CD8^+$ T cell expansion[42].

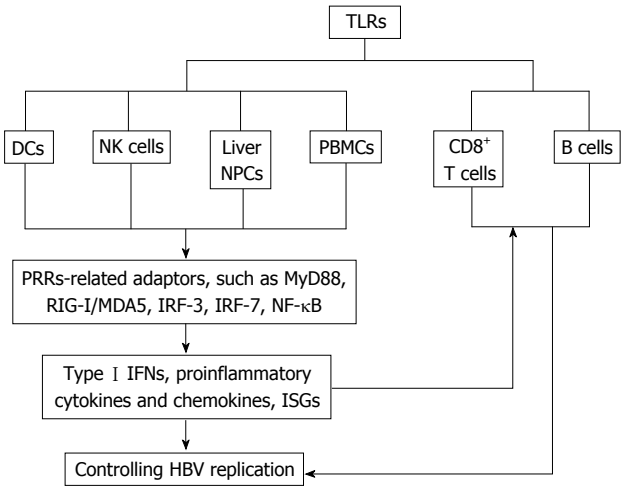

Figure 1 Expressions and activation of toll-like receptors in innate and adaptive immune cells in controlling hepatitis B virus infection. HBV: Hepatitis B virus; NPCs: Non-parenchymal cells; NK: Natural killer; IFN: Interferon; TLRs: Toll-like receptors; ISG: Interferon-stimulated genes; RIG-I: Retinoic acid inducible gene I; IRF: Interferon-regulatory factors; NF-κB: Nuclear factor κB; PBMCs: Peripheral blood mononuclear cells; MDA5: Melanoma differentiation associated gene 5; DCs: Dendritic cells.

B cells represent an important link between the adaptive and innate immune systems in that they express both antigen-specific B cell receptors (BCRs) as well as various TLRs[43]. Conventionally, signaling through the BCR initiates a sequence of events that are necessary for B cell activation and differentiation of. In combination with BCR signaling, TLR signaling plays multiple roles in B cell differentiation and activation and the outcome is largely context-dependent[44]. However, activation of resting B cells by simultaneous involvement of TLR-2 and the costimulatory molecules CD40 and CD86 could be BCR-independent[45,46]. Expressions and activation of TLRs in immune cells in HBV infection are illustrated in Figure 1.

CYTIKINES

Cytokines and chemokines play a crucial role in initiating, maintaining, and regulating immunological homeostasis and inflammatory processes. Cytokines are released by many different cell types and activate cells of both the innate and adaptive immune system[47]. Cytokine-mediated immune responses play a pivotal role in determining the clinical outcome of HBV infection. Different patterns of serum cytokines and chemokines are associated with different phases of HBV infection. Non-cytolytic intracellular viral inactivation by IFNγ and TNF-α play an important role in the clearance of HBV in resolved acute HBV infection without killing infected cells. The recognition of PAMPs by PRRs such as TLRs, RIG-I like receptors, NOD-like receptors results in activation of intracellular pathways and leads to the production of antiviral, immunoregulatory and proinflammatory molecules[48].

IFNs

IFNs represent one of the first lines of host defense against invading pathogens. As key components of the innate immune system, IFNs have been demonstrated to restrict HBV replication by affecting multiple steps in the viral life cycle, including HBV RNA synthesis, pgRNA encapsulation, the turnover rate of viral proteins, and modulation of covalently closed circular (ccc)DNA formation[49,50] by inducing numerous IFN-stimulated genes[51]. IFNs are classified into three groups, types Ⅰ, Ⅱ and Ⅲ, based on the structure of their receptors on the cell surface[47]. The early phase of viral infection is characterized mainly by the production of type Ⅰ IFNα/β, and NK cell activation. The production of type Ⅰ IFNs can be triggered directly by virus replication through cellular mechanisms that detect the presence of viral RNA or DNA. The main sources of IFNα/β are infected cells and pDCs, whereas IFNγ is produced primarily by NK and NKT cells[52]. IFNβ has also been identified as a major antiviral factor produced by NPCs in response to TLR3[36]. Recombinant IFN (rIFN)-α has been approved and successfully used as a standard treatment for chronic HBV infection[48]. Furthermore, treatment of the HBV-producing hepatocytes with rIFN-γ and rTNF-α efficiently suppresses HBV replication without cytolysis[53]. In addition, IFNs have immunomodulatory functions as indicated by the ability of IFNα treatment to recover HBV-impaired hepatocyte-intrinsic innate immunity[54].

TNF-α

TNF-α is another major antiviral cytokine which, like IFNγ, also stimulates adaptive immunity and the antiviral effects of CTLs[53,55]. The absence of TNF-α or early treatment with a TNF receptor blocker reduces viral clearance, persistently maintains elevated HBV viral load and increases expression of the inhibitory receptor, programmed death-1 (PD-1) in $CD8^+$ T cells in a mouse model[56,57]. These results suggest that HBV is reactivated during therapy with TNF-α-blocking agents in clinical practice. In addition to the induction of non-cytopathic suppression and clearance of HBV in animal models, TNF-α rapidly blocks HBV replication by promoting destabilization of pre-existing cytoplasmic viral nucleocapsids containing viral RNA and DNA, as well as of empty nucleocapsids[57].

IL-6

Sodium-taurocholate cotransporting polypeptide (NTCP) has been identified as an HBV-specific receptor. Studies have shown that NTCP-mediates HBV entry is markedly inhibited by IL-6, with a strong inhibition of long-term HBsAg secretion and a profound reduction in intracellular HBV cccDNA[58]. Hösel *et al*[59] demonstrated that recognition of HBV patterns by liver NPCs results in IL-6-mediated control of HBV infection at the transcriptional level. In the early phase of infection, IL-6 rather than IFN mediates control of the virus, limiting activation of the adaptive immune response and preventing death of

HBV-infected hepatocytes[59].

IL-12

IL-12 is an immunomodulatory cytokine that promotes cellular immunity. Research suggests that IL-12-based vaccination therapy strongly enhances hepatic HBV-specific $CD8^+$ T cell responses, restores systemic HBV-specific $CD4^+$ T cell responses and activates HBsAg-specific follicular Th-germinal center B cell responses, resulting in IFNγ secretion and anti-HBs antibody production[55]. Studies have also shown that IL-12 initiates LSEC-mediated $CD8^+$ T cell immunity[35].

IL-18

IL-18 is produced mainly by activated macrophages, and like IL-12, induces IFNγ and TNF-α. It has been shown that IL-18 inhibits HBV replication in hepatoma cell lines and in the liver through induction of IFN-γ production by NK cells and T cells. HBeAg protein may suppress IL-18-mediated NF-κB signaling in NK and hepatoma cells and inhibit expression of IFNγ[60], which contributes to the establishment of HBV persistent infection. Studies have shown that *IL-18* gene polymorphisms affect susceptibility to HBV infection and are associated with different outcomes of HBV infection. However, the results from other studies are conflicting. Motavaf suggested that the IL-18 genotype -607 A/A is associated with susceptibility to chronic HBV infection[61], while Karra indicated that it may be protective against HBV infection and associated with spontaneous clearance[62]. Thus, the effects of this IL-18 genotype on HBV infection remain to be fully elucidated.

IL-22

Despite hepatoprotective and anti-fibrotic functions in acute liver injury models, IL-22 exacerbates liver inflammation and fibrosis in chronic HBV-infected patients and HBV transgenic (Tg) mice by recruiting Th17 cells into the liver. IL-22 also induces upregulation of numerous IL-22 pathway-associated proinflammatory genes in HBV-infected liver tissues and exerts mitogenic and anti-apoptotic effects on hepatocytes[63]. Furthermore, IL-22 depletion was shown to significantly inhibit recruitment of antigen-non-specific inflammatory cells into the liver in HBV Tg mice, while, IFNγ mediated non-cytopathic inhibition of virus replication initiated by HBV-specific cytotoxic T cells was not affected[64]. This indicates that IL-22 has no direct inhibitory effects on virus replication.

Transforming growth factor-β and IL-10

Transforming growth factor (TGF)-β is an important cytokine for the maturation and differentiation of many different immune cells in the liver. This cytokine mediates dual immunoregulatory functions involving induction of proinflammatory or anti-inflammatory responses in cooperation with other soluble factors. It suppresses differentiation of Th1 and Th2 cells and promotes development of the Th17, Th9, and the Treg phenotypes[64]. Thus, TGF-β plays a dual role in HBV infection by suppressing immune responses against viral infection and inhibiting viral replication. TGF-β1 suppresses HBV replication primarily through transcriptional inhibition of pre-genomic RNA[65]. KCs in HBV-carrier mice express high levels of IL-10 and mediate the induction of systemic tolerance in an IL-10-dependent manner[66]. Blockade of IL-10 restores NK cell effector function in acute HBV infection, indicating that the immunosuppressive cytokine environment in chronic HBV infection may inhibit the ability of NK cells to produce IFNγ and subsequent activation of $CD8^+$ T cells[67]. NK cells and regulatory B (Breg) cells also produce elevated IL-10 in CHB[68].

Other cytokines

IL-21, derived from HBV-specific $CD4^+$ T cells plays key roles in sustaining $CD8^+$ T cells and promoting B cell responses that are essential for effective HBV control[69]. IL-21 is not only mediates direct and effective suppression of HBV replication, but also reduces HBV replication by inhibiting IL-10 secretion[70]. However, as a mediator of inflammation, IL-21 is also involved in the development of HBV-induced liver cirrhosis and exacerbating liver injury[71].

IL-35 is a recently identified potent immunosuppressive cytokine of the IL-12 family, which is secreted by regulatory T (Treg) cells and the newly reported Breg cells. IL-35 suppresses the proliferation of HBV antigen-specific cytotoxic T-lymphocytes and IFNγ production *in vitro* and decreases the proliferation of $CD4^+CD45RA^+$ naïve T cells and the expansion of $CD11c^+$ DCs *ex vivo*. High expression of IL-35 in $CD4^+$ T cells may be one of the factors involved in the inhibition of cellular immune responses in chronic HBV infection[71-73].

DCS AND OTHER IMMUNE CELLS

DCs are the most efficient professional APCs, which stimulate the initial T cell activation and proliferation. Typically, immature DCs capture and process antigens to peptides which are then presented in the context of MHC class Ⅱ or class Ⅰ molecules. It is generally accepted that the function of DCs of patients with chronic HBV infection is impaired, resulting in more tolerogenic rather than immunogenic responses, which may contribute to viral persistence. However, whether DCs in chronic HBV patients are phenotypically and functionally equal to DCs from healthy donors is still open to discussion. A few studies have shown that the frequency and function of *ex vivo*-analyzed mDCs and pDCs are largely intact in patients with HBV infection and similar to those of healthy donor DCs, with the exception of reduced IFNα production by pDC from CHB patients[74]. Treatment of MoDCs with HBsAg resulted in enhanced cell surface expression of CD80, CD83, CD86 and MHC class Ⅱ, and increased production of IL-12 p40, IL-12 p70, and IL-10[75]. Nevertheless, other studies showed that the pDCs isolated from CHB patients have lower expression

of HLA-DR and the costimulatory molecules CD80 and CD40, leading to low allo-stimulatory function, and lower levels of IFN-α and IL-12 production[76-78]. The major role of DCs in CHB immunopathogenesis mainly involves their interaction with other cells of the innate or adaptive immune systems.

DC s and NK cells

NK cell functions are closely related to those of DCs. DCs play a crucial role in the NK cell activation and a reciprocal functional interaction between NK cells and either pDCs or mDCs may play an important physiological role in the regulation of both innate and adaptive immune responses[79-81]. DCs efficiently enhances NK cell expression of CD69, proliferation, IFNγ secretion and cytotoxic activity. Studies have suggested that membrane-associated molecules, as well as soluble factors such as IL-12, TNF-α and type Ⅰ IFNs, contribute to DC-mediated NK cell activation[82] and subsequent adaptive immune responses. CHB patients display a diminished functional interaction between poly(I:C)/IFNγ activated mDC and NK cells due to impaired mDC function and reduced IFNγ production compared to those of healthy individuals. Furthermore, restoration of TLR3-activated mDC activity leads to improved NK cell function, which underlies the impaired DC-induced NK cell dysfunction in CHB[83].

NK cells also promote the DCs maturation and markedly augment their capacity to produce proinflammatory cytokines and to stimulate T cell responses. The NK cell-mediated effects on DCs are dependent on cell membrane-associated molecules, such as NKp30 and soluble factors, such as TNF-α and IFNγ[82]. The intrahepatic pool of NK cells also plays a key role in the regulation of DC function in CHB patients[80]. Therefore, it can be speculated that enhancing this reciprocal interaction will reinforce the innate and thus, the adaptive immune response, which may contribute significantly to achieving effective antiviral immunity[81].

DCs and HBV-specific CD8$^+$ T cells

Experimental evidence has shown that HBV-specific T cell responses are essential for the control of HBV infection. In chronic HBV infection, virus-specific CD8$^+$ T cells are recruited to the liver, but are functionally or quantitatively impaired[84]. Typically, DCs activate resting T cells to initiate immune responses. Impaired DC function in patients with CHB may lead to insufficient T cell responses to HBV, which may be associated with persistent viral infection. HBV particles and purified HBsAg both contribute to the mDC dysfunction[85,86] and inhibit the antiviral function of autologous lymphocytes manifested by decreased IFNγ and IL-2 production and increased IL-10 secretion. A recent study demonstrated that HBcAg-pulsed DCs derived from CHB patients exhibited a stronger capacity to stimulate autologous CD4$^+$ and CD8$^+$ T cells to release IFNγ and induce HBV core 18-27 specific CTLs[87]. Furthermore, CpG-activated pDCs act synergistically *in vitro* with HBcAg-pulsed moDCs (core-DC) in inducing autologous HBV-specific CD8$^+$ T cell proliferation and IFNγ production[88]. Thus, mature DCs efficiently induce Th1 polarization of T cells and generate HBcAg-specific CTLs. In addition, liver-resident CD103$^+$ DCs are also highly immunogenic in hepatotropic viral infections and serve as a major APC to support the local CD8$^+$ T cell responses[89].

DCs and Treg cells

Circulating CD4$^+$ CD25$^+$ Tregs have been demonstrated to maintain immunotolerance and suppress antigen-specific or antigen-non-specific T cell responses. In CHB patients, the frequency of CD4$^+$ CD25 (high) Tregs is increased and correlates positively with serum viral load and has been shown to suppress HBV antigen-stimulated autologous PBMC proliferation and IFNγ production *in vitro*[90]. In CHB patients, DCs induce the expansion of Tregs, which continue to express high levels of forkhead box P3 (Foxp3) protein[91]. Furthermore, Tregs induced by NK-primed DCs are capable of inducing a suppressor effect *via* the negative co-stimulation of PD-1[92]. On the other hand, when triggered by a specific antigen, Tregs act on immature DCs *via* a feedback mechanism to block the upregulation of the costimulatory molecules, CD80 and CD86[91].

NK CELLS, NKT CELLS AND ADAPTIVE CELLS

NK cells represent the main effector cell population involved in innate immune responses against intracellular pathogens and tumor cells through their cytolytic activity and production of cytokines. NK cells are enriched in the liver, with a frequency of 30%-50% of intrahepatic lymphocytes in humans, which is 10-12-fold higher in CHB patients compared to healthy controls[93]. NKT cells share characteristics with innate lymphocytes and classic NK cell markers that link innate and adaptive immunity[94,95]. CD1d-restricted invariant NKT (iNKT) cells are a group of innate-like regulatory T cells, which play a central role in the regulation of the liver environment[96]. In addition to the direct killing of viral-infected cells without antigen-specific priming, NK cells regulate adaptive immune responses by producing interferon IFNγ, TNF-α and immunoregulatory cytokines[97]. The ability of NK cells to modulate T cell responses can be mediated through direct T-NK interactions, cytokine production, or indirectly through DCs and other cell types. Early NK cell interactions with other immune cells can have long-lasting effects on the number and quality of memory T cells, as well as impacting the exhaustion of T cells during chronic infections[98]. Evidence supporting the role of NK cells in acute HBV infection is conflicting. One study demonstrated that the activation and cytokine-producing function of NK cells was impaired in acute HBV patients[69], while another study demonstrated that NK cell activation and the development of NK and NKT cell responses is earlier

than that of HBV-specific T cells, which may contribute to limiting the spread of HBV and lead to the timely induction of adaptive responses[99].

It is becoming increasingly apparent that NK cells exert a detrimental effect on the host during chronic HBV infection. As reviewed by Schuch *et al*[100], NK cells regulate adaptive immune responses by exhaustion of HBV-specific $CD8^+$ T cells, probably by producing IL-10 and TGF-β on activation[101-104], upregulation of tumor necrosis factor-related apoptosis-inducing ligand[105] and by diminishing APC function during persistent virus infection[106]. NK cell depletion can improve memory T cell formation[107] and control persistent infection[108]. The absence of the inhibitory receptor 2B4 on NK cells resulted in a reduced virus-specific $CD8^+$ T cell response that led to prolonged viral persistence[109]. Human regulatory NK cells (NKreg), which are a subgroup of NK cells, have been shown to produce IL-10 and reduce the proliferation of antigen-specific $CD4^+$ T cells *in vitro*[110]. NKreg cells can also limit virus-specific $CD8^+$ T cell immunity and promote chronic virus infection or immune pathology[92]. Furthermore, in a mouse model of acute infection, NK cells have also been shown to inhibit the generation of virus-specific memory T- and B-cells as well as virus-specific antibody production in a perforin-dependent manner[111]. iNKT cells play a central role in the regulation of the liver environment. Upon activation, iNKT cells secret large amounts of both Th1 and Th2 cytokines and play key regulatory roles in antimicrobial immunity[96]. One report showed that the number and cytokine-producing function of iNKT cells were comparable in CHB patients and healthy controls, while another study showed that iNKT cell frequency decreased with disease progression in CHB patients[112]. When activated by the ligand, alpha-galactosylceramide (alpha-GalCer), Vα14-positive NKT cells strongly enhance the induction and proliferation of HBsAg-specific CTLs in mouse models and promote the disruption of tolerance to HBV-specific $CD8^+$ T cell antigens[113].

ADAPTIVE IMMUNE CELLS

B cells and T cells

Anti-HBs antibodies play an important role in the clearance of HBV particles in the blood and protection against reinfection of hepatocytes[114]. Memory B cell responses are indicative of a resolved previous infection[115] because the appearance of anti-HBs antibodies occurs relatively late after HBV exposure, and are usually absent in the clinical symptomatic phase of infection as well as in the chronic stage. The role of anti-HBs-positive B cells in the resolution or the pathogenesis of infection has been underestimated. In addition to anti-HBs production, B cells can act as APCs for antiviral $CD4^+$ T cells[116]. A number of studies have yielded contradictory findings. Xu *et al*[117] suggested that expression of CD80, serum HBs antibody levels and the frequency of HBsAg-specific B cells were significantly decreased in CHB patients compared with healthy control subjects. In contrast, another study showed that there were no differences in the frequencies of B-lymphocytes expressing CD80 and CD86 between CHB patients and healthy controls[118]. Some data indicated that interactions between B and T cells may contribute to immunotolerance in mouse models with B cells as predominant APCs[119]. Sustained exposure to viral antigens can lead to an increase in the frequency of B cells with an exhausted phenotype in the liver[120] as well as the induction of negative costimulatory molecules, such as PD-1 and CTLA-4[121]. In contrast, some evidence demonstrates that an overwhelming B cell response plays a key role in HBV-associated acute liver failure[122,123]. Therefore, the function of B cells in HBV infection requires further investigation.

$CD4^+$ T cells and $CD8^+$ T cells

Evidence of the role of $CD4^+$ T cells in the control of HBV infection is conflicting. Some data show that, similar to $CD8^+$ T cells, the $CD4^+$ T cell response in the acute phase of self-limiting infection is significantly greater and multi-specific than in the chronic phase. Furthermore, the induction of functional HBV-specific $CD8^+$ T cell responses is dependent on early $CD4^+$ T cell priming prior to HBV spread[114]. While $CD4^+$ T cell depletion at the peak of HBV infection had no effect on viral replication in infected chimpanzees[124], depletion prior to HBV infection resulted in quantitatively and functionally impaired HBV-specific $CD8^+$ T cell responses[125]. In the absence of early $CD4^+$ T cell responses, intrahepatic $CD8^+$ T cell priming results in T cell inactivation, tolerance or apoptosis[126,127]. Functional impairment of T cells may also contribute to hyperactivation of regulatory $CD4^+$ $FoxP3^+$ T cells that suppress virus-specific T cells, thereby affecting the quality and intensity of antiviral responses. In CHB patients, the frequency of circulating $CD4^+CD25^+$ Treg cells correlates significantly with serum viral load and liver injury[128]. Th17 cells, another $CD4^+$ T cell subset, may contribute to disease progression and the pathogenesis of liver injury in HBV-infected patients. An increased Treg/Th17 ratio and the Th17 frequency at onset have significant predictive value for survival of patients with HBV-related acute-on-chronic liver failure[129,130].

LIVER CELLS AND ADAPTIVE IMMUNE

Liver cells include NPCs and hepatocytes. Under normal conditions, resident liver LSECs and KCs secrete IL-10 and TGF-β, maintaining a tolerogenic environment and restraining inflammatory responses to foreign antigens, such as HBV[67,131]. LSECs, as one type of local APC, are capable of antigen cross-presentation and subsequent tolerization of naive $CD8^+$ T cells. Under certain conditions, LSECs can switch from a tolerogenic to an immunogenic state and promote the development of T cell immunity[131]. As in the setting of acute HBV infection, liver cells might be able to

sense HBV infection and mount antiviral effects *via* an IFN response[132]. Furthermore, LSEC-mediated cross-presentation of soluble, circulating or hepatocyte-derived antigens to naïve $CD8^+$ T cells results in the development of antigen-experienced memory-like T cells[133]. LSECs and KCs can reduce TLR expression leading to the inactivation of innate immunity[134]. LSECs are the major liver cell type responsible for the induction of TGF-β-dependent hepatic $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells, which contribute to the tolerogenic features of the intrahepatic microenvironment[135,136]. In contrast to activated professional APCs, intrahepatic antigen presentation by HBV-positive hepatocytes suppresses HBV-specific $CD8^+$ T cell responses or mediates T cell apoptosis *via* the PD-1/ PD-L1 pathway[137]. This may in part, explain the development of the tolerogenic hepatic microenvironment and the occurrence of persistent HBV infection in the liver. Thus, precise quantitative and qualitative regulation of $CD4^+$ T responses is required to initiate the activation of $CD8^+$ T cells to control the infection.

CONCLUSION

The innate immune system is the first line of host defense against infection immediately after the pathogen invasion. Its functions depend largely on multiple regulators, including TLRs and cytokines, mainly type Ⅰ IFN and subsequent activation of adaptive immune responses. Initiation of effective adaptive immune responses, especially HBV-specific $CD8^+$ T cell responses, is central to the control of HBV infection. Efficient clearance of viral infections requires the synergistic interaction of both innate and adaptive immune responses, which is vital for the development of long-lasting immunity. A better understanding of these complex interactions and their role in HBV infection is essential for designing effective immunotherapeutic regimens for CHB and designing new combination treatment strategies for the eradication HBV[138].

REFERENCES

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

2 **Lai CL**, Ratziu V, Yuen MF, Poynard T. Viral hepatitis B. *Lancet* 2003; **362**: 2089-2094 [PMID: 14697813]

3 **Chen J**, Yuan Z. Interplay between hepatitis B virus and the innate immune responses: implications for new therapeutic strategies. *Virol Sin* 2014; **29**: 17-24 [PMID: 24452540 DOI: 10.1007/s12250-014-3412-3]

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

5 **Yang PL**, Althage A, Chung J, Maier H, Wieland S, Isogawa M, Chisari FV. Immune effectors required for hepatitis B virus clearance. *Proc Natl Acad Sci USA* 2010; **107**: 798-802 [PMID: 20080755 DOI: 10.1073/pnas.0913498107]

6 **Bertoletti A**, Tan AT, Gehring AJ. HBV-Specific Adaptive Immunity. *Viruses* 2009; **1**: 91-103 [PMID: 21994540 DOI: 10.3390/v1020091]

7 **Kondo Y**, Kobayashi K, Asabe S, Shiina M, Niitsuma H, Ueno Y, Kobayashi T, Shimosegawa T. Vigorous response of cytotoxic T lymphocytes associated with systemic activation of CD8 T lymphocytes in fulminant hepatitis B. *Liver Int* 2004; **24**: 561-567 [PMID: 15566505]

8 **Busca A**, Kumar A. Innate immune responses in hepatitis B virus (HBV) infection. *Virol J* 2014; **11**: 22 [PMID: 24507433 DOI: 10.1186/1743-422X-11-22]

9 **Sarkar N**, Panigrahi R, Pal A, Biswas A, Singh SP, Kar SK, Bandopadhyay M, Das D, Saha D, Kanda T, Sugiyama M, Chakrabarti S, Banerjee A, Chakravarty R. Expression of microRNA-155 correlates positively with the expression of Toll-like receptor 7 and modulates hepatitis B virus via C/EBP-β in hepatocytes. *J Viral Hepat* 2015; **22**: 817-827 [PMID: 25720442 DOI: 10.1111/jvh.12390]

10 **Wu JF**, Chen CH, Ni YH, Lin YT, Chen HL, Hsu HY, Chang MH. Toll-like receptor and hepatitis B virus clearance in chronic infected patients: a long-term prospective cohort study in Taiwan. *J Infect Dis* 2012; **206**: 662-668 [PMID: 22740716 DOI: 10.1093/infdis/jis420]

11 **Wu JF**, Hsu HY, Chiu YC, Chen HL, Ni YH, Chang MH. The effects of cytokines on spontaneous hepatitis B surface antigen seroconversion in chronic hepatitis B virus infection. *J Immunol* 2015; **194**: 690-696 [PMID: 25505294 DOI: 10.4049/jimmunol.1401659]

12 **Kondo Y**, Ueno Y, Shimosegawa T. Toll-like receptors signaling contributes to immunopathogenesis of HBV infection. *Gastroenterol Res Pract* 2011; **2011**: 810939 [PMID: 22190911 DOI: 10.1155/2011/810939]

13 **Akira S**, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006; **124**: 783-801 [PMID: 16497588]

14 **Ma Z**, Zhang E, Yang D, Lu M. Contribution of Toll-like receptors to the control of hepatitis B virus infection by initiating antiviral innate responses and promoting specific adaptive immune responses. *Cell Mol Immunol* 2015; **12**: 273-282 [PMID: 25418467 DOI: 10.1038/cmi.2014.112]

15 **Gay NJ**, Gangloff M. Structure and function of Toll receptors and their ligands. *Annu Rev Biochem* 2007; **76**: 141-165 [PMID: 17362201]

16 **Takeda K**, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol* 2003; **21**: 335-376 [PMID: 12524386]

17 **Miyake K**. Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. *Semin Immunol* 2007; **19**: 3-10 [PMID: 17275324]

18 **Seki E**, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. *Hepatology* 2008; **48**: 322-335 [PMID: 18506843 DOI: 10.1002/hep.22306]

19 **Zhang E**, Lu M. Toll-like receptor (TLR)-mediated innate immune responses in the control of hepatitis B virus (HBV) infection. *Med Microbiol Immunol* 2015; **204**: 11-20 [PMID: 25550115 DOI: 10.1007/s00430-014-0370-1]

20 **Isogawa M**, Robek MD, Furuichi Y, Chisari FV. Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. *J Virol* 2005; **79**: 7269-7272 [PMID: 15890966]

21 **Fitzgerald-Bocarsly P**, Dai J, Singh S. Plasmacytoid dendritic cells and type I IFN: 50 years of convergent history. *Cytokine Growth Factor Rev* 2008; **19**: 3-19 [PMID: 18248767 DOI: 10.1016/j.cytogfr.2007.10.006]

22 **Wu J**, Meng Z, Jiang M, Zhang E, Trippler M, Broering R, Bucchi A, Krux F, Dittmer U, Yang D, Roggendorf M, Gerken G, Lu M, Schlaak JF. Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. *Immunology* 2010; **129**: 363-374 [PMID: 19922426 DOI: 10.1111/j.1365-2567.2009.03179.x]

23 **Sajadi SM**, Mirzaei V, Hassanshahi G, Khorramdelazad H, Daredor HY, Hosseini SM, Moogooi M, Ravary A, Arababadi MK, Kennedy D. Decreased expressions of Toll-like receptor 9 and its signaling molecules in chronic hepatitis B virus-infected patients. *Arch Pathol Lab Med* 2013; **137**: 1674-1679 [PMID: 24168509 DOI: 10.5858/arpa.2012-0415-OA]

24 **Momeni M**, Zainodini N, Bidaki R, Hassanshahi G, Daneshvar H, Khaleghinia M, Ebrahim M, Karimi-Googheri M, Askari A, Arababadi MK, Kennedy D. Decreased expression of toll like receptor signaling molecules in chronic HBV infected patients. *Hum Immunol* 2014; **75**: 15-19 [PMID: 24120739 DOI: 10.1016/j.humimm.2013.09.015]

25 **Shahrakyvahed A**, Sanchooli J, Sanadgol N, Arababadi MK, Kennedy D. TLR9: an important molecule in the fight against hepatitis B virus. *Postgrad Med J* 2014; **90**: 396-401 [PMID: 24942353 DOI: 10.1136/postgradmedj-2013-132309]

26 **Xie Q**, Shen HC, Jia NN, Wang H, Lin LY, An BY, Gui HL, Guo SM, Cai W, Yu H, Guo Q, Bao S. Patients with chronic hepatitis B infection display deficiency of plasmacytoid dendritic cells with reduced expression of TLR9. *Microbes Infect* 2009; **11**: 515-523 [PMID: 19289178 DOI: 10.1016/j.micinf.2009.02.008]

27 **Chen Z**, Cheng Y, Xu Y, Liao J, Zhang X, Hu Y, Zhang Q, Wang J, Zhang Z, Shen F, Yuan Z. Expression profiles and function of Toll-like receptors 2 and 4 in peripheral blood mononuclear cells of chronic hepatitis B patients. *Clin Immunol* 2008; **128**: 400-408 [PMID: 18565796 DOI: 10.1016/j.clim.2008.04.006]

28 **Lu X**, Xu Q, Bu X, Ma X, Zhang F, Deng Q, Zhang Y, Ding J. Relationship between expression of toll-like receptors 2/4 in dendritic cells and chronic hepatitis B virus infection. *Int J Clin Exp Pathol* 2014; **7**: 6048-6055 [PMID: 25337250]

29 **Adib-Conquy M**, Scott-Algara D, Cavaillon JM, Souza-Fonseca-Guimaraes F. TLR-mediated activation of NK cells and their role in bacterial/viral immune responses in mammals. *Immunol Cell Biol* 2014; **92**: 256-262 [PMID: 24366517 DOI: 10.1038/icb.2013.99]

30 **Hart OM**, Athie-Morales V, O'Connor GM, Gardiner CM. TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-gamma production. *J Immunol* 2005; **175**: 1636-1642 [PMID: 16034103]

31 **Girart MV**, Fuertes MB, Domaica CI, Rossi LE, Zwirner NW. Engagement of TLR3, TLR7, and NKG2D regulate IFN-gamma secretion but not NKG2D-mediated cytotoxicity by human NK cells stimulated with suboptimal doses of IL-12. *J Immunol* 2007; **179**: 3472-3479 [PMID: 17804388]

32 **Ratnam DT**, Sievert W, Visvanathan K. Natural killer cells display impaired responses to toll like receptor 9 that support viral persistence in chronic hepatitis B. *Cell Immunol* 2012; **279**: 109-115 [PMID: 23123793 DOI: 10.1016/j.cellimm.2012.09.005]

33 **Limmer A**, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. *Nat Med* 2000; **6**: 1348-1354 [PMID: 11100119]

34 **Kern M**, Popov A, Scholz K, Schumak B, Djandji D, Limmer A, Eggle D, Sacher T, Zawatzky R, Holtappels R, Reddehase MJ, Hartmann G, Debey-Pascher S, Diehl L, Kalinke U, Koszinowski U, Schultze J, Knolle PA. Virally infected mouse liver endothelial cells trigger CD8+ T-cell immunity. *Gastroenterology* 2010; **138**: 336-346 [PMID: 19737567 DOI: 10.1053/j.gastro.2009.08.057]

35 **Liu J**, Jiang M, Ma Z, Dietze KK, Zelinskyy G, Yang D, Dittmer U, Schlaak JF, Roggendorf M, Lu M. TLR1/2 ligand-stimulated mouse liver endothelial cells secrete IL-12 and trigger CD8+ T cell immunity in vitro. *J Immunol* 2013; **191**: 6178-6190 [PMID: 24227786 DOI: 10.4049/jimmunol.1301262]

36 **Wu J**, Lu M, Meng Z, Trippler M, Broering R, Szczeponek A, Krux F, Dittmer U, Roggendorf M, Gerken G, Schlaak JF. Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice. *Hepatology* 2007; **46**: 1769-1778 [PMID: 17929296]

37 **Jiang M**, Broering R, Trippler M, Poggenpohl L, Fiedler M, Gerken G, Lu M, Schlaak JF. Toll-like receptor-mediated immune responses are attenuated in the presence of high levels of hepatitis B virus surface antigen. *J Viral Hepat* 2014; **21**: 860-872 [PMID: 24498958 DOI: 10.1111/jvh.12216]

38 **Reynolds JM**, Dong C. Toll-like receptor regulation of effector T lymphocyte function. *Trends Immunol* 2013; **34**: 511-519 [PMID: 23886621 DOI: 10.1016/j.it.2013.06.003]

39 **Geng D**, Zheng L, Srivastava R, Asprodites N, Velasco-Gonzalez C, Davila E. When Toll-like receptor and T-cell receptor signals collide: a mechanism for enhanced CD8 T-cell effector function. *Blood* 2010; **116**: 3494-3504 [PMID: 20696947 DOI: 10.1182/blood-2010-02-268169]

40 **Tabiasco J**, Devêvre E, Rufer N, Salaun B, Cerottini JC, Speiser D, Romero P. Human effector CD8+ T lymphocytes express TLR3 as a functional coreceptor. *J Immunol* 2006; **177**: 8708-8713 [PMID: 17142772]

41 **Babu S**, Blauvelt CP, Kumaraswami V, Nutman TB. Cutting edge: diminished T cell TLR expression and function modulates the immune response in human filarial infection. *J Immunol* 2006; **176**: 3885-3889 [PMID: 16547219]

42 **Mandraju R**, Murray S, Forman J, Pasare C. Differential ability of surface and endosomal TLRs to induce CD8 T cell responses in vivo. *J Immunol* 2014; **192**: 4303-4315 [PMID: 24688022 DOI: 10.4049/jimmunol.1302244]

43 **Crampton SP**, Voynova E, Bolland S. Innate pathways to B-cell activation and tolerance. *Ann N Y Acad Sci* 2010; **1183**: 58-68 [PMID: 20146708 DOI: 10.1111/j.1749-6632.2009.05123.x]

44 **Hua Z**, Hou B. TLR signaling in B-cell development and activation. *Cell Mol Immunol* 2013; **10**: 103-106 [PMID: 23241902 DOI: 10.1038/cmi.2012.61]

45 **Jain S**, Chodisetti SB, Agrewala JN. Combinatorial signaling through TLR-2 and CD86 augments activation and differentiation of resting B cells. *PLoS One* 2013; **8**: e54392 [PMID: 23365665 DOI: 10.1371/journal.pone.0054392]

46 **Jain S**, Chodisetti SB, Agrewala JN. CD40 signaling synergizes with TLR-2 in the BCR independent activation of resting B cells. *PLoS One* 2011; **6**: e20651 [PMID: 21674065]

47 **Lin FC**, Young HA. Interferons: Success in anti-viral immunotherapy. *Cytokine Growth Factor Rev* 2014; **25**: 369-376 [PMID: 25156421 DOI: 10.1016/j.cytogfr.2014.07.015]

48 **Pei RJ**, Chen XW, Lu MJ. Control of hepatitis B virus replication by interferons and Toll-like receptor signaling pathways. *World J Gastroenterol* 2014; **20**: 11618-11629 [PMID: 25206268 DOI: 10.3748/wjg.v20.i33.11618]

49 **Guidotti LG**, Morris A, Mendez H, Koch R, Silverman RH, Williams BR, Chisari FV. Interferon-regulated pathways that control hepatitis B virus replication in transgenic mice. *J Virol* 2002; **76**: 2617-2621 [PMID: 11861827]

50 **Belloni L**, Allweiss L, Guerrieri F, Pediconi N, Volz T, Pollicino T, Petersen J, Raimondo G, Dandri M, Levrero M. IFN-α inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. *J Clin Invest* 2012; **122**: 529-537 [PMID: 22251702 DOI: 10.1172/JCI58847]

51 **Samuel CE**. Antiviral actions of interferons. *Clin Microbiol Rev* 2001; **14**: 778-809, table of contents [PMID: 11585785]

52 **Katze MG**, He Y, Gale M. Viruses and interferon: a fight for supremacy. *Nat Rev Immunol* 2002; **2**: 675-687 [PMID: 12209136]

53 **Phillips S**, Chokshi S, Riva A, Evans A, Williams R, Naoumov NV. CD8(+) T cell control of hepatitis B virus replication: direct comparison between cytolytic and noncytolytic functions. *J Immunol* 2010; **184**: 287-295 [PMID: 19949099 DOI: 10.4049/jimmunol.0902761]

54 **Lan P**, Zhang C, Han Q, Zhang J, Tian Z. Therapeutic recovery of hepatitis B virus (HBV)-induced hepatocyte-intrinsic immune defect reverses systemic adaptive immune tolerance. *Hepatology* 2013; **58**: 73-85 [PMID: 23447417 DOI: 10.1002/hep.26339]

55 **Bertoletti A**, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. *Postgrad Med J* 2013; **89**: 294-304 [PMID: 23596257 DOI: 10.1136/postgradmedj-2011-301073rep]

56 **Tzeng HT**, Tsai HF, Chyuan IT, Liao HJ, Chen CJ, Chen PJ, Hsu PN. Tumor necrosis factor-alpha induced by hepatitis B virus core mediating the immune response for hepatitis B viral clearance in mice model. *PLoS One* 2014; **9**: e103008 [PMID: 25047809 DOI: 10.1371/journal.pone.0103008]

57 **Chyuan IT**, Tsai HF, Tzeng HT, Sung CC, Wu CS, Chen PJ, Hsu

PN. Tumor necrosis factor-alpha blockage therapy impairs hepatitis B viral clearance and enhances T-cell exhaustion in a mouse model. *Cell Mol Immunol* 2015; **12**: 317-325 [PMID: 25661729 DOI: 10.1038/cmi.2015.01]

58 **Bouezzedine F**, Fardel O, Gripon P. Interleukin 6 inhibits HBV entry through NTCP down regulation. *Virology* 2015; **481**: 34-42 [PMID: 25765005 DOI: 10.1016/j.virol.2015.02.026]

59 **Hösel M**, Quasdorff M, Wiegmann K, Webb D, Zedler U, Broxtermann M, Tedjokusumo R, Esser K, Arzberger S, Kirschning CJ, Langenkamp A, Falk C, Büning H, Rose-John S, Protzer U. Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology* 2009; **50**: 1773-1782 [PMID: 19937696 DOI: 10.1002/hep.23226]

60 **Jegaskanda S**, Ahn SH, Skinner N, Thompson AJ, Ngyuen T, Holmes J, De Rose R, Navis M, Winnall WR, Kramski M, Bernardi G, Bayliss J, Colledge D, Sozzi V, Visvanathan K, Locarnini SA, Kent SJ, Revill PA. Downregulation of interleukin-18-mediated cell signaling and interferon gamma expression by the hepatitis B virus e antigen. *J Virol* 2014; **88**: 10412-10420 [PMID: 24872585 DOI: 10.1128/JVI.00111-14]

61 **Motavaf M**, Safari S, Alavian SM. Interleukin 18 gene promoter polymorphisms and susceptibility to chronic hepatitis B infection: a review study. *Hepat Mon* 2014; **14**: e19879 [PMID: 25031585 DOI: 10.5812/hepatmon.19879]

62 **Karra VK**, Gumma PK, Chowdhury SJ, Ruttala R, Polipalli SK, Chakravarti A, Kar P. IL-18 polymorphisms in hepatitis B virus related liver disease. *Cytokine* 2015; **73**: 277-282 [PMID: 25802197 DOI: 10.1016/j.cyto.2015.02.015]

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

64 **Schon HT**, Weiskirchen R. Immunomodulatory effects of transforming growth factor-β in the liver. *Hepatobiliary Surg Nutr* 2014; **3**: 386-406 [PMID: 25568862 DOI: 10.3978/j.issn.2304-3881.2014.11.06]

65 **Chou YC**, Chen ML, Hu CP, Chen YL, Chong CL, Tsai YL, Liu TL, Jeng KS, Chang C. Transforming growth factor-beta1 suppresses hepatitis B virus replication primarily through transcriptional inhibition of pregenomic RNA. *Hepatology* 2007; **46**: 672-681 [PMID: 17580335]

66 **Xu L**, Yin W, Sun R, Wei H, Tian Z. Kupffer cell-derived IL-10 plays a key role in maintaining humoral immune tolerance in hepatitis B virus-persistent mice. *Hepatology* 2014; **59**: 443-452 [PMID: 23929689 DOI: 10.1002/hep.26668]

67 **Dunn C**, Peppa D, Khanna P, Nebbia G, Jones M, Brendish N, Lascar RM, Brown D, Gilson RJ, Tedder RJ, Dusheiko GM, Jacobs M, Klenerman P, Maini MK. Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. *Gastroenterology* 2009; **137**: 1289-1300 [PMID: 19591831 DOI: 10.1053/j.gastro.2009.06.054]

68 **Gong Y**, Zhao C, Zhao P, Wang M, Zhou G, Han F, Cui Y, Qian J, Zhang H, Xiong H, Sheng J, Jiang T. Role of IL-10-Producing Regulatory B Cells in Chronic Hepatitis B Virus Infection. *Dig Dis Sci* 2015; **60**: 1308-1314 [PMID: 25260658 DOI: 10.1007/s10620-014-3358-1]

69 **Li Y**, Tang L, Hou J. Role of interleukin-21 in HBV infection: friend or foe? *Cell Mol Immunol* 2015; **12**: 303-308 [PMID: 25363528 DOI: 10.1038/cmi.2014.109]

70 **Li HJ**, Kang FB, Li BS, Yang XY, Zhang YG, Sun DX. Interleukin-21 inhibits HBV replication in vitro. *Antivir Ther* 2015; **20**: 583-590 [PMID: 25774942 DOI: 10.3851/IMP2950]

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

72 **Li X**, Tian L, Dong Y, Zhu Q, Wang Y, Han W, Liu X, Ni Q, Chen Y, Li L. IL-35 inhibits HBV antigen-specific IFN-γ-producing CTLs in vitro. *Clin Sci* (Lond) 2015; **129**: 395-404 [PMID: 25869609 DOI: 10.1042/CS20140511]

73 **Zhou Y**, Zhang H, Li Y. IL-35 expression in peripheral blood CD4(+) T cells from chronic hepatitis B virus-infected patients directly correlates with virus load. *Cytokine* 2015; **73**: 169-175 [PMID: 25752977 DOI: 10.1016/j.cyto.2015.02.003]

74 **Tavakoli S**, Mederacke I, Herzog-Hauff S, Glebe D, Grün S, Strand D, Urban S, Gehring A, Galle PR, Böcher WO. Peripheral blood dendritic cells are phenotypically and functionally intact in chronic hepatitis B virus (HBV) infection. *Clin Exp Immunol* 2008; **151**: 61-70 [PMID: 18031557]

75 **Jan RH**, Lin YL, Chen CJ, Lin TY, Hsu YC, Chen LK, Chiang BL. Hepatitis B virus surface antigen can activate human monocyte-derived dendritic cells by nuclear factor kappa B and p38 mitogen-activated protein kinase mediated signaling. *Microbiol Immunol* 2012; **56**: 719-727 [PMID: 22853328 DOI: 10.1111/j.1348-0421.2012.00496.x]

76 **Wang FS**, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY. Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2001; **7**: 537-541 [PMID: 11819824]

77 **Gehring AJ**, Ann D'Angelo J. Dissecting the dendritic cell controversy in chronic hepatitis B virus infection. *Cell Mol Immunol* 2015; **12**: 283-291 [PMID: 25363524 DOI: 10.1038/cmi.2014.95]

78 **Lin C**, Zou H, Wang S. Hepatitis B e Antigen Seroconversion Is Related with the Function of Dendritic Cells in Chronic Hepatitis B Virus Infection. *Gastroenterol Res Pract* 2014; **2014**: 413952 [PMID: 25574162 DOI: 10.1155/2014/413952]

79 **Gerosa F**, Gobbi A, Zorzi P, Burg S, Briere F, Carra G, Trinchieri G. The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. *J Immunol* 2005; **174**: 727-734 [PMID: 15634892]

80 **Gerosa F**, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. *J Exp Med* 2002; **195**: 327-333 [PMID: 11828007]

81 **Degli-Esposti MA**, Smyth MJ. Close encounters of different kinds: dendritic cells and NK cells take centre stage. *Nat Rev Immunol* 2005; **5**: 112-124 [PMID: 15688039]

82 **Wehner R**, Dietze K, Bachmann M, Schmitz M. The bidirectional crosstalk between human dendritic cells and natural killer cells. *J Innate Immun* 2011; **3**: 258-263 [PMID: 21411969 DOI: 10.1159/000323923]

83 **Tjwa ET**, van Oord GW, Biesta PJ, Boonstra A, Janssen HL, Woltman AM. Restoration of TLR3-activated myeloid dendritic cell activity leads to improved natural killer cell function in chronic hepatitis B virus infection. *J Virol* 2012; **86**: 4102-4109 [PMID: 22318141 DOI: 10.1128/JVI.07000-11]

84 **Maini MK**, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, Williams R, Vergani D, Naoumov NV, Ferrari C, Bertoletti A. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000; **191**: 1269-1280 [PMID: 10770795]

85 **Apostolopoulos V**, Thalhammer T, Tzakos AG, Stojanovska L. Targeting antigens to dendritic cell receptors for vaccine development. *J Drug Deliv* 2013; **2013**: 869718 [PMID: 24228179 DOI: 10.1155/2013/869718]

86 **Op den Brouw ML**, Binda RS, van Roosmalen MH, Protzer U, Janssen HL, van der Molen RG, Woltman AM. Hepatitis B virus surface antigen impairs myeloid dendritic cell function: a possible immune escape mechanism of hepatitis B virus. *Immunology* 2009; **126**: 280-289 [PMID: 18624732 DOI: 10.1111/j.1365-2567.2008.02896.x]

87 **Chen W**, Shi M, Shi F, Mao Y, Tang Z, Zhang B, Zhang H, Chen L, Chen L, Xin S, Wang FS. HBcAg-pulsed dendritic cell vaccine induces Th1 polarization and production of hepatitis B virus-specific cytotoxic T lymphocytes. *Hepatol Res* 2009; **39**: 355-365 [PMID: 19889049 DOI: 10.1111/j.1872-034X.2008.00468.x]

88 **Chen W**, Zhang Z, Shi M, Chen L, Fu J, Shi F, Zhang B, Zhang H, Jin L, Wang FS. Activated plasmacytoid dendritic cells act synergistically with hepatitis B core antigen-pulsed monocyte-derived dendritic cells in the induction of hepatitis B virus-specific

CD8 T-cell response. *Clin Immunol* 2008; **129**: 295-303 [PMID: 18774748 DOI: 10.1016/j.clim.2008.07.026]
89 **Krueger PD**, Kim TS, Sung SS, Braciale TJ, Hahn YS. Liver-resident CD103+ dendritic cells prime antiviral CD8+ T cells in situ. *J Immunol* 2015; **194**: 3213-3222 [PMID: 25712214 DOI: 10.4049/jimmunol.1402622]
90 **Peng G**, Li S, Wu W, Sun Z, Chen Y, Chen Z. Circulating CD4+ CD25+ regulatory T cells correlate with chronic hepatitis B infection. *Immunology* 2008; **123**: 57-65 [PMID: 17764450]
91 **Yamazaki S**, Inaba K, Tarbell KV, Steinman RM. Dendritic cells expand antigen-specific Foxp3+ CD25+ CD4+ regulatory T cells including suppressors of alloreactivity. *Immunol Rev* 2006; **212**: 314-329 [PMID: 16903923]
92 **Lang PA**, Lang KS, Xu HC, Grusdat M, Parish IA, Recher M, Elford AR, Dhanji S, Shaabani N, Tran CW, Dissanayake D, Rahbar R, Ghazarian M, Brüstle A, Fine J, Chen P, Weaver CT, Klose C, Diefenbach A, Häussinger D, Carlyle JR, Kaech SM, Mak TW, Ohashi PS. Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. *Proc Natl Acad Sci USA* 2012; **109**: 1210-1215 [PMID: 22167808 DOI: 10.1073/pnas.1118834109]
93 **Sitia G**, Isogawa M, Iannacone M, Campbell IL, Chisari FV, Guidotti LG. MMPs are required for recruitment of antigen-nonspecific mononuclear cells into the liver by CTLs. *J Clin Invest* 2004; **113**: 1158-1167 [PMID: 15085195]
94 **Boni C**, Fisicaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, Laccabue D, Zerbini A, Cavalli A, Missale G, Bertoletti A, Ferrari C. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. *J Virol* 2007; **81**: 4215-4225 [PMID: 17287266]
95 **Villanueva AI**, Haeryfar SM, Mallard BA, Kulkarni RR, Sharif S. Functions of invariant NK T cells are modulated by TLR ligands and IFN-α. *Innate Immun* 2015; **21**: 275-288 [PMID: 24934453 DOI: 10.1177/1753425914527327]
96 **Lawrenczyk A**, Kim S, Wen X, Xiong R, Yuan W. Exploring the Therapeutic Potentials of iNKT Cells for Anti-HBV Treatment. *Pathogens* 2014; **3**: 563-576 [PMID: 25438012 DOI: 10.3390/pathogens3030563]
97 **Pollicino T**, Koumbi L. Role natural killer group 2D-ligand interactions in hepatitis B infection. *World J Hepatol* 2015; **7**: 819-824 [PMID: 25937859 DOI: 10.4254/wjh.v7.i6.819]
98 **Cook KD**, Waggoner SN, Whitmire JK. NK cells and their ability to modulate T cells during virus infections. *Crit Rev Immunol* 2014; **34**: 359-388 [PMID: 25404045]
99 **Fisicaro P**, Valdatta C, Boni C, Massari M, Mori C, Zerbini A, Orlandini A, Sacchelli L, Missale G, Ferrari C. Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. *Gut* 2009; **58**: 974-982 [PMID: 19201769 DOI: 10.1136/gut.2008.163600]
100 **Schuch A**, Hoh A, Thimme R. The role of natural killer cells and CD8(+) T cells in hepatitis B virus infection. *Front Immunol* 2014; **5**: 258 [PMID: 24917866 DOI: 10.3389/fimmu.2014.00258]
101 **Lee SH**, Kim KS, Fodil-Cornu N, Vidal SM, Biron CA. Activating receptors promote NK cell expansion for maintenance, IL-10 production, and CD8 T cell regulation during viral infection. *J Exp Med* 2009; **206**: 2235-2251 [PMID: 19720840 DOI: 10.1084/jem.20082387]
102 **Brooks DG**, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence in vivo. *Nat Med* 2006; **12**: 1301-1309 [PMID: 17041596]
103 **Gray JD**, Hirokawa M, Ohtsuka K, Horwitz DA. Generation of an inhibitory circuit involving CD8+ T cells, IL-2, and NK cell-derived TGF-beta: contrasting effects of anti-CD2 and anti-CD3. *J Immunol* 1998; **160**: 2248-2254 [PMID: 9498764]
104 **Conroy MJ**, Mac Nicholas R, Grealy R, Taylor M, Otegbayo JA, O'Dea S, Mulcahy F, Ryan T, Norris S, Doherty DG. Circulating CD56dim natural killer cells and CD56+ T cells that produce interferon-γ or interleukin-10 are expanded in asymptomatic, E antigen-negative patients with persistent hepatitis B virus infection. *J Viral Hepat* 2015; **22**: 335-345 [PMID: 25186004 DOI: 10.1111/jvh.12299]
105 **Peppa D**, Gill US, Reynolds G, Easom NJ, Pallett LJ, Schurich A, Micco L, Nebbia G, Singh HD, Adams DH, Kennedy PT, Maini MK. Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. *J Exp Med* 2013; **210**: 99-114 [PMID: 23254287 DOI: 10.1084/jem.20121172]
106 **Cook KD**, Whitmire JK. The depletion of NK cells prevents T cell exhaustion to efficiently control disseminating virus infection. *J Immunol* 2013; **190**: 641-649 [PMID: 23241878 DOI: 10.4049/jimmunol.1202448]
107 **Soderquest K**, Walzer T, Zafirova B, Klavinskis LS, Polić B, Vivier E, Lord GM, Martín-Fontecha A. Cutting edge: CD8+ T cell priming in the absence of NK cells leads to enhanced memory responses. *J Immunol* 2011; **186**: 3304-3308 [PMID: 21307295 DOI: 10.4049/jimmunol.1004122]
108 **Waggoner SN**, Daniels KA, Welsh RM. Therapeutic depletion of natural killer cells controls persistent infection. *J Virol* 2014; **88**: 1953-1960 [PMID: 24284324 DOI: 10.1128/JVI.03002-13]
109 **Sun C**, Fu B, Gao Y, Liao X, Sun R, Tian Z, Wei H. TGF-β1 down-regulation of NKG2D/DAP10 and 2B4/SAP expression on human NK cells contributes to HBV persistence. *PLoS Pathog* 2012; **8**: e1002594 [PMID: 22438812 DOI: 10.1371/journal.ppat.1002594]
110 **Deniz G**, Erten G, Kücüksezer UC, Kocacik D, Karagiannidis C, Aktas E, Akdis CA, Akdis M. Regulatory NK cells suppress antigen-specific T cell responses. *J Immunol* 2008; **180**: 850-857 [PMID: 18178824]
111 **Rydyznski C**, Daniels KA, Karmele EP, Brooks TR, Mahl SE, Moran MT, Li C, Sutiwisesak R, Welsh RM, Waggoner SN. Generation of cellular immune memory and B-cell immunity is impaired by natural killer cells. *Nat Commun* 2015; **6**: 6375 [PMID: 25721802 DOI: 10.1038/ncomms7375]
112 **Zhu H**, Zhang Y, Liu H, Zhang Y, Kang Y, Mao R, Yang F, Zhou D, Zhang J. Preserved Function of Circulating Invariant Natural Killer T Cells in Patients With Chronic Hepatitis B Virus Infection. *Medicine* (Baltimore) 2015; **94**: e961 [PMID: 26091463 DOI: 10.1097/MD.0000000000000961]
113 **Ito H**, Ando K, Ishikawa T, Nakayama T, Taniguchi M, Saito K, Imawari M, Moriwaki H, Yokochi T, Kakumu S, Seishima M. Role of Valpha14+ NKT cells in the development of Hepatitis B virus-specific CTL: activation of Valpha14+ NKT cells promotes the breakage of CTL tolerance. *Int Immunol* 2008; **20**: 869-879 [PMID: 18487227 DOI: 10.1093/intimm/dxn046]
114 **Isogawa M**, Tanaka Y. Immunobiology of hepatitis B virus infection. *Hepatol Res* 2015; **45**: 179-189 [PMID: 25331910 DOI: 10.1111/hepr.12439]
115 **Wang Q**, Sachse P, Semmo M, Lokhande M, Montani M, Dufour JF, Zoulim F, Klenerman P, Semmo N. T- and B-cell responses and previous exposure to hepatitis B virus in 'anti-HBc alone' patients. *J Viral Hepat* 2015; **22**: 1068-1078 [PMID: 26075501 DOI: 10.1111/jvh.12428]
116 **Mitchison NA**. T-cell-B-cell cooperation. *Nat Rev Immunol* 2004; **4**: 308-312 [PMID: 15057789]
117 **Xu X**, Shang Q, Chen X, Nie W, Zou Z, Huang A, Meng M, Jin L, Xu R, Zhang JY, Fu J, Wang L, Tang Z, Xie Y, Yang X, Zhang Z, Wang FS. Reversal of B-cell hyperactivation and functional impairment is associated with HBsAg seroconversion in chronic hepatitis B patients. *Cell Mol Immunol* 2015; **12**: 309-316 [PMID: 25849120 DOI: 10.1038/cmi.2015.25]
118 **Wongjitrat C**, Sukwit S, Chuenchitra T, Seangjaruk P, Rojanasang P, Romputtan P, Srisurapanon S. CTLA-4 and its ligands on the surface of T- and B-lymphocyte subsets in chronic hepatitis B virus infection. *J Med Assoc Thai* 2013; **96** Suppl 1: S54-S59 [PMID: 23724456]
119 **Raimondi G**, Zanoni I, Citterio S, Ricciardi-Castagnoli P, Granucci F. Induction of peripheral T cell tolerance by antigen-presenting B cells. II. Chronic antigen presentation overrules antigen-presenting B cell activation. *J Immunol* 2006; **176**: 4021-4028 [PMID: 16547237]
120 **Mohamadkhani A**, Naderi E, Sotoudeh M, Katoonizadeh

A, Montazeri G, Poustchi H. Clinical feature of intrahepatic B-lymphocytes in chronic hepatitis B. *Int J Inflam* 2014; **2014**: 896864 [PMID: 24579055 DOI: 10.1155/2014/896864]
121 **Frommer F**, Heinen TJ, Wunderlich FT, Yogev N, Buch T, Roers A, Bettelli E, Müller W, Anderton SM, Waisman A. Tolerance without clonal expansion: self-antigen-expressing B cells program self-reactive T cells for future deletion. *J Immunol* 2008; **181**: 5748-5759 [PMID: 18832734]
122 **Farci P**, Diaz G, Chen Z, Govindarajan S, Tice A, Agulto L, Pittaluga S, Boon D, Yu C, Engle RE, Haas M, Simon R, Purcell RH, Zamboni F. B cell gene signature with massive intrahepatic production of antibodies to hepatitis B core antigen in hepatitis B virus-associated acute liver failure. *Proc Natl Acad Sci USA* 2010; **107**: 8766-8771 [PMID: 20421498 DOI: 10.1073/pnas.1003854107]
123 **Dao DY**, Hynan LS, Yuan HJ, Sanders C, Balko J, Attar N, Lok AS, Word RA, Lee WM. Two distinct subtypes of hepatitis B virus-related acute liver failure are separable by quantitative serum immunoglobulin M anti-hepatitis B core antibody and hepatitis B virus DNA levels. *Hepatology* 2012; **55**: 676-684 [PMID: 21987355 DOI: 10.1002/hep.24732]
124 **Thimme R**, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, Chisari FV. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J Virol* 2003; **77**: 68-76 [PMID: 12477811]
125 **Asabe S**, Wieland SF, Chattopadhyay PK, Roederer M, Engle RE, Purcell RH, Chisari FV. The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. *J Virol* 2009; **83**: 9652-9662 [PMID: 19625407 DOI: 10.1128/JVI.00867-09]
126 **Isogawa M**, Chung J, Murata Y, Kakimi K, Chisari FV. CD40 activation rescues antiviral CD8+ T cells from PD-1-mediated exhaustion. *PLoS Pathog* 2013; **9**: e1003490 [PMID: 23853599 DOI: 10.1371/journal.ppat.1003490]
127 **Bertolino P**, Bowen DG, McCaughan GW, Fazekas de St Groth B. Antigen-specific primary activation of CD8+ T cells within the liver. *J Immunol* 2001; **166**: 5430-5438 [PMID: 11313380]
128 **Xu D**, Fu J, Jin L, Zhang H, Zhou C, Zou Z, Zhao JM, Zhang B, Shi M, Ding X, Tang Z, Fu YX, Wang FS. Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006; **177**: 739-747 [PMID: 16785573]
129 **Xue-Song L**, Cheng-Zhong L, Ying Z, Mo-Bin W. Changes of Treg and Th17 cells balance in the development of acute and chronic hepatitis B virus infection. *BMC Gastroenterol* 2012; **12**: 43 [PMID: 22548790 DOI: 10.1186/1471-230X-12-43]
130 **Liang XS**, Li CZ, Zhou Y, Yin W, Liu YY, Fan WH. Changes in circulating Foxp3(+) regulatory T cells and interleukin-17-producing T helper cells during HBV-related acute-on-chronic liver failure. *World J Gastroenterol* 2014; **20**: 8558-8571 [PMID: 25024610 DOI: 10.3748/wjg.v20.i26.8558]
131 **You Q**, Cheng L, Kedl RM, Ju C. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. *Hepatology* 2008; **48**: 978-990 [PMID: 18712788 DOI: 10.1002/hep.22395]
132 **Schurich A**, Berg M, Stabenow D, Böttcher J, Kern M, Schild HJ, Kurts C, Schuette V, Burgdorf S, Diehl L, Limmer A, Knolle PA. Dynamic regulation of CD8 T cell tolerance induction by liver sinusoidal endothelial cells. *J Immunol* 2010; **184**: 4107-4114 [PMID: 20212092 DOI: 10.4049/jimmunol.0902580]
133 **Ait-Goughoulte M**, Lucifora J, Zoulim F, Durantel D. Innate antiviral immune responses to hepatitis B virus. *Viruses* 2010; **2**: 1394-1410 [PMID: 21994686 DOI: 10.3390/v2071394]
134 **Knolle PA**, Böttcher J, Huang LR. The role of hepatic immune regulation in systemic immunity to viral infection. *Med Microbiol Immunol* 2015; **204**: 21-27 [PMID: 25523194 DOI: 10.1007/s00430-014-0371-0]
135 **Chang JJ**, Lewin SR. Immunopathogenesis of hepatitis B virus infection. *Immunol Cell Biol* 2007; **85**: 16-23 [PMID: 17130898]
136 **Carambia A**, Freund B, Schwinge D, Heine M, Laschtowitz A, Huber S, Wraith DC, Korn T, Schramm C, Lohse AW, Heeren J, Herkel J. TGF-β-dependent induction of CD4+CD25+Foxp3+ Tregs by liver sinusoidal endothelial cells. *J Hepatol* 2014; **61**: 594-599 [PMID: 24798620 DOI: 10.1016/j.jhep.2014.04.027]
137 **Mühlbauer M**, Fleck M, Schütz C, Weiss T, Froh M, Blank C, Schölmerich J, Hellerbrand C. PD-L1 is induced in hepatocytes by viral infection and by interferon-alpha and -gamma and mediates T cell apoptosis. *J Hepatol* 2006; **45**: 520-528 [PMID: 16876901]
138 **Fazle Akbar SM**, Al-Mahtab M, Hiasa Y. Designing immune therapy for chronic hepatitis B. *J Clin Exp Hepatol* 2014; **4**: 241-246 [PMID: 25755566 DOI: 10.1016/j.jceh.2014.06.008]

P- Reviewer: Ito H, Rodriguez-Frias F, Tai DI, Vaughan G
S- Editor: Wang JL **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.i30.2992

World J Hepatol 2015 December 28; 7(30): 2992-2998
ISSN 1948-5182 (online)

MINIREVIEWS

Management of rectal varices in portal hypertension

Kawtar Al Khalloufi, Adeyinka O Laiyemo

Kawtar Al Khalloufi, Adeyinka O Laiyemo, Division of Gastroenterology, Department of Medicine, Howard University College of Medicine, Washington, DC 20060, United States

Author contributions: All authors contributed to this manuscript.

Supported by The National Center for Advancing Translational Science, Nos. KL2TR000102-04 and UL1RT000101; the National Institute for Diabetes; Digestive Diseases and Kidney, No. R21DK100875; and National Institutes of Health.

Conflict-of-interest statement: The authors declare no conflicts of interest regarding this manuscript.

Correspondence to: Adeyinka O Laiyemo, MD, MPH, Division of Gastroenterology, Department of Medicine, Howard University College of Medicine, 2041 Georgia Avenue, NW, Washington, DC 20060,
United States. adeyinka.laiyemo@howard.edu
Telephone: +1-202-8657186
Fax: +1-202-8654607

Received: July 28, 2015
Peer-review started: July 29, 2015
First decision: September 29, 2015
Revised: October 13, 2015
Accepted: December 9, 2015
Article in press: December 11, 2015
Published online: December 28, 2015

Abstract

Rectal varices are portosystemic collaterals that form as a complication of portal hypertension, their prevalence has been reported as high as 94% in patients with extrahepatic portal vein obstruction. The diagnosis is typically based on lower endoscopy (colonoscopy or sigmoidoscopy). However, endoscopic ultrasonography has been shown to be superior to endoscopy in diagnosing rectal varices. Color Doppler ultrasonography is a better method because it allows the calculation of the velocity of blood flow in the varices and can be used to predict the bleeding risk in the varices. Although rare, bleeding from rectal varices can be life threatening. The management of patients with rectal variceal bleeding is not well established. It is important to ensure hemodynamic stability with blood transfusion and to correct any coagulopathy prior to treating the bleeding varices. Endoscopic injection sclerotherapy has been reported to be more effective in the management of active bleeding from rectal varices with less rebleeding rate as compared to endoscopic band ligation. Transjugular intrahepatic portsystemic shunt alone or in combination with embolization is another method used successfully in control of bleeding. Balloon-occluded retrograde transvenous obliteration is an emerging procedure for management of gastric varices that has also been successfully used to treat bleeding rectal varices. Surgical procedures including suture ligation and porto-caval shunts are considered when other methods have failed.

Key words: Rectal varices; Portal hypertension; Liver cirrhosis; Colonoscopy; Gastrointestinal bleeding

Core tip: Rectal varices complicate portal hypertension. Although rare, bleeding from rectal varices can be life threatening. There are no established guidelines for the treatment of rectal varices. In this article, the authors review endoscopic, radiological, and surgical techniques which have been suggested to be effective in the management of bleeding rectal varices.

Al Khalloufi K, Laiyemo AO. Management of rectal varices in

portal hypertension. *World J Hepatol* 2015; 7(30): 2992-2998 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i30/2992.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i30.2992

INTRODUCTION

In 1954, Cabot *et al*[1] discussed the first reported case of bleeding rectal varices. Ganguly *et al*[2] defined rectal varices as dilated veins that originate more than 4 cm above the anal verge, clearly distinct from hemorrhoids, and not contiguous with the anal columns and/or pectinate line. The incidence of rectal varices in cirrhotic and non-cirrhotic patient varies in different reports and ranges between 38% and 94%[3,4]. Their prevalence in patients with cirrhosis is between 38% and 56%, whereas their prevalence in extrahepatic portal vein obstruction was reported between 63% and 94%[3,5]. Despite the high prevalence of rectal varices, clinically significant bleeding is rare and occurs in 0.5%-5% of patients[6].

While the management of esophageal varices has been well established[7], the optimal treatment of rectal varices remains to be determined. Endoscopic therapies, Transjugular Intrahepatic Portosystemic Shunt placement (TIPS), balloon-occluded retrograde transvenous obliteration (BRTO), and surgical management are some of the therapeutic options for management of rectal varices. The purpose of this article is to provide an updated review of current management of rectal varices.

PATHOGENESIS

Rectal varices are collaterals between the portal and systemic circulations that manifest as a dilation of the submucosal veins and constitute a pathway for portal venous flow between the superior rectal veins which branch from the inferior mesenteric system and the middle inferior rectal veins from the iliac system[8].

The normal hepatic venous pressure gradient (HVPG) ranges between 1 and 5 mmHg, becomes clinically significant when it reaches 10 mmHg and varices usually develop when the value of HVPG increases to at least 12 mmHg[9,10]. In the western hemisphere, sinusoidal portal hypertension secondary to liver cirrhosis is the most common cause of portal hypertension. There is a direct correlation between the progression of cirrhosis reflected by the Child Pugh or MELD scores and the degree of hyperdynamic circulation[11,12]. Hosking *et al*[13] studied 100 patients with cirrhosis and reported that the overall prevalence of rectal varices was 44%, this prevalence increased with the degree of portal hypertension. The authors described rectal varices in 19% of patients with cirrhosis without esophageal varices, 39% in patients with esophageal varices without history of bleeding, and 59% in patients with esophageal varices and history of bleeding. In this study, hemorrhoids occurred independently of the presence of rectal varices and 30% of patients had rectal varices and coexistent hemorrhoids[13].

There have been conflicting reports regarding the occurrence of rectal varices after obliteration of esophageal varices. However, a large study conducted in Japan by Watanabe *et al*[14] reported that 95% of patients with rectal varices had a history of esophageal varices and 87% of these patients had previously undergone endoscopic variceal obliteration for esophageal varices. The mechanism of rectal varices after treatment of esophageal or gastric varices is thought to be the result of obliteration of supplying vessels such as the left gastric, posterior gastric and short gastric veins leading to development of collateral vessels of the inferior mesenteric venous system and thus the formation of rectal varices. In this nationally representative study in Japan, the most frequent afferent vessel to the rectal varices was the inferior mesenteric vein, followed by the superior rectal vein and the efferent vessels included the internal iliac vein and the inferior rectal vein[14].

DIAGNOSIS

Endoscopy

Endoscopy is the main method for diagnosing rectal varices. They are visualized as blue tinted submucosal elevations located near the anus[15]. Rectal varices may be confused with internal hemorrhoids because of their location. However, hemorrhoids are not related to portal hypertension. Hemorrhoids result from a displacement of the anal cushions and hyperperfusion of the arteriovenous plexus vascular cushions without direct communication with any of the major branches of the portal venous system[16].

According to the general rules for recording endoscopic findings of esophago-gastric varices prepared by the Japanese Research Committee on Portal Hypertension, all codes for esophageal varices are used to describe ectopic varices including rectal varices[15]. Varices are classified into four groups according to their shapes and sizes. When there are no varices (F0), small and straight (F1), enlarged and tortuous (F2) and large and coil-shaped (F3). The color (C) of the varices is classified as either white (Cw) or blue (Cb). The dilated, small vessels or telangiectasia on the variceal surface is referred to as the red color sign (RC) which endoscopically indicates a high risk of bleeding. RC signs are graded as 0, 1, 2 or 3 according to their density and distribution. RC0 refers to no RC sign, RC1 to only a few RC signs, RC2 to several RC signs and RC3 to many RC signs. The bleeding signs as well as the mucosal findings can also be evaluated and described by endoscopy[15] (Table 1).

Endoscopic ultrasound

Conventional endoscopic ultrasound (EUS) reveals rectal varices as rounded, oval, or longitudinal echo free structures in the submucosa and also shows perirectal collateral veins outside the rectal wall. EUS can detect deep rectal varices in a large proportion of patients who do not have identified varices on routine endoscopy[17].

Table 1 The general rules for recording endoscopic findings of varices prepared by the Japanese research committee on portal hypertension[15]

Form (F)	F0: No varicose appearance
	F1: Straight, small-caliber varices
	F2: Moderately enlarged, beady varices
	F3: Markedly enlarged, nodular or tumor-shaped varices
Color (C)	Cw: White varices
	Cb: Blue varices
	Cw-Th: Thrombosed white varices
	Cb-Th: Thrombosed blue varices
Red color signs (RC)	RWM: Red wale markings
	CRS: Cherry red spots
	HCS: Hematocystic spots
	RC(-): Absent
	RC(+): Small in number and localized
	RC(++): Intermediate between (+) and (+++)
	RC(++ +): Large in number and circumferential
	Te: Telangiectasia
Bleeding signs	Gushing bleeding
	Spurting bleeding
	Oozing bleeding
	Red plug
	White plug
Mucosal findings	E: Erosion
	Ul: Ulcer
	S: Scar

Dhiman *et al*[18] showed that EUS is better than endoscopy in detecting rectal varices (85% *vs* 45%) and in determining their number. Sato *et al*[19] demonstrated that intramural rectal varices, perirectal collateral veins, and the communicating veins between intramural rectal varices and perirectal collateral veins could be observed clearly with an ultrasonic microprobe. They also showed that the mean velocity of blood flow in rectal varices in the patients with rectal bleeding was significantly higher than in those cases experiencing no bleeding which indicates that the color doppler ultrasonography may be helpful in identifying high-risk group for rectal variceal rupture *via* the measurement of velocity[20]. Endoscopic color Doppler ultrasonography is better equipped than conventional EUS to evaluate the hemodynamics of varices, it can detect rectal varices through color flow images, calculate the velocity of blood flow in rectal varices for an effective and safe endoscopic variceal management[21].

TREATMENT

Medical management

The management of bleeding rectal varices essentially includes prompt resuscitation and correction of coagulopathy. The intravascular volume repletion is done with crystalloids and packed red blood cells. The Asian Pacific Association for the Study of the Liver recommends to maintain systolic blood pressure between 90-100 mmhg, and the heart rate below 100 beats/min[22]. The goal of blood transfusion is a hemoglobin level approximately 8 g/dL (hematocrit of 24)[7]. A short course of prophylactic antibiotic therapy should be administered to improve survival and decrease the risk of spontaneous bacterial peritonitis in all patients presenting with cirrhosis and gastrointestinal bleed including rectal bleeding[23]. There are no randomized control trials to recommend the use of vasoactive drugs such as vasopressin, terlipressin or octreotide in bleeding rectal varices. However, these drugs have a proven benefit in the management of bleeding gastro-oesophageal varices and hence can be considered for use in bleeding rectal varices[24].

Endoscopic management

Endoscopic injection sclerotherapy: Endoscopic injection sclerotherapy (EIS) was first reported to be useful for treatment of rectal bleeding in 1985[25]. Later on, other case reports of successful EIS for treatment of bleeding rectal varices were published[26-28]. Sato *et al*[29] performed EIS using 5% ethanolamine oleate with iopamidol, which was injected intermittently under fluoroscopy in 32 patients. The patients were successfully treated without serious complications. The authors suggested the necessity to evaluate the hemodynamics of the rectal varices before EIS to avoid severe complications such as pulmonary embolism. They also recommended injecting the sclerosant slowly under fluoroscopy. The recurrence rate in this series was 24% over the 1-year follow-up period.

Endoscopic band ligation: Endoscopic band ligation (EBL) has been well studied and its efficiency in treating bleeding esophageal varices is well known[30,31]. EBL has also been used in treatment of gastric varices. However, its efficacy in this regard is equivocal[32,33]. In 1996, Kojima *et al*[34] used EBL in the management of bleeding rectal varices. Subsequently, Uno *et al*[35] reported a successful use of EBL to treat bleeding rectal varices after failure of sclerotherapy in a child with extrahepatic portal hypertension. Long term follow up of 46 mo after successful use of EBL in treatment of bleeding and obliteration of rectal varices as the initial therapy in an adult patient was reported by Firoozi *et al*[36] EBL is a safe and effective therapy for rectal varices, however the risk of recurrence is high[34,37]. Sato *et al*[38] compared EIS to EBL in the management of rectal varices. EIS appeared to be superior to EBL with regard to effectiveness. The recurrence rate was less with EIS 33.3% *vs* 55.6% with EBL. No complications were noted with EIS, however one patient who received EBL developed bleeding ulcer[38].

Cyanoacrylate injection: Cyanoacrylate glue is an accepted therapeutic method for gastric varices, although its use is off-label in the United States[39]. It was first described by Soehendra *et al*[40]. This glue preparations work by immediate polymerization upon contact with blood, causing vascular obstruction and is eventually extruded into the gastric lumen, typically about 1 mo after injection[41,42]. Weilert *et al*[43] reported a case of rectal varix managed successfully with EUS-guided cyanoacrylate injection and embolization coils.

The use of coils is believed to provide a scaffold to retain glue within the varix, thereby minimizing the risk of embolization and allowing for a decreased volume of glue injection for variceal obliteration[43]. Color Doppler-EUS has been used to diagnose submucosal endoscopically inevident rectal varices bleeding and to manage it by histoacryl glue injection[44]. The most serious adverse events of glue injection therapy is systemic embolization and sepsis which has been reported secondary to embolized glue acting as a septic focus[45]. Embolization into the arterial circulation (*via* a patent foramen ovale or arteriovenous pulmonary shunt) can result in stroke and multiorgan infarction[45].

Interventional radiology

TIPS: TIPS is a minimally invasive and effective method used for management of rectal varices during active bleeding. It can serve both as a bridge to transplantation and as the definitive therapy in patients who are not good candidates for surgery[24]. TIPS was first used in 1993 by Katz *et al*[46] in a patient with repeated bleeding from anorectal varices (ARV) with marked decompression of the varices 24 h after placement of the TIPS. The patient had no recurrent bleeding after 6 mo of follow up. Several case reports and small case series of bleeding ARV successfully managed with TIPS have been described in the literature[47-55]. Kochar *et al*[56] reported in 2008 the largest series of patients (n = 28) with bleeding ectopic varices, 12 of them were rectal varices treated by TIPS placement. Hemostasis was effectively achieved in 67% of the patients. This was achieved solely with TIPS without concomitant embolization in 21 of the 22 (95%) patients and in three of the five (60%) patients who had TIPS and concomitant variceal embolization. Rebleeding from ectopic varices occurred in five (21%) patients. In two (40%) patients, the rebleeding was secondary to shunt dysfunction and responded to revision of the shunt. However, rebleeding occurred in three patients despite a functioning shunt with low portal pressure gradients[56].

Embolization: Embolization is a procedure performed by interventional radiologist to occlude the feeding vein to the rectal varices. It can either be performed alone or in combination with band ligation or TIPS[53,57]. When used alone, embolization results in high 1 year rebleeding rates[54]. The combination of TIPS and embolization has been described as efficient in the prevention of recurrent bleeding from esophagogastric varices[58]. After embolization, the communication of the portal vein and the rectal veins remains partially interrupted even after shunt stenosis. Hence, the increase of the pressure in the portal vein is not directly transmitted into the rectal plexus[53]. Ahn *et al*[59] reported recently a case of recurrent bleeding after successful TIPS treated with variceal embolization. Various embolization materials are used, including coils, gelfoam, thrombin, collagen, autologous blood clot and ethanol[60,61].

BRTO: Developed by Kanagawa *et al*[62] in the early 1990s, the BRTO procedure is an endovascular technique that causes occlusion of outflow portosystemic shunt, such as a gastrorenal shunt, using an occlusion balloon followed by the endovascular injection of a sclerosing agent directly into the gastro-variceal system[57,62]. For the past two decades, this procedure has become common practice in Asia for the management of gastric varices. It is now becoming more popular in the United States. It has been shown to be effective in controlling gastric variceal bleeding with low rebleeding rates. BRTO has many advantages over TIPS. It is less invasive and can be performed on patients with poor hepatic reserve and those with encephalopathy[63]. Anan *et al*[64] reported a case of successful treatment of colonic varices by means of BRTO in a patient with hepatic encephalopathy leading to resolution of the encephalopathy and worsening of preexisting esophageal varices. This reflects postprocedural increased portal hypertension. A more recent article reported the success of BRTO as an additional therapy to surgical suture in controlling bleeding rectal varices with 1.26 cm feeding vessel. However, the patient died 6 mo later from liver failure[65].

Surgical management

Surgery has been used for treatment of rectal varices mainly when endoscopic management has failed. Surgical methods include simple suture ligation, inferior mesenteric vein occlusion and porto-caval shunt surgery. The later has been shown to be effective in controlling life threatening bleeding. However, the majority of patients presenting with bleeding rectal varices have a poor general condition and are not good candidate for these major surgical procedures[66]. The mortality in these patients is high and is mainly secondary to liver failure. Bittinger *et al*[66] reported 80% mortality within 2 mo despite adequate local treatment of the rectal varices.

Direct suture ligation is a technically challenging option and often not successful. However, the stapled approach seems to be a suitable alternative. Stapled procedure for the control of bleeding varices was first reported in 2002 by Botterill *et al*[67]. The authors reported a circumferential stapling device was used to successfully control bleeding ano-rectal varices after failure of injection sclerotherapy and band ligation. In 2005, another case report also demonstrated that stapled procedure may be an effective means of bleeding control[68]. A case series of nine patients was published by Kaul *et al*[69] with successful control of bleeding following a circumferential stapled procedure. Four of the nine patients were previously treated with endoscopic therapy (three with banding and one with injection sclerotherapy). No further rebleeding was noted during the follow up period of 4 to 24 mo.

CONCLUSION

Bleeding rectal varices can be a life threatening

condition in patients with portal hypertension and should be considered in the differential diagnosis of these patients when they present with lower gastrointestinal bleeding.

The management of rectal varices is multidisciplinary and involves gastroenterologists, interventional radiologists and surgeons. There are no established guidelines to define the appropriate management strategies for rectal varices. Published studies consist mainly of case reports and series. This article provides a review of the literature summarizing the different therapeutic options to manage rectal varices.

REFERENCES

1 **Cabot RC**, Castleman B, Towne VW. Case 40102. *N Engl J Med* 1954; **250**: 434-438 [DOI: 10.1056/NEJM195403112501008]
2 **Ganguly S**, Sarin SK, Bhatia V, Lahoti D. The prevalence and spectrum of colonic lesions in patients with cirrhotic and noncirrhotic portal hypertension. *Hepatology* 1995; **21**: 1226-1231 [PMID: 7737627 DOI: 10.1016/0270-9139(95)90041-1]
3 **Chawla Y**, Dilawari JB. Anorectal varices--their frequency in cirrhotic and non-cirrhotic portal hypertension. *Gut* 1991; **32**: 309-311 [PMID: 2013427 DOI: 10.1136/gut.32.3.309]
4 **Misra SP**, Dwivedi M, Misra V. Prevalence and factors influencing hemorrhoids, anorectal varices, and colopathy in patients with portal hypertension. *Endoscopy* 1996; **28**: 340-345 [PMID: 8813499 DOI: 10.1055/s-2007-1005477]
5 **Misra SP**, Dwivedi M, Misra V, Dharmani S, Kunwar BK, Arora JS. Colonic changes in patients with cirrhosis and in patients with extrahepatic portal vein obstruction. *Endoscopy* 2005; **37**: 454-459 [PMID: 15844025 DOI: 10.1055/s-2005-861252]
6 **Shudo R**, Yazaki Y, Sakurai S, Uenishi H, Yamada H, Sugawara K. Clinical study comparing bleeding and nonbleeding rectal varices. *Endoscopy* 2002; **34**: 189-194 [PMID: 11870567]
7 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907]
8 **Sato T**, Akaike J, Toyota J, Karino Y, Ohmura T. Clinicopathological features and treatment of ectopic varices with portal hypertension. *Int J Hepatol* 2011; **2011**: 960720 [PMID: 21994879 DOI: 10.4061/2011/960720]
9 **Groszmann RJ**, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Gao H, Makuch R. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med* 2005; **353**: 2254-2261 [PMID: 16306522 DOI: 10.1056/NEJMoa044456]
10 **Iwakiri Y**, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. *Hepatology* 2006; **43**: S121-S131 [PMID: 16447289 DOI: 10.1002/hep.20993]
11 **Llach J**, Ginès P, Arroyo V, Rimola A, Titó L, Badalamenti S, Jiménez W, Gaya J, Rivera F, Rodés J. Prognostic value of arterial pressure, endogenous vasoactive systems, and renal function in cirrhotic patients admitted to the hospital for the treatment of ascites. *Gastroenterology* 1988; **94**: 482-487 [PMID: 3335320]
12 **Møller S**, Hobolth L, Winkler C, Bendtsen F, Christensen E. Determinants of the hyperdynamic circulation and central hypovolaemia in cirrhosis. *Gut* 2011; **60**: 1254-1259 [PMID: 21504996 DOI: 10.1136/gut.2010.235473]
13 **Hosking SW**, Smart HL, Johnson AG, Triger DR. Anorectal varices, haemorrhoids, and portal hypertension. *Lancet* 1989; **1**: 349-352 [PMID: 2563507 DOI: 10.1016/S0140-6736(89)91724-8]
14 **Watanabe N**, Toyonaga A, Kojima S, Takashimizu S, Oho K, Kokubu S, Nakamura K, Hasumi A, Murashima N, Tajiri T. Current status of ectopic varices in Japan: Results of a survey by the Japan Society for Portal Hypertension. *Hepatol Res* 2010; **40**: 763-776 [PMID: 20649816 DOI: 10.1111/j.1872-034X.2010.00690.x]
15 **Idezuki Y**. General rules for recording endoscopic findings of esophagogastric varices (1991). Japanese Society for Portal Hypertension. *World J Surg* 1995; **19**: 420-422; discussion 423 [PMID: 7638999 DOI: 10.1007/BF00299178]
16 **Lohsiriwat V**. Hemorrhoids: from basic pathophysiology to clinical management. *World J Gastroenterol* 2012; **18**: 2009-2017 [PMID: 22563187 DOI: 10.3748/wjg.v18.i17.2009]
17 **Wiechowska-Kozłowska A**, Białek A, Milkiewicz P. Prevalence of 'deep' rectal varices in patients with cirrhosis: an EUS-based study. *Liver Int* 2009; **29**: 1202-1205 [PMID: 19508616 DOI: 10.1111/j.1478-3231.2009.02047.x]
18 **Dhiman RK**, Choudhuri G, Saraswat VA, Mukhopadhyay DK, Khan EM, Pandey R, Naik SR. Endoscopic ultrasonographic evaluation of the rectum in cirrhotic portal hypertension. *Gastrointest Endosc* 1993; **39**: 635-640 [PMID: 8224684 DOI: 10.1016/S0016-5107(93)70214-X]
19 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Suga T. The value of the ultrasonic microprobe in the detection and treatment of rectal varices: a case report. *Hepatol Res* 2003; **27**: 158-162 [PMID: 14563431 DOI: 10.1016/S1386-6346(03)00203-1]
20 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Akaike J. Diagnosis of rectal varices via color Doppler ultrasonography. *Am J Gastroenterol* 2007; **102**: 2253-2258 [PMID: 17561969 DOI: 10.1111/j.1572-0241.2007.01340.x]
21 **Sato T**, Yamazaki K, Akaike J. Evaluation of the hemodynamics of rectal varices by endoscopic ultrasonography. *J Gastroenterol* 2006; **41**: 588-592 [PMID: 16868808 DOI: 10.1007/s00535-006-1815-0]
22 **Sarin SK**, Kumar A, Angus PW, Baijal SS, Baik SK, Bayraktar Y, Chawla YK, Choudhuri G, Chung JW, de Franchis R, de Silva HJ, Garg H, Garg PK, Helmy A, Hou MC, Jafri W, Jia JD, Lau GK, Li CZ, Lui HF, Maruyama H, Pandey CM, Puri AS, Rerknimitr R, Sahni P, Saraya A, Sharma BC, Sharma P, Shiha G, Sollano JD, Wu J, Xu RY, Yachha SK, Zhang C. Diagnosis and management of acute variceal bleeding: Asian Pacific Association for Study of the Liver recommendations. *Hepatol Int* 2011; **5**: 607-624 [PMID: 21484145 DOI: 10.1007/s12072-010-9236-9]
23 **Soares-Weiser K**, Brezis M, Tur-Kaspa R, Leibovici L. Antibiotic prophylaxis for cirrhotic patients with gastrointestinal bleeding. *Cochrane Database Syst Rev* 2002; (**2**): CD002907 [PMID: 12076458 DOI: 10.1002/14651858.CD002907]
24 **Maslekar S**, Toh EW, Adair R, Bate JP, Botterill I. Systematic review of anorectal varices. *Colorectal Dis* 2013; **15**: e702-e710 [PMID: 24020839 DOI: 10.1111/codi.12417]
25 **Wang M**, Desigan G, Dunn D. Endoscopic sclerotherapy for bleeding rectal varices: a case report. *Am J Gastroenterol* 1985; **80**: 779-780 [PMID: 3876025]
26 **Weiserbs DB**, Zfass AM, Messmer J. Control of massive hemorrhage from rectal varices with sclerotherapy. *Gastrointest Endosc* 1986; **32**: 419-421 [PMID: 3492406 DOI: 10.1016/S0016-5107(86)71928-7]
27 **Richon J**, Berclaz R, Schneider PA, Marti MC. Sclerotherapy of rectal varices. *Int J Colorectal Dis* 1988; **3**: 132-134 [PMID: 3411184 DOI: 10.1007/BF01645319]
28 **Yamanaka T**, Shiraki K, Ito T, Sugimoto K, Sakai T, Ohmori S, Takase K, Nakano T, Oohashi Y, Okuda Y. Endoscopic sclerotherapy (ethanolamine oleate injection) for acute rectal varices bleeding in a patient with liver cirrhosis. *Hepatogastroenterology* 2002; **49**: 941-943 [PMID: 12143248]
29 **Sato T**. Efficacy of Endoscopic Injection Sclerotherapy for Rectal Varices. *Clin Exp Gastroenterol* 2010; **2**: 159-163
30 **Laine L**, el-Newihi HM, Migikovsky B, Sloane R, Garcia F. Endoscopic ligation compared with sclerotherapy for the treatment of bleeding esophageal varices. *Ann Intern Med* 1993; **119**: 1-7 [PMID: 8498757 DOI: 10.7326/0003-4819-119-1-199307010-00001]
31 **Lo GH**, Lai KH, Cheng JS, Hwu JH, Chang CF, Chen SM, Chiang HT. A prospective, randomized trial of sclerotherapy versus ligation in the management of bleeding esophageal varices. *Hepatology* 1995; **22**: 466-471 [PMID: 7635414 DOI: 10.1002/hep.1840220215]
32 **Jutabha R**, Jensen DM, Egan J, Machicado GA, Hirabayashi

K. Randomized, prospective study of cyanoacrylate injection, sclerotherapy, or rubber band ligation for endoscopic hemostasis of bleeding canine gastric varices. *Gastrointest Endosc* 1995; **41**: 201-205 [PMID: 7789677 DOI: 10.1016/S0016-5107(95)70338-1]

33 **Sarin SK**, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. *Hepatology* 1992; **16**: 1343-1349 [PMID: 1446890 DOI: 10.1002/hep.1840160607]

34 **Kojima T**, Onda M, Tajiri T, Kim DY, Toba M, Masumori K, Umehara M, Yoshida H, Mamada Y, Taniai N, Nishikubo H, Yokoyama S, Matsuzaki S, Tanaka N, Yamashita K, Aramaki T, Tetsuoh Y. [A case of massive bleeding from rectal varices treated with endoscopic variceal ligation (EVL)]. *Nihon Shokakibyo Gakkai Zasshi* 1996; **93**: 114-119 [PMID: 8865752]

35 **Uno Y**, Munakata A, Ishiguro A, Fukuda S, Sugai M, Munakata H. Endoscopic ligation for bleeding rectal varices in a child with primary extrahepatic portal hypertension. *Endoscopy* 1998; **30**: S107-S108 [PMID: 9932774 DOI: 10.1055/s-2007-1001436]

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

37 **Sato T**. Two Cases of Rectal Varices Treated by Endoscopic Variceal Ligation. *Dig Endosc* 1999; **11**: 66–69 [DOI: 10.1111/j.1443-1661.1999.tb00196.x]

38 **Sato T**, Yamazaki K, Akaike J, Toyota J, Karino Y, Ohmura T. Retrospective analysis of endoscopic injection sclerotherapy for rectal varices compared with band ligation. *Clin Exp Gastroenterol* 2010; **3**: 159-163 [PMID: 21694861 DOI: 10.2147/CEG.S15401]

39 **Ljubicić N**, Bisćanin A, Nikolić M, Supanc V, Hrabar D, Pavić T, Boban M. A randomized-controlled trial of endoscopic treatment of acute esophageal variceal hemorrhage: N-butyl-2-cyanoacrylate injection vs. variceal ligation. *Hepatogastroenterology* 2011; **58**: 438-443 [PMID: 21661410]

40 **Soehendra N**, Grimm H, Nam VC, Berger B. N-butyl-2-cyano-acrylate: a supplement to endoscopic sclerotherapy. *Endoscopy* 1987; **19**: 221-224 [PMID: 3500847 DOI: 10.1055/s-2007-1018288]

41 **Nguyen AJ**, Baron TH, Burgart LJ, Leontovich O, Rajan E, Gostout CJ. 2-Octyl-cyanoacrylate (Dermabond), a new glue for variceal injection therapy: results of a preliminary animal study. *Gastrointest Endosc* 2002; **55**: 572-575 [PMID: 11923777 DOI: 10.1067/mge.2002.122032]

42 **Wang YM**, Cheng LF, Li N, Wu K, Zhai JS, Wang YW. Study of glue extrusion after endoscopic N-butyl-2-cyanoacrylate injection on gastric variceal bleeding. *World J Gastroenterol* 2009; **15**: 4945-4951 [PMID: 19842227 DOI: 10.3748/wjg.15.4945]

43 **Weilert F**, Shah JN, Marson FP, Binmoeller KF. EUS-guided coil and glue for bleeding rectal varix. *Gastrointest Endosc* 2012; **76**: 915-916 [PMID: 22172480]

44 **Sharma M**, Somasundaram A. Massive lower GI bleed from an endoscopically inevident rectal varices: diagnosis and management by EUS (with videos). *Gastrointest Endosc* 2010; **72**: 1106-1108 [PMID: 20579995 DOI: 10.1016/j.gie.2010.02.054]

45 **Seewald S**, Ang TL, Imazu H, Naga M, Omar S, Groth S, Seitz U, Zhong Y, Thonke F, Soehendra N. A standardized injection technique and regimen ensures success and safety of N-butyl-2-cyanoacrylate injection for the treatment of gastric fundal varices (with videos). *Gastrointest Endosc* 2008; **68**: 447-454 [PMID: 18760173 DOI: 10.1016/j.gie.2008.02.050]

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

47 **Godil A**, McCracken JD. Rectal variceal bleeding treated by transjugular intrahepatic portosystemic shunt. Potentials and pitfalls. *J Clin Gastroenterol* 1997; **25**: 460-462 [PMID: 9412951 DOI: 10.1097/00004836-199709000-00014]

48 **Vangeli M**, Patch D, Terreni N, Tibballs J, Watkinson A, Davies N, Burroughs AK. Bleeding ectopic varices--treatment with transjugular intrahepatic porto-systemic shunt (TIPS) and embolisation. *J Hepatol* 2004; **41**: 560-566 [PMID: 15464235 DOI: 10.1016/j.jhep.2004.06.024]

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

50 **Ory G**, Spahr L, Megevand JM, Becker C, Hadengue A. The long-term efficacy of the intrahepatic portosystemic shunt (TIPS) for the treatment of bleeding anorectal varices in cirrhosis. A case report and review of the literature. *Digestion* 2001; **64**: 261-264 [PMID: 11842284]

51 **Vidal V**, Joly L, Perreault P, Bouchard L, Lafortune M, Pomier-Layrargues G. Usefulness of transjugular intrahepatic portosystemic shunt in the management of bleeding ectopic varices in cirrhotic patients. *Cardiovasc Intervent Radiol* 2006; **29**: 216-219 [PMID: 16284702 DOI: 10.1007/s00270-004-0346-4]

52 **Haskal ZJ**, Scott M, Rubin RA, Cope C. Intestinal varices: treatment with the transjugular intrahepatic portosystemic shunt. *Radiology* 1994; **191**: 183-187 [PMID: 8134568 DOI: 10.1148/radiology.191.1.8134568]

53 **Hidajat N**, Stobbe H, Hosten N, Schroeder RJ, Fauth M, Vogl T, Felix R. Transjugular intrahepatic portosystemic shunt and transjugular embolization of bleeding rectal varices in portal hypertension. *AJR Am J Roentgenol* 2002; **178**: 362-363 [PMID: 11804893 DOI: 10.2214/ajr.178.2.1780362]

54 **Demirel H**, Pieterman H, Laméris JS, van Buuren HR. Transjugular embolization of the inferior mesenteric vein for bleeding anorectal varices after unsuccessful transjugular intrahepatic portosystemic shunt. *Am J Gastroenterol* 1997; **92**: 1226-1227 [PMID: 9219809]

55 **Shibata D**, Brophy DP, Gordon FD, Anastopoulos HT, Sentovich SM, Bleday R. Transjugular intrahepatic portosystemic shunt for treatment of bleeding ectopic varices with portal hypertension. *Dis Colon Rectum* 1999; **42**: 1581-1585 [PMID: 10613477 DOI: 10.1007/BF02236211]

56 **Kochar N**, Tripathi D, McAvoy NC, Ireland H, Redhead DN, Hayes PC. Bleeding ectopic varices in cirrhosis: the role of transjugular intrahepatic portosystemic stent shunts. *Aliment Pharmacol Ther* 2008; **28**: 294-303 [PMID: 19086235 DOI: 10.1111/j.1365-2036.2008.03719.x]

57 **Ibukuro K**, Kojima K, Kigawa I, Tanaka R, Fukuda H, Abe S, Tobe K, Tagawa K. Embolization of rectal varices via a paraumbilical vein with an abdominal wall approach in a patient with massive ascites. *J Vasc Interv Radiol* 2009; **20**: 1259-1261 [PMID: 19729137 DOI: 10.1016/j.jvir.2009.05.041]

58 **Hidajat N**, Vogl T, Stobbe H, Schmidt J, Wex C, Lenzen R, Berg T, Neuhaus P, Felix R. Transjugular intrahepatic portosystemic shunt. Experiences at a liver transplantation center. *Acta Radiol* 2000; **41**: 474-478 [PMID: 11016769 DOI: 10.1080/028418500127345712]

59 **Ahn SS**, Kim EH, Kim MD, Lee WJ, Kim SU. Successful hemostasis of intractable rectal variceal bleeding using variceal embolization. *World J Gastroenterol* 2015; **21**: 2558-2562 [PMID: 25741168 DOI: 10.3748/wjg.v21.i8.2558]

60 **Helmy A**, Al Kahtani K, Al Fadda M. Updates in the pathogenesis, diagnosis and management of ectopic varices. *Hepatol Int* 2008; **2**: 322-334 [PMID: 19669261 DOI: 10.1007/s12072-008-9074-1]

61 **Almadi MA**, Almessabi A, Wong P, Ghali PM, Barkun A. Ectopic varices. *Gastrointest Endosc* 2011; **74**: 380-388 [PMID: 21612777 DOI: 10.1016/j.gie.2011.03.1177]

62 **Kanagawa H**, Mima S, Kouyama H, Gotoh K, Uchida T, Okuda K. Treatment of gastric fundal varices by balloon-occluded retrograde transvenous obliteration. *J Gastroenterol Hepatol* 1996; **11**: 51-58 [PMID: 8672742 DOI: 10.1111/j.1440-1746.1996.tb00010.x]

63 **Saad WE**. Balloon-occluded retrograde transvenous obliteration of gastric varices: concept, basic techniques, and outcomes. *Semin Intervent Radiol* 2012; **29**: 118-128 [PMID: 23729982 DOI: 10.1055/s-0032-1312573]

64 **Anan A**, Irie M, Watanabe H, Sohda T, Iwata K, Suzuki N, Yoshikane M, Nakane H, Hashiba T, Yokoyama M, Higashihara H, Okazaki M, Sakisaka S. Colonic varices treated by balloon-occluded retrograde transvenous obliteration in a cirrhotic patient

with encephalopathy: a case report. *Gastrointest Endosc* 2006; **63**: 880-884 [PMID: 16650568 DOI: 10.1016/j.gie.2005.11.038]

65 **Yoshino K**, Imai Y, Nakazawa M, Chikayama T, Ando S, Sugawara K, Hamaoka K, Inao M, Oka M, Mochida S. Therapeutic strategy for patients with bleeding rectal varices complicating liver cirrhosis. *Hepatol Res* 2014; **44**: 1088-1094 [PMID: 24033909 DOI: 10.1111/hepr.12232]

66 **Bittinger M**. Bleeding from Rectal Varices in Patients with Liver Cirrhosis - an Ominous Event. *Gastrointest Endosc* 2004; **59**: 270 [DOI: 10.1016/S0016-5107(04)01197-6]

67 **Botterill ID**, Jayne DG, Snelling AP, Ambrose NS. Correction of symptomatic ano-rectal varices with circumferential stapled anoplasty. *Colorectal Dis* 2002; **4**: 217 [PMID: 12780622 DOI: 10.1046/j.1463-1318.2002.00343.x]

68 **Parvaiz A**, Azeem S, Singh RK, Lamparelli M. Stapled hemorrhoidopexy: an alternative technique for the treatment of bleeding anorectal varices. Report of a case. *Dis Colon Rectum* 2006; **49**: 411-412 [PMID: 16322963 DOI: 10.1007/s10350-005-0255-x]

69 **Kaul AK**, Skaife PG. Circumferential stapled procedure for bleeding ano-rectal varices is an effective treatment--experience in nine patients. *Colorectal Dis* 2009; **11**: 420-423 [PMID: 18624814 DOI: 10.1111/j.1463-1318.2008.01616.x]

P- Reviewer: Nakayama Y, Zielinski J **S- Editor**: Qiu S
L- Editor: A **E- Editor**: Liu SQ

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