ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2015 November 28; 7(27): 2703-2780

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 27 November 28, 2015

World Journal of Hepatology
Volume 7 Number 27 November 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
November 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.i27.2703

World J Hepatol 2015 November 28; 7(27): 2703-2715
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

2015 Advances in Alcoholic Liver Disease

Binge drinking: Burden of liver disease and beyond

Susana Llerena, María Teresa Arias-Loste, Angela Puente, Joaquín Cabezas, Javier Crespo, Emilio Fábrega

Susana Llerena, María Teresa Arias-Loste, Angela Puente, Joaquín Cabezas, Javier Crespo, Emilio Fábrega, Gastroenterology and Hepatology Unit, Marqués de Valdecilla University Hospital, Instituto de Investigación Marqués de Valdecilla, 39008 Santander, Cantabria, Spain

Author contributions: Llerena S, Arias-Loste MT, Puente A, Cabezas J, Crespo J and Fábrega E contributed equally to this work; Llerena S and Fábrega E wrote the paper.

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

Correspondence to: Emilio Fábrega, MD, PhD, Gastroenterology and Hepatology Unit, Marqués de Valdecilla University Hospital, Instituto de Investigación Marqués de Valdecilla, Avenida Valdecilla s/n, 39008 Santander, Cantabria, Spain. digfge@humv.es
Telephone: +34-734-42202544
Fax: +34-734-42202544

Received: April 27, 2015
Peer-review started: May 4, 2015
First decision: October 21, 2015
Revised: October 26, 2015
Accepted: November 10, 2015
Article in press: November 11, 2015
Published online: November 28, 2015

Abstract

The consumption of alcoholic beverages is harmful to human health. In recent years, consumption patterns of alcoholic beverages have changed in our society, and binge drinking has generalized. It is considered to be a socio-sanitary problem with few known consequences in terms of individual and third-party social impacts (in the form of violence or traffic accidents) and its organic impact (affects the liver and other organs and systems, such as the nervous and cardiovascular systems) and represents an important financial burden due to its increasing economic impact. This review provides a global approach to binge drinking and emphasizes its epidemiological character, the effect of this type of consumption and the possible management of a problem with an increasing tendency in our society.

Key words: Binge drinking; Binge drinking adolescent; Binge drinking brain; Binge drinking cardiovascular; Alcohol binge; Binge drinking liver

Core tip: Binge drinking is an alcohol consumption conduct that is primarily performed during the weekend by 24% of teenagers and young adults. Although the consequences of this habit are not well known, they have a social and organic impact on individuals. Binge drinking is considered to be a public health issue that should be addressed with primary prevention programs and a comprehensive intervention of the problem.

Llerena S, Arias-Loste MT, Puente A, Cabezas J, Crespo J, Fábrega E. Binge drinking: Burden of liver disease and beyond. *World J Hepatol* 2015; 7(27): 2703-2715 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i27/2703.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i27.2703

INTRODUCTION

The consumption of alcoholic beverages is harmful to human health. Excessive alcohol intake is a major global and public health challenge that has been

identified as one of the main determinants of a variety of noncommunicable diseases[1]. The excessive consumption of alcohol is the leading global cause of preventable morbidity and mortality and a major problem in Western countries. According to the World Health Organization (WHO), it is the cause of 4.5% of the diseases in the world and 4% of the deaths in the world and is considered the main cause of death among men between 15 and 59 years of age, especially in Eastern Europe countries[2,3]. In the United States, this excessive alcohol consumption causes 75000 deaths each year and is the third leading preventable cause of death[4]. Alcohol is the main cause of cirrhosis and indication for liver transplants in Europe, and accounts for 1.8% of all deaths caused by liver disease[5]. When the data are adjusted by age, alcohol is the main risk factor for impairment (*i.e.*, life-years lost at early ages) in young populations between 10-24 years of age[6].

In recent years, consumption patterns of alcoholic beverages have changed in our society, and binge drinking has generalized. The reason for this change and its implications for the individual and the society are not well known. For this reason, we present this review using a comprehensive approach to the binge-drinking problem.

DEFINITION OF BINGE DRINKING

A unified definition of binge drinking is necessary to effectively approach this subject and to analyze the risk factors of binge drinking, its socio-sanitary implications and its relation to alcohol dependence. We review the controversial term of binge drinking, which lacks a consensus among the different studies. The controversy stems from the following items: (1) its inadequate definition; (2) the minimum amount of consumption that is considered to be a problem has not been established; (3) a standard drinking unit (SDU) that is common to all countries has not been established; and (4) the unspecified period of time that is considered to be "a single event".

Consequently, epidemiological studies describe important methodological problems; the prevalence of this type of consumption in young populations varies between 7% and 40% due to the lack of uniform cut-off points[7]. This variability is attributed to the lack of consensus in determining the harmful consumption levels of alcohol and the differences in pure ethanol in an SDU for each country. Therefore, the cut-off points for the number of SDUs ingested in each event (*i.e.*, five alcoholic beverages and six alcoholic beverages) and the frequency intervals (*i.e.*, in the last week, 15 d, and 30 d) in which the episodes of heavy consumption occur vary in the different studies[8]. Regarding the term binge drinking, several authors suggest that this definition traditionally refers to a pattern of consuming large amounts of alcohol in a few hours and primarily during weekend nights that is conducted by younger age groups without a differentiation of gender[8]; they primarily correlate it with clinical definitions of abuse or dependence[9-11].

To prevent confusion, alternate terms have been suggested, such as heavy drinking[12-16], heavy episodic drinking[17-22], heavy sessional drinking, risky single-occasion drinking[23], dangerous drinking[24], or high-risk drinking[25]. In Spain, the First Conference in Health Prevention and Promotion in the Clinical Practice in 2007 proposed the term heavy episodic drinking of alcohol.

Although binge drinking cannot be identified with the common criteria for the harmful consumption of alcohol, many authors have stressed its social and health consequences, which may exceed the social and health consequences of regular alcohol consumption[26-29].

In the 1990s, the effect of alcohol consumption regarding the sex of the patient was determined in the Harvard School of Public Health College Alcohol Study[30,31]. Wechsler's group employed a questionnaire to evaluate the habits of alcohol consumption. The group discovered that significant problems of alcohol consumption occur in men after the intake of five beverages in one event, whereas similar problems occur after the intake of four beverages by women. The term heavy alcohol consumption (HAC) evolved and was understood as the consumption of five or more drinks by men and four or more drinks by women in a single occasion, at least once in the last two weeks[31].

Regarding the "single-event" discussion that is referenced in the binge drinking definition, several authors consider including the concentration of alcohol in the blood to determine the adequate threshold for the binge-drinking pattern. This threshold is explained by the difference in the effect of the intake of one alcoholic beverage in one hour during five continuous hours in an adult with an average body weight and the intake of the same amount of alcohol (five beverages) over a shorter period, for example, two hours.

Accordingly, the National Institute on Alcohol Abuse and Alcoholism (NIAAA)[32] redefined the term HAC by considering the level of concentration of alcohol in the blood. HAC considers minimum levels of 0.08 g/L of alcohol in the blood when determining the pattern of alcohol consumption. In adults, this level would correspond to the intake of five or more beverages by men and four or more beverages by women in approximately two hours. The NIAAA considers duration (two hours) in the HAC definition.

To consolidate a definition that includes alcohol levels in the blood, several studies have employed different variants of Widmark's formula, which was developed in the 1930s and has proven adequate reliability[33]. This equation establishes that the maximum concentration of alcohol in the blood is $A/(p \times r)$, where A = amount of alcohol consumed (in grams); p = body weight and r = fat/water ratio (0.7 for men and 0.6 for women).

Recently, another study[34] determined that the definition by Wechsler's group[30,31] and the NIAAA proposal[33] are strongly correlated with a similar pattern of association among the variables of sex, race and

Table 1 Prevalence of binge drinking

Ref.	Prevalence
Galán *et al*[41]	13.35%
Slutske *et al*[150]	19.50%
Bartoli *et al*[42]	37.85%
Delegación del Gobierno para el Plan Nacional sobre Drogas[40]	18%
CDC[44]	17.10%
Grucza *et al*[45]	50%
Hanewinkel *et al*[151]	27.00%
Lee *et al*[152]	46.30%

Table 2 Factors associated with binge drinking[42]

	OR (95%CI)
Female gender	1.57
Living with parents	0.57
High financial availability for each weekend	1.33
Cannabis use	1.61
Smoking e-cigarettes	2.49
Positive alcohol expectancies	1.11
Peer influence	2.4
Interest for discos and parties	1.53
High educational level	3.63

age and the initiation of consumption. These authors believe that quantity and duration should be considered, as suggested by the NIAAA, because the sole inclusion of the quantity variable underestimates the HAC prevalence and is insufficiently sensitive in discriminating between problematic and nonproblematic patterns of consumption.

An additional and more adequate definition for the clinical environment may be the consumption of six or more alcoholic beverages by men (60 g) and five or more alcoholic beverages by women (50 g) in a single occasion (in a two-hour period) at least once in the last 30 d. This definition is similar to the approach by the NIAAA[32] and Wechsler's group[30,31] and gathers all proven relevant variables of quantity and frequency but requires customization to the country in which the research will be conducted.

EPIDEMIOLOGY

Since Strauss and Bacon's epidemiological study, which was performed in the United States during the 1950s[30], several authors have reported an alarming increase in alcohol consumption among young global populations and consider it to be a risk pattern of consumption in this population (Table 1)[35-38]. At the European level (Eurobarometer, 2007), approximately 80 million Europeans who are aged 15 years or older [over one-fifth of the adult European Union (EU) population] reported binge drinking at least once a week in 2006; this proportion has increased since 2003 in the adult population of the EU 15 (Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, The Netherlands, Portugal, Spain, Sweden, and the United Kingdom). Binge drinking is not the prerogative of the young. Eighteen percent of persons aged 55 years of age and older reported binge drinking at least once a week in 2006 compared with 24% of persons aged between 15 and 24 years. Eastern Europe had the highest pattern of drinking score of 4.9, which indicated that people in this region frequently consumed large quantities of alcohol and frequently drank to intoxication, engaged in prolonged binges, and primarily consumed alcohol outside of mealtime[39]. Traditionally, alcohol consumption in Spain has been associated with the adult population; its regular consumption was primarily linked with gastronomy and social events. In the last 20 years in the remaining Mediterranean countries, important changes have occurred regarding the quantity, patterns and meaning of consumption that are similar to the increased binge drinking in the rest of the world[8]. One of the most recent household surveys on alcohol and drug use in Spain showed that 18% of the population between 15 and 34 years of age (with a mean age of first contact with alcohol at the age of 16.8 years) indicated that they consumed five or more alcoholic beverages in one single occasion (occasion refers to the intake of several glasses in a couple of hours) in the last 30 d[40]. A cross-sectional study[41] with a significant number of participants (n = 20608) of 15 years of age and older that employed the 2011-2012 National Health Survey as a source of information (Servicio Nacional de Salud, by its initials in Spanish) considered the intake of $\geqslant$ 40 g/d of alcohol in men or $\geqslant$ 24 g/d in women to be high-risk consumption. Binge drinking was defined as the consumption of $\geqslant$ 6 (men) and $\geqslant$ 5 (women) standard beverages of alcohol in 4-6 h in the last 12 mo. A total of 1.3% of the surveyed subjects were average high-risk drinkers; 19.6% of the men and 7.1% of the women had performed binge drinking in the last year. This pattern decreased with age but increased with educational level in both sexes, with beer as the most-consumed beverage. A study in Italy[42] of 654 individuals with a mean age of 20.6 years showed that 38% of the subjects had recently engaged in binge drinking. By performing a multivariate analysis, a relation was observed between this type of consumption and higher educational expectations, a larger amount of money available to spend during the weekends, interests in parties and discos, a higher prevalence in women (despite the reports from Anglo-Saxon countries), the use of cannabis, a greater influence of friends and the use of electronic cigarettes. Conversely, living with parents produced a protective factor (Table 2). In another Italian study based on the CAGE questionnaire or Alcohol Use Disorders Identification Test showed that 19.5% of the 1520 patients who attended an emergency service during the five months of the study had problems with alcoholism; the most frequent attendees were young males (18-20 years of age), divorced or single patients, and unemployed, homeless or immigrant patients[43].

In the United States, approximately 38 million adults binge drink, according to a 2010 survey by the Centers for Disease Control. The total prevalence of binge drinking among adults in the 48 states and the District of Columbia was 17.1%[44]. Epidemiological studies have identified that binge drinking is prevalent on college campuses; some studies indicate that approximately 50% of students reported binge drinking in recent weeks[45]. A recent study noted that approximately 500000 college students are injured and 1700 college students die each year from alcohol-related injuries[46].

Binge drinkers have a greater risk for developing alcohol dependence[47]. In addition, binge drinking has been associated with unplanned and unsafe sexual activity, assaults, falls, injuries, criminal violations, automobile crashes, and total poor neuropsychological functioning[48]. Each year, two thousand homicides are registered in Europe due to excessive alcohol consumption.

The importance of this problem translates to high healthcare costs. In the United States, the estimated annual expenditure for binge drinking is 168 billion dollars[44]. The estimated cost of binge drinking for the English Public Health System was £1.7 billion in 2003, which reflects the physical and psychological health problems that are associated with this type of excessive drinking[49].

ALCOHOL AND TOBACCO

Similar to alcohol, tobacco is considered to be a major cause of morbidity and mortality[50,51]. Tobacco has been directly responsible for 100 million deaths in the XX century[50,51]. Cigarette smoking is strongly associated with alcohol consumption[52-58]. Conversely, drinkers, especially binge drinkers, are more likely to smoke than nondrinkers[52,55]. This tobacco-alcohol relationship involves the pleasure-reward dopamine brain systems, as evidenced in murine models. In a recent study of mice, the rodents that were exposed to nicotine tended to ingest alcohol more frequently than rodents that were not administered nicotine due to a reduction in the dopamine response of the reward-response system in the brain, which decreased the pleasurable response to alcohol[59].

Young adults perceive an increased enjoyment of and desire for cigarettes while drinking alcohol[53,60], which may explain why smokers smoke more cigarettes while under the influence of alcohol[53,61-63], especially during binge drinking episodes[53,61]. If the frequency of alcohol consumption, binge drinking and being a smoker are associated, we are experiencing a global health issue with an early beginning in adolescence because both substances synergically increase the future risk to a level that exceeds the usual risk for liver, cardiovascular and neoplastic diseases posed by the individual use of either of these substances[64]. Young adults smoke cigarettes at rates that are higher than any other age group. According to the 2010 National Survey on Drug Use and Health survey, 34.2% of young adults aged 18 to 26 are current smokers, compared with 22.8% of adults aged 26 or older. In a recent study[65], teenagers who attend bars and discos showed a higher rate of tobacco consumption; this consumption was highly associated with the intake of alcoholic beverages.

TOXICITY OF ALCOHOL

The factors that affect the susceptibility to alcohol toxicity include genetics, gender, lifestyle/nutrition, exposure to environmental chemicals and drugs, and comorbidities. Toxic and other adverse effects of alcohol on organs and tissues in humans are a consequence of its metabolism to acetaldehyde, the associated formation of reactive oxygen and nitrogen species, the depletion of co-factors (*e.g.*, NAD+), and the impairment in energy homeostasis[66]. Due to the considerable redundancy in the oxidative enzymatic pathways (alcohol dehydrogenases, CYP2E1 and catalase) that can convert alcohol to acetaldehyde, the majority of tissues are capable of alcohol metabolism even though the liver is the primary site. Similarly, acetaldehyde dehydrogenases are ubiquitous in mitochondria. A minor and non-oxidative pathway of alcohol metabolism is *via* fatty acid ethyl ester (*via* fatty acid ethyl ester synthase) and phosphatidyl ethanol (*via* phospholipase D). Alcohol impacts the integrity of the gastrointestinal mucosal barrier, resulting in the translocation of the gut bacteria-derived lipopolysaccharide (endotoxin) and other molecules to the liver *via* the portal blood flow and the activation of the innate immune response. The molecular and cellular sequelae of the toxic mediators of alcoholic injury assume many forms. Acetaldehyde and oxidants are highly reactive molecules that can damage deoxyribonucleic acid (DNA), proteins and lipids. Changes in hepatic respiration and lipid metabolism can cause tissue hypoxia and impairment in the mitochondrial function. Secondary effects include the disruption of signaling pathways and ion channel function, the unfolded-protein response and oxidative stress as well as the activation of adaptive immune response that is significantly triggered by acetaldehyde protein adducts. Cell death triggers additional innate immune response, activation of fibrogenesis, and tissue repair. In addition to pro-inflammatory mediators, other signaling molecules, such as neurotransmitters, are affected by alcohol. Depending on the affected tissue, gross pathological changes that are associated with alcohol drinking include most or all of the following conditions: Fat accumulation (steatosis), inflammation, necrosis and fibrosis and functional deterioration[67]. Alcohol *via* acetaldehyde also favors carcinogenesis and has been considered to be a class 1 carcinogen by the WHO[68].

CONSEQUENCES

Compared with nonbinge drinkers, frequent binge drinkers were more likely to report fair or poor health and experience a greater number of sick days. These findings appear to reflect the generally negative consequences

Table 3 Summary of the organic effects of alcohol-binge drinking

Hepatic	Neurocognitive	Renal
Steatosis	Impaired verbal memory	Glomerulonephritis
Steatohepatitis	Impaired episodic memory	Acute nephropathy
Fibrosis	Deficits language and attentional tasks	Kidney graft failure
Cirrhosis	Prospective memory	
Hepatocellular carcinoma	Executive functions	
Oncogenic	Cardiovascular	Others
Oral cavity	Hypertension	Acute pancreatitis
Pharynx	Ischemic heart disease	Chronic pancreatitis
Larynx	Stroke	Major depression
Esophagus	Cardiomyopathy	Impaired fertility
Colorectum	Myocarditis	Premature and low weigh births
Breast	Arrhythmias	Fetal alcohol syndrome
Pancreas	Atherosclerosis	

of alcohol abuse but at an earlier stage in poor health development[69]. Binge drinking is associated with the deterioration of work performance, brain damage, alcohol dependence, stroke, heart rhythm disturbances, coronary disease, sexually transmitted diseases and premature death[35]. Table 3 summarized the organic effects of binge drinking on different organs and systems.

Effect on the liver

The epidemiological evidence demonstrates that binge drinking in chronic alcoholics augments liver injury[70]. A recent study showed that frequent consumers (5-7 d/wk) have a higher mortality rate compared with persons with lower rates of consumption (1-4 d/wk)[71]. A heavy binge drinking episode in patients who chronically consume alcohol is the most common trigger for the admission of patients with steatohepatitis[72]. A study of a large cohort of drinkers with consecutive biopsies suggested the concept of multiple hits of alcoholic hepatitis in the same patients as the prime determinant in the progression of alcoholic liver injury[73]. Mathews *et al*[74] have recently developed a chronic plus binge alcohol feeding model in mice, which is similar to the drinking patterns of many alcoholic hepatitis patients: A history of chronic drinking and recent excessive alcohol consumption have begun to identify novel mechanisms that participate in the pathogenesis of alcoholic liver injury. Chronic binge ethanol feeding induces higher levels of steatosis, serum alanine transaminase, and liver inflammation[74]. Binge alcohol consumption aggravates oxidative stress and promotes the pathogenesis of nonalcoholic steatohepatitis from obesity-induced simple steatosis. Alcohol and high fat diets synergistically induce nitrosative, endoplasmic reticulum, and mitochondrial stress and an up-regulation of hepatic toll-like receptor 4 (TLR4), thereby contributing to steatohepatitis[75,76]. Additionally, high fat diet plus binge ethanol synergistically exacerbates acute steatohepatitis through the induction of CXCL1 and subsequent hepatic neutrophil infiltration[77]. Moderate ethanol binges induce significant liver damage (hepatocyte apoptosis) in genetically obese (*ob*/*ob*) mice by increasing tumor necrosis factor α and decreasing nuclear factor κB activity[78]. Individuals with fatty liver are predisposed to increased liver injury by chronic binge alcohol drinking. This finding has been proven in studies involving rats, where repeated alcohol binges in the context of mild steatosis may promote the activation of stellate cells and contribute to liver injury[79].

Despite these findings, note that the majority of experimental data concerning the impact of binge drinking on the pathogenesis of a liver injury may not be completely extrapolated to humans because the majority of the studies are based on animal models that do not completely mimic liver injury in humans. Note that ethanol sensitivity in human, rat, mice, and other animal models (*e.g.*, drosophila, zebrafish) can also vary due to differences in populations, species, and strains. In animal models, several approaches have been considered to examine the effect of binge ethanol, including the single binge, the intermittent repeat binge, and chronic ethanol exposure followed by episodes of binging. Evidence from these animal studies provide mechanistic information on the binge ethanol effect relevant to alcoholic liver disease. For example, the cellular effects of ethanol are increasingly attributed to the modulation of immunological, metabolic, signaling, and epigenetic pathways[80-82]. Binge alcohol alters the levels of several cellular components and dramatically amplifies liver injury in chronically alcohol exposed liver. Evidence exists that acute alcohol exposure inhibits hepatic mitochondrial DNA synthesis and also impairs mitochondrial metabolism and dynamics. Alcohol intoxication inhibits the inflammatory response by inhibiting signaling through TLRs when a potent external TLR stimulus is provided during alcohol intoxication[83]. As previously reported, binge drinking promotes the activation of stellate cells and contributes to liver injury *via* a pro-fibrogenic response[79].

Other factors involved in the toxicity of alcohol to the liver are obesity, resistance to insulin, chronic infection with hepatitis C virus, being female, and tobacco consumption[84]. A priori, tobacco appeared to have a minor role in fibrosis and chronic liver disease; however, additional studies have suggested its deleterious role in the course of chronic liver disease. Tobacco and alcohol may have a synergic and deleterious impact on chronic liver disease[85]. Tobacco may accelerate the progression to cirrhosis in patients with alcoholic chronic liver disease and increase liver decompensations in individuals with established cirrhosis[86]. Approximately 90% of the patients with advanced alcoholic chronic liver disease are smokers[87]. Tobacco seems to be involved in the risk of developing hepatocarcinoma[88] by increasing aflatoxin B1, which is a known hepatic carcinogen[89].

The mechanisms by which smoking promotes the progression of chronic diseases are substantially unknown. Smoking may accelerate the progression of "fibrogenic" conditions, such as chronic renal, cardiac or pancreatic diseases[90,91]. Cigarette smoke induces an

array of pathogenic effects that are potentially involved in tissue fibrogenesis, including systemic inflammation, thrombogenesis and oxidative stress[92]. Smoking exerts powerful immunoregulatory actions that can produce an impaired wound healing response to injury. These effects may be more pronounced in susceptible individuals as suggested by genetic epidemiological studies[93]. The strongest evidence to support a fibrogenic effect of smoking is the fact that smoking cessation has beneficial effects on the progression of chronic renal diseases[94,95].

Smoking increases the production of pro-inflammatory cytokines (interleukin 1, 6 and 13) and tumor necrosis factor α, angiogenic factors (vascular endothelial growth factor-A) and fibrogenic mediators (leptin, transforming growth factor β1 and angiotensin Ⅱ) also induces oxidative stress by stimulating nicotinamide adenine dinucleotide phosphate oxidase and decreasing antioxidant defenses, which cause lipid peroxidation[92]. These effects can cause an increase in hepatocellular damage and the subsequent activation of resident hepatic stellate cells, which comprise a major fibrogenic cell type. Another potential mechanism by which smoking causes liver fibrogenesis may be iron deposition. Smoking also induces profound changes in the microvasculature, such as endothelial dysfunction, smooth muscle cell proliferation and vasoconstriction, which cause impaired delivery of nitric oxide and tissue hypoxia[96]. These events are potentially implicated in the wound healing response of the liver to chronic injury. Heavy smokers commonly exhibit several features of the insulin resistance syndrome and develop an increased risk for type 2 diabetes[97]. Because insulin resistance promotes liver fibrogenesis, it can participate in the fibrogenic effect of tobacco in the liver. Therefore, we can conclude that the interaction between alcohol and tobacco synergistically elevates the disease risk to a level above the risk posed by the individual use of either of these substances[64].

Oncogenic effects

Alcoholic beverages and ethanol in alcoholic beverages are classified by the WHO International Agency for Research on Cancer as "carcinogenic to humans" (group 1)[68]. Probable mechanisms for the association between alcohol drinking and upper digestive tract cancer have been presented in several studies[98,99]. The carcinogen of esophageal cancer, with regard to alcohol consumption, is acetaldehyde[100,101], which is a highly reactive and toxic alcohol metabolite. Acetaldehyde interferes with DNA repair machinery and directly inhibits O6 methylguanyltransferase, which is an enzyme that is deemed important for the repair[102]. The inhalation of acetaldehyde has been known to cause bronchial cancer and esophageal cancer. Several studies have reported the hazards of binge drinking using experiments. After *in vivo* administration of ethanol in the stomach of rats, which is analogous to the binge drinking condition, histone H3 modification, which primarily affects histone methylation in the liver, lung and spleen, was detected in the histone of rat tissues[103]. A study in a South Korean population that included 2677 men of 55 years of age, with a follow-up of 20.8 years, associated severe binge drinking and its frequency with mortality due to oral and esophageal cancer. A higher mortality was observed in these cancers for patients with a daily binge drinking habit compared with nondrinkers. The alcohol dose and mortality due to esophageal cancer and the mortality and the frequency of alcohol consumption are highly associated, whereas the volume of consumed alcohol is not highly associated. Note that tobacco consumption was an important confounding factor in this study[104]. Binge alcohol consumption seems to be a risk factor for pancreatic cancer. After adjusting for sex and age in a case-control population study[105] in San Francisco (United States) with 532 cases and 1701 controls, the risk of pancreatic cancer was determined to be higher in binge drinking patients when a higher amount of units were consumed and a longer consumption had occurred. This finding supports the notion that a high consumption of alcohol, including binge drinking, is a risk factor for the development of pancreatic cancer. Alcohol is also involved in the development of a hepatocarcinoma. Acetaldehyde, a reactive metabolite of ethanol, binds to nucleic acids, proteins such as enzymes, microsomal proteins and microtubules. The generated reactive oxygen species can also activate or repress the epigenetic elements such as chromatin remodeling, non-coding RNAs (micro-RNAs), DNA (de) methylation and histone modification that affect gene expression, hence leading to hepatocarcinoma[106].

We should consider that smoking is a known risk factor for upper digestive tract cancer, including oral cavity, pharynx and esophagus cancers. Therefore, the interaction of alcohol and tobacco synergistically elevates the disease risk to a level above the risk posed by the individual use of either of these substances.

Neurocognitive effects

The consequences on the memory of alcoholic beverage binge drinking have been explored in animal models. The results show that binge doses of alcohol cause a disruption in the growth of new brain cells; this lack of new growth may cause the long-term deficits detected in key areas of the brain (such as hippocampal structure and function) that are induced by binge drinking[107,108]. The increasing interest in performing studies to analyze the neurotoxic effect of alcohol due to the increased practice of binge drinking in adolescence is not surprising. A "safe" alcohol dose for the developing brain of an adolescent is unknown. The prefrontal cortex and limbic system, which includes the hippocampus, undergoes prominent reorganization during the late teenage years[109,110]; these cognitive processes, which are dependent on these areas of the brain, such as memorial processes, are very sensitive to any damage caused by excessive alcohol ingestion. Different studies have reported poorer performance in neurocognitive tests with the worst verbal memory and poorer episodic memory[111]. Binge

drinking affects the executive functions and the working memory from the Brodmann areas 46 and 9 of the dorsomedial prefrontal cortex. Studies of neurocognitive function in teenagers aged 15-19 years with a history of alcohol abuse have revealed deficits for a range of language and attentional tasks, verbal and non-verbal memory tasks, and specific working memory impairments[112,113]. Compared with nonalcohol drinkers, binge drinkers evinced cognitive impairments in the Paced Auditory Serial Addition Test regarding executive planning function and episodic memory tasks-these findings were similar to frontal function deficits observed in Korsakoff alcoholics[105]. Using magnetic resonance, several studies[114] have correlated binge drinking in post-adolescence and early adulthood with brain structural alterations. These results showed a greater decrease in the gray matter of the dorsomedial prefrontal cortex in binge drinking subjects compared with the control subjects. A positive correlation between the increased gray matter in binge drinkers and the results from the Self-Ordered Pointing Test (SOPT), which is an error test, was observed[109]. The measure of the prefrontal cortex was also correlated with the volume and the rate of alcohol intake[109].

A Spanish cohort study[115] evaluated the binge drinking habits of 89 university students with a two-year follow-up. The neuropsychological performance was measured using several scales; binge drinkers yielded the worst scores in the Wechsler Memory Scale-Ⅲ and the SOPT and demonstrated a worse verbal memory compared with nonbinge drinkers. Another study, with a longer follow-up of ten years, of adolescents with abusive alcohol consumption revealed that verbal memory deteriorated with time for adolescents who presented the habit to a young adult age[116]. At a neuropsychological level, binge drinking subjects show deficiencies in the assessment tests for the frontal executing functions of attention, planning, cognitive flexibility, work memory, decision-making, verbal fluency, decision-changing and inhibitory control tasks[117].

Regarding the effect of prospective memory, a study[118] showed similar results on the Prospective and Retrospective Memory Questionnaire test for binge drinkers and nonbinge drinkers. These findings contrast with another study by the same author[119]. A higher number of short- and long-term prospective memory lapses were observed in this group. This study excluded consumers of other substances and lacked the control of the age, type of alcohol consumption, hours after the last intake or period of consumption. However, a lower score in the prospective remembering video procedure (PRVP) was observed in binge drinkers, which revealed differences when consumers of other substances and consumers who had drank alcohol in the last 48 h were excluded. Nonsignificant differences were observed between the groups regarding age, anxiety or depression levels, and years of alcohol consumption. Subjects with a higher intake of alcohol units per week demonstrated lower results in the PRVP test.

Effect on the cardiovascular system

Approximately 10% of cardiovascular disease-related deaths are attributable to alcohol[120]. The probability of coronary heart disease and cardiovascular mortality increases with heavy consumption[121]. Studies suggest that a binge pattern of drinking may precipitate myocardial ischemia or infarction[122], and evidence of an association between binge alcohol consumption and a two-fold greater mortality after acute myocardial infarction also exists[123]. In addition to the volume of consumption, the *pattern* of drinking must be considered. Recently, Liu *et al*[124] demonstrated that binge patterns in mice increase the development of atherosclerosis compared with no alcohol controls. The results from retrospective studies of adults who range in age between 40 and 60 years have indicated that binge drinking is associated with a heightened risk of cardiovascular (CV) events, such as stroke, sudden death, myocardial infarction, and increased mortality after myocardial infarction[123,125-127]. In addition, an alcohol binge drinking pattern is associated with the progression of carotid atherosclerosis[128]. Endothelial dysfunction is an early indicator of blood vessel damage and atherosclerosis and a strong prognostic factor for future CV events[129-131]. In binge drinkers, cardioprotective changes in high density lipoproteins are not observed, and adverse changes in low-density lipoproteins are acquired. Binge drinking seems capable of predisposing the heart to arrhythmia by reducing the threshold for ventricular fibrillation and by causing scarring of the myocardium. The myocardium may be especially sensitive during withdrawal, as will occur after weekend binges. In addition, irregular drinking is associated with an increased risk of thrombosis, which is most likely to occur after heavy drinking stops. These physiological mechanisms may explain the observed increase in cardiovascular events during the weekend and on Mondays. In countries with known weekend binge drinking, the Monday peak is pronounced and is accompanied by slight increases in mortality on Saturdays and Sundays. This finding has been observed in countries of the former Soviet Union and in Scotland[132-134]. Chenet *et al*[135] hypothesize that alcohol, particularly when drunk in binges, serves as a catalyst in acute ischemic heart diseases by being synergetic to other triggering factors.

In an experimental animal model in which binge alcohol was administered after chronic alcohol treatment, binges caused a decrease in the messenger ribonucleic acid (mRNA) of low-density lipoprotein-receptor (LDL-R) and increased mRNA levels of the angiotensinogen gene in the liver. Binge ethanol intake in chronically exposed rat liver decreased LDL-R and increased angiotensinogen gene expression[136]. Note that increases in plasma LDL cholesterol and angiotensin are cardiovascular risk factors in human alcoholics. In a recent study performed in ApoE KO mice, the arterial lumen was reduced and the deposits of macrophages were more evident, which confirms the aterogenic capacity of alcohol binge drinking[124]. These results imply that binge alcohol-

induced alterations in liver have consequences on the cardiovascular system. Thus, binge drinking affects interorgan cross-talk. This finding is further supported by increases in the plasminogen activator inhibitor (PAI). PAI-1 serves a major role in fibrin metabolism by blocking fibrinolysis. The role of PAI-1 in fibrin accumulation in vascular disease is well understood to contribute to endothelial dysfunction and inflammation.

Thus, these findings provide strong evidence to support a health message that discourages binge drinking. The provision to healthcare professionals of scientific evidence that binge drinking can accelerate atherosclerosis may encourage them to perform brief interventions for individuals with at-risk drinking behaviors.

Other effects

The effect of alcohol on other organs and systems varies. Binge drinking is one of the main causes of pancreatitis[137] and is involved in a higher mortality from a duodenal ulcer[138]. It is also the cause of neuropsychiatric conditions, such as depression[139]. In the kidney[140], binge drinking has been correlated with glomerulonephritis, acute nephropathies, and the loss of kidney transplants. It is the cause of fertility disorders, prematurity, low weight and newborn alcoholic syndrome[141].

APPROACHING THE PROBLEM

Numerous social and political interventions are available to decrease this type of consumption, such as laws against driving under the effect of alcohol, increased taxes, restriction of access and availability of alcohol, and brief interventions, such as medical advice and control *via* publicity.

Our main weapon against this problem is primary prevention, which is difficult to develop due to established alcohol consumption among different cultures, which is primarily associated with social events. In this manner, the WHO has developed a strategic plan to approach the harmful consumption of alcohol, based on preventive interventions[142,143] with the help of health services, to reduce access to alcoholic beverages and prohibit its marketing and by increasing prices.

A substantial amount of evidence across different countries to support making alcohol more expensive, primarily *via* taxation, and to reduces the extensive range of harm due to intoxication and binge drinking, including road traffic accidents and fatalities, intentional and unintentional injuries, rapes and robberies, homicides, crime, and violence[144]. Another issue in this plan is the marketing control of the illicit production of beverages with regulation systems.

Similarly, a substantial amount of evidence to support raising the minimum purchasing age to reduce alcohol-related road traffic accidents and to reduce the density of alcohol outlets to reduce drunkenness, assaults, and road traffic fatalities. However, these strategies will only be effective if it is not backed up with a credible threat to remove the licenses of outlets that repeatedly sell to under-aged customers. These strategies are also more effective when supported by community-based prevention programs. Some of these measures are effective in decreasing the damage caused by alcohol but are also cost-effective from a revenue point of view due to increases in the price and taxes of alcoholic beverages[143,145].

Preliminary data support the intriguing possibility that integrated intervention may enhance smoking cessation and reduce binge drinking[146].

Decreased smoking and improved maintenance of abstinence may result from a behavioral intervention to reduce binge drinking. This hypothesis is supported by several lines of evidence, including conditioning mechanisms in which the craving to smoke is elicited by higher levels of alcohol consumption[147,148], and environmental factors, such as parental and peer influence for concurrent use of cigarettes while engaging in binge drinking[149].

Smoke-free bar policies may not be sufficient to influence the association between smoking and drinking, particularly if tobacco marketing continues in these venues or in the absence of programs that specifically address the co-use of tobacco and alcohol. Tobacco interventions should prioritize bars and other social venues that are popular among young adults to reach persons who are at greatest risk. The strong and consistent association between smoking and drinking indicates that public health efforts and clinical cessation programs need to address the paired use of tobacco and alcohol among the young adult bar-going population.

CONCLUSION

Binge drinking is an increasing public health issue that affects teenagers and young adults. Although its consequences are not well known, relevant hepatic, cardiovascular, neurocognitive and oncogenic effects may be present. Binge drinking also has a significant social and economic impact. Interventions should be globally approached to address the consumption of alcohol and tobacco.

REFERENCES

1 **Parry CD**, Patra J, Rehm J. Alcohol consumption and non-communicable diseases: epidemiology and policy implications. *Addiction* 2011; **106**: 1718-1724 [PMID: 21819471 DOI: 10.1111/j.1360-0443.2011.03605.x]

2 **Chick J**. The WHO global strategy to reduce the harmful use of alcohol. *Alcohol Alcohol* 2011; **46**: 223 [PMID: 21508193 DOI: 10.1093/alcalc/agr035]

3 **Zaridze D**, Brennan P, Boreham J, Boroda A, Karpov R, Lazarev A, Konobeevskaya I, Igitov V, Terechova T, Boffetta P, Peto R. Alcohol and cause-specific mortality in Russia: a retrospective case-control study of 48,557 adult deaths. *Lancet* 2009; **373**: 2201-2214 [PMID: 19560602 DOI: 10.1016/S0140-6736(09)61034-5]

4 **Centers for Disease Control and Prevention (CDC)**. Alcohol-attributable deaths and years of potential life lost--United States, 2001. *MMWR Morb Mortal Wkly Rep* 2004; **53**: 866-870 [PMID:

15385917]
5 **Zatoński WA**, Sulkowska U, Mańczuk M, Rehm J, Boffetta P, Lowenfels AB, La Vecchia C. Liver cirrhosis mortality in Europe, with special attention to Central and Eastern Europe. *Eur Addict Res* 2010; **16**: 193-201 [PMID: 20606444 DOI: 10.1159/000317248]
6 **Gore FM**, Bloem PJ, Patton GC, Ferguson J, Joseph V, Coffey C, Sawyer SM, Mathers CD. Global burden of disease in young people aged 10-24 years: a systematic analysis. *Lancet* 2011; **377**: 2093-2102 [PMID: 21652063 DOI: 10.1016/S0140-6736(11)60512-6]
7 **White AM**, Kraus CL, Swartzwelder H. Many college freshmen drink at levels far beyond the binge threshold. *Alcohol Clin Exp Res* 2006; **30**: 1006-1010 [PMID: 16737459 DOI: 10.1111/j.1530-0277.2006.00122.x]
8 **Parada M**, Corral M, Caamaño-Isorna F, Mota N, Crego A, Rodríguez Holguín S, Cadaveira F. [Definition of adolescent binge drinking]. *Adicciones* 2011; **23**: 53-63 [PMID: 21503564]
9 **Epstein EE**, Kahler CW, McCrady BS, Lewis KD, Lewis S. An empirical classification of drinking patterns among alcoholics: binge, episodic, sporadic, and steady. *Addict Behav* 1995; **20**: 23-41 [PMID: 7785480]
10 **Gill JS**. Reported levels of alcohol consumption and binge drinking within the UK undergraduate student population over the last 25 years. *Alcohol Alcohol* 2002; **37**: 109-120 [PMID: 11912065]
11 **Lange JE**, Clapp JD, Turrisi R, Reavy R, Jaccard J, Johnson MB, Voas RB, Larimer M. College binge drinking: what is it? Who does it? *Alcohol Clin Exp Res* 2002; **26**: 723-730 [PMID: 12045482]
12 **Christiansen M**, Vik PW, Jarchow A. College student heavy drinking in social contexts versus alone. *Addict Behav* 2002; **27**: 393-404 [PMID: 12118627]
13 **LaBrie JW**, Pedersen ER, Tawalbeh S. Classifying risky-drinking college students: another look at the two-week drinker-type categorization. *J Stud Alcohol Drugs* 2007; **68**: 86-90 [PMID: 17149521]
14 **Sher KJ**, Rutledge PC. Heavy drinking across the transition to college: predicting first-semester heavy drinking from precollege variables. *Addict Behav* 2007; **32**: 819-835 [PMID: 16860940 DOI: 10.1016/j.addbeh.2006.06.024]
15 **Turrisi R**, Mallett KA, Mastroleo NR, Larimer ME. Heavy drinking in college students: who is at risk and what is being done about it? *J Gen Psychol* 2006; **133**: 401-420 [PMID: 17128959 DOI: 10.3200/GENP.133.4.401-420]
16 **Vik PW**, Carrello P, Tate SR, Field C. Progression of consequences among heavy-drinking college students. *Psychol Addict Behav* 2000; **14**: 91-101 [PMID: 10860108]
17 **Bendtsen P**, Johansson K, Akerlind I. Feasibility of an email-based electronic screening and brief intervention (e-SBI) to college students in Sweden. *Addict Behav* 2006; **31**: 777-787 [PMID: 15996827 DOI: 10.1016/j.addbeh.2005.06.002]
18 **Collins SE**, Carey KB. The theory of planned behavior as a model of heavy episodic drinking among college students. *Psychol Addict Behav* 2007; **21**: 498-507 [PMID: 18072832 DOI: 10.1037/0893-164X.21.4.498]
19 **Dawson DA**, Grant BF, Stinson FS, Chou PS. Another look at heavy episodic drinking and alcohol use disorders among college and noncollege youth. *J Stud Alcohol* 2004; **65**: 477-488 [PMID: 15378804]
20 **Mäkelä P**, Fonager K, Hibell B, Nordlund S, Sabroe S, Simpura J. Episodic heavy drinking in four Nordic countries: a comparative survey. *Addiction* 2001; **96**: 1575-1588 [PMID: 11784455 DOI: 10.1080/09652140120080714]
21 **Oesterle S**, Hill KG, Hawkins JD, Guo J, Catalano RF, Abbott RD. Adolescent heavy episodic drinking trajectories and health in young adulthood. *J Stud Alcohol* 2004; **65**: 204-212 [PMID: 15151351]
22 **Wilsnack RW**, Vogeltanz ND, Wilsnack SC, Harris TR, Ahlström S, Bondy S, Csémy L, Ferrence R, Ferris J, Fleming J, Graham K, Greenfield T, Guyon L, Haavio-Mannila E, Kellner F, Knibbe R, Kubicka L, Loukomskaia M, Mustonen H, Nadeau L, Narusk A, Neve R, Rahav G, Spak F, Teichman M, Trocki K, Webster I, Weiss S. Gender differences in alcohol consumption and adverse drinking consequences: cross-cultural patterns. *Addiction* 2000; **95**: 251-265 [PMID: 10723854]
23 **Murgraff V**, Parrott A, Bennett P. Risky single-occasion drinking amongst young people--definition, correlates, policy, and intervention: a broad overview of research findings. *Alcohol Alcohol* 1999; **34**: 3-14 [PMID: 10075396]
24 **Lederman LC**, Stewart LP, Goodhart FW, Laitman L. A case against "binge" as the term of choice: convincing college students to personalize messages about dangerous drinking. *J Health Commun* 2003; **8**: 79-91 [PMID: 12635812 DOI: 10.1080/10810730305734]
25 **Baer JS**, Kivlahan DR, Blume AW, McKnight P, Marlatt GA. Brief intervention for heavy-drinking college students: 4-year follow-up and natural history. *Am J Public Health* 2001; **91**: 1310-1316 [PMID: 11499124]
26 **Bloomfield K**, Stockwell T, Gmel G, Rehn N. International comparisons of alcohol consumption. *Alcohol Res Health* 2003; **27**: 95-109 [PMID: 15301404]
27 **Kuntsche E**, Rehm J, Gmel G. Characteristics of binge drinkers in Europe. *Soc Sci Med* 2004; **59**: 113-127 [PMID: 15087148 DOI: 10.1016/j.socscimed.2003.10.009]
28 **Perkins HW**. Surveying the damage: a review of research on consequences of alcohol misuse in college populations. *J Stud Alcohol Suppl* 2002; (**14**): 91-100 [PMID: 12022733]
29 **Room R**, Babor T, Rehm J. Alcohol and public health. *Lancet* 2005; **365**: 519-530 [PMID: 15705462 DOI: 10.1016/S0140-6736(05)17870-2]
30 **Wechsler H**, Davenport A, Dowdall G, Moeykens B, Castillo S. Health and behavioral consequences of binge drinking in college. A national survey of students at 140 campuses. *JAMA* 1994; **272**: 1672-1677 [PMID: 7966895]
31 **Wechsler H**, Dowdall GW, Davenport A, Rimm EB. A gender-specific measure of binge drinking among college students. *Am J Public Health* 1995; **85**: 982-985 [PMID: 7604925]
32 **Warren KR**, Hewitt BG. NIAAA: advancing alcohol research for 40 years. *Alcohol Res Health* 2010; **33**: 5-17 [PMID: 23579932]
33 **Hustad JT**, Carey KB. Using calculations to estimate blood alcohol concentrations for naturally occurring drinking episodes: a validity study. *J Stud Alcohol* 2005; **66**: 130-138 [PMID: 15830913]
34 **Cranford JA**, McCabe SE, Boyd CJ. A new measure of binge drinking: prevalence and correlates in a probability sample of undergraduates. *Alcohol Clin Exp Res* 2006; **30**: 1896-1905 [PMID: 17067355 DOI: 10.1111/j.1530-0277.2006.00234.x]
35 **Farke W**, Anderson P. Binge drinking in Europe. *Adicciones* 2007; **19**: 333-339 [PMID: 18173097]
36 **O'Malley PM**, Bachman JG, Johnston LD. Period, age, and cohort effects on substance use among American youth, 1976-82. *Am J Public Health* 1984; **74**: 682-688 [PMID: 6742254]
37 **Wechsler H**, Lee JE, Kuo M, Seibring M, Nelson TF, Lee H. Trends in college binge drinking during a period of increased prevention efforts. Findings from 4 Harvard School of Public Health College Alcohol Study surveys: 1993-2001. *J Am Coll Health* 2002; **50**: 203-217 [PMID: 11990979 DOI: 10.1080/07448480209595713]
38 **Mathurin P**, Deltenre P. Effect of binge drinking on the liver: an alarming public health issue? *Gut* 2009; **58**: 613-617 [PMID: 19174416 DOI: 10.1136/gut.2007.145573]
39 **Popova S**, Rehm J, Patra J, Zatonski W. Comparing alcohol consumption in central and eastern Europe to other European countries. *Alcohol Alcohol* 2007; **42**: 465-473 [PMID: 17287207 DOI: 10.1093/alcalc/agl124]
40 **Delegación del Gobierno para el Plan Nacional sobre Drogas.** Informe de la encuesta domiciliaria sobre alcohol y drogas de España. 2009
41 **Galán I**, González MJ, Valencia-Martín JL. [Alcohol drinking patterns in Spain: a country in transition]. *Rev Esp Salud Publica* 2014; **88**: 529-540 [PMID: 25090408 DOI: 10.4321/S1135-57272014000400007]
42 **Bartoli F**, Carretta D, Crocamo C, Schivalocchi A, Brambilla G,

Clerici M, Carrà G. Prevalence and correlates of binge drinking among young adults using alcohol: a cross-sectional survey. *Biomed Res Int* 2014; **2014**: 930795 [PMID: 25101300 DOI: 10.1155/2014/930795]
43 **Peraro L**, Lobello S, Rosa-Rizzotto E, Caroli D, Polato F, De Lazzari F. 982 binge drinking, alcohol abuse and dependence: screening study in an emergency department. *J hepatology* 2013; **58** Supplement 1: S404-S405 [DOI: 10.1016/S0168-8278(13)60984-8]
44 **CDC**. Binge drinking prevalence, frequency, and intensity among adults-United States. 2012
45 **Grucza RA**, Norberg KE, Bierut LJ. Binge drinking among youths and young adults in the United States: 1979-2006. *J Am Acad Child Adolesc Psychiatry* 2009; **48**: 692-702 [PMID: 19465879 DOI: 10.1097/CHI.0b013e3181a2b32f]
46 **Hingson R**, Heeren T, Winter M, Wechsler H. Magnitude of alcohol-related mortality and morbidity among U.S. college students ages 18-24: changes from 1998 to 2001. *Annu Rev Public Health* 2005; **26**: 259-279 [PMID: 15760289 DOI: 10.1146/annurev.publhealth.26.021304.144652]
47 **Robin RW**, Long JC, Rasmussen JK, Albaugh B, Goldman D. Relationship of binge drinking to alcohol dependence, other psychiatric disorders, and behavioral problems in an American Indian tribe. *Alcohol Clin Exp Res* 1998; **22**: 518-523 [PMID: 9581662]
48 **Courtney KE**, Polich J. Binge drinking in young adults: Data, definitions, and determinants. *Psychol Bull* 2009; **135**: 142-156 [PMID: 19210057 DOI: 10.1037/a0014414]
49 **Statistics OfN**. Statistics on alcohol. 2003
50 **He J**, Gu D, Wu X, Reynolds K, Duan X, Yao C, Wang J, Chen CS, Chen J, Wildman RP, Klag MJ, Whelton PK. Major causes of death among men and women in China. *N Engl J Med* 2005; **353**: 1124-1134 [PMID: 16162883 DOI: 10.1056/NEJMsa050467]
51 **Pham TM**, Fujino Y, Ide R, Shirane K, Tokui N, Kubo T, Mizoue T, Ogimoto I, Yoshimura T. Mortality attributable to cigarette smoking in a cohort study in Japan. *Eur J Epidemiol* 2007; **22**: 599-605 [PMID: 17653602 DOI: 10.1007/s10654-007-9161-y]
52 **Bobo JK**, Husten C. Sociocultural influences on smoking and drinking. *Alcohol Res Health* 2000; **24**: 225-232 [PMID: 15986717]
53 **McKee SA**, Hinson R, Rounsaville D, Petrelli P. Survey of subjective effects of smoking while drinking among college students. *Nicotine Tob Res* 2004; **6**: 111-117 [PMID: 14982695 DOI: 10.1080/14622200310001656939]
54 **Schorling JB**, Gutgesell M, Klas P, Smith D, Keller A. Tobacco, alcohol and other drug use among college students. *J Subst Abuse* 1994; **6**: 105-115 [PMID: 8081105]
55 **Weitzman ER**, Chen YY. The co-occurrence of smoking and drinking among young adults in college: national survey results from the United States. *Drug Alcohol Depend* 2005; **80**: 377-386 [PMID: 16009507 DOI: 10.1016/j.drugalcdep.2005.05.008]
56 **Harrison EL**, Desai RA, McKee SA. Nondaily smoking and alcohol use, hazardous drinking, and alcohol diagnoses among young adults: findings from the NESARC. *Alcohol Clin Exp Res* 2008; **32**: 2081-2087 [PMID: 18828805 DOI: 10.1111/j.1530-0277.2008.00796.x]
57 **McKee SA**, Falba T, O'Malley SS, Sindelar J, O'Connor PG. Smoking status as a clinical indicator for alcohol misuse in US adults. *Arch Intern Med* 2007; **167**: 716-721 [PMID: 17420431 DOI: 10.1001/archinte.167.7.716]
58 **Acosta MC**, Eissenberg T, Nichter M, Nichter M, Balster RL; Tobacco Etiology Research Network (TERN). Characterizing early cigarette use episodes in novice smokers. *Addict Behav* 2008; **33**: 106-121 [PMID: 17913378 DOI: 10.1016/j.addbeh.2007.09.005]
59 **Doyon WM**, Dong Y, Ostroumov A, Thomas AM, Zhang TA, Dani JA. Nicotine decreases ethanol-induced dopamine signaling and increases self-administration via stress hormones. *Neuron* 2013; **79**: 530-540 [PMID: 23871233 DOI: 10.1016/j.neuron.2013.06.006]
60 **Gilpin EA**, White VM, Pierce JP. How effective are tobacco industry bar and club marketing efforts in reaching young adults? *Tob Control* 2005; **14**: 186-192 [PMID: 15923469 DOI: 10.1136/tc.2004.009712]
61 **Harrison EL**, McKee SA. Young adult non-daily smokers: patterns of alcohol and cigarette use. *Addict Behav* 2008; **33**: 668-674 [PMID: 18093745 DOI: 10.1016/j.addbeh.2007.11.012]
62 **Glautier S**, Clements K, White JA, Taylor C, Stolerman IP. Alcohol and the reward value of cigarette smoking. *Behav Pharmacol* 1996; **7**: 144-154 [PMID: 11224406]
63 **Ajani UA**, Gaziano JM, Lotufo PA, Liu S, Hennekens CH, Buring JE, Manson JE. Alcohol consumption and risk of coronary heart disease by diabetes status. *Circulation* 2000; **102**: 500-505 [PMID: 10920060]
64 **Blot WJ**, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF. Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Res* 1988; **48**: 3282-3287 [PMID: 3365707]
65 **Jiang N**, Lee YO, Ling PM. Association between tobacco and alcohol use among young adult bar patrons: a cross-sectional study in three cities. *BMC Public Health* 2014; **14**: 500 [PMID: 24886521 DOI: 10.1186/1471-2458-14-500]
66 **Rusyn I**, Bataller R. Alcohol and toxicity. *J Hepatol* 2013; **59**: 387-388 [PMID: 23391479 DOI: 10.1016/j.jhep.2013.01.035]
67 **Gao B**, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011; **141**: 1572-1585 [PMID: 21920463 DOI: 10.1053/j.gastro.2011.09.002]
68 **Baan R**, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Cogliano V; WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of alcoholic beverages. *Lancet Oncol* 2007; **8**: 292-293 [PMID: 17431955]
69 **Okoro CA**, Brewer RD, Naimi TS, Moriarty DG, Giles WH, Mokdad AH. Binge drinking and health-related quality of life: do popular perceptions match reality? *Am J Prev Med* 2004; **26**: 230-233 [PMID: 15026103 DOI: 10.1016/j.amepre.2003.10.022]
70 **Reuben A**. Alcohol and the liver. *Curr Opin Gastroenterol* 2007; **23**: 283-291 [PMID: 17414844 DOI: 10.1097/MOG.0b013e3280f27582]
71 **Marugame T**, Yamamoto S, Yoshimi I, Sobue T, Inoue M, Tsugane S; Japan Public Health Center-based Prospective Study Group. Patterns of alcohol drinking and all-cause mortality: results from a large-scale population-based cohort study in Japan. *Am J Epidemiol* 2007; **165**: 1039-1046 [PMID: 17344205 DOI: 10.1093/aje/kwk112]
72 **Crosse KI**, Anania FA. Alcoholic Hepatitis. *Curr Treat Options Gastroenterol* 2002; **5**: 417-423 [PMID: 12408778]
73 **Mathurin P**, Beuzin F, Louvet A, Carrié-Ganne N, Balian A, Trinchet JC, Dalsoglio D, Prevot S, Naveau S. Fibrosis progression occurs in a subgroup of heavy drinkers with typical histological features. *Aliment Pharmacol Ther* 2007; **25**: 1047-1054 [PMID: 17439505 DOI: 10.1111/j.1365-2036.2007.03302.x]
74 **Mathews S**, Xu M, Wang H, Bertola A, Gao B. Animals models of gastrointestinal and liver diseases. Animal models of alcohol-induced liver disease: pathophysiology, translational relevance, and challenges. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G819-G823 [PMID: 24699333 DOI: 10.1152/ajpgi.00041.2014]
75 **Gäbele E**, Dostert K, Dorn C, Patsenker E, Stickel F, Hellerbrand C. A new model of interactive effects of alcohol and high-fat diet on hepatic fibrosis. *Alcohol Clin Exp Res* 2011; **35**: 1361-1367 [PMID: 21463337 DOI: 10.1111/j.1530-0277.2011.01472.x]
76 **Xu J**, Lai KK, Verlinsky A, Lugea A, French SW, Cooper MP, Ji C, Tsukamoto H. Synergistic steatohepatitis by moderate obesity and alcohol in mice despite increased adiponectin and p-AMPK. *J Hepatol* 2011; **55**: 673-682 [PMID: 21256905 DOI: 10.1016/j.jhep.2010.12.034]
77 **Chang B**, Xu MJ, Zhou Z, Cai Y, Li M, Wang W, Feng D, Bertola A, Wang H, Kunos G, Gao B. Short- or long-term high-fat diet feeding plus acute ethanol binge synergistically induce acute liver injury in mice: An important role for CXCL1. *Hepatology* 2015; **62**: 1070-1085 [PMID: 26033752 DOI: 10.1002/hep.27921]
78 **Robin MA**, Demeilliers C, Sutton A, Paradis V, Maisonneuve C, Dubois S, Poirel O, Lettéron P, Pessayre D, Fromenty B. Alcohol

increases tumor necrosis factor alpha and decreases nuclear factor-kappab to activate hepatic apoptosis in genetically obese mice. *Hepatology* 2005; **42**: 1280-1290 [PMID: 16317704 DOI: 10.1002/hep.20949]
79 **Nieto N**, Rojkind M. Repeated whiskey binges promote liver injury in rats fed a choline-deficient diet. *J Hepatol* 2007; **46**: 330-339 [PMID: 17156887 DOI: 10.1016/j.jhep.2006.09.010]
80 **Beier JI**, McClain CJ. Mechanisms and cell signaling in alcoholic liver disease. *Biol Chem* 2010; **391**: 1249-1264 [PMID: 20868231 DOI: 10.1515/BC.2010.137]
81 **Gao B**, Seki E, Brenner DA, Friedman S, Cohen JI, Nagy L, Szabo G, Zakhari S. Innate immunity in alcoholic liver disease. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G516-G525 [PMID: 21252049 DOI: 10.1152/ajpgi.00537.2010]
82 **Shukla SD**, Velazquez J, French SW, Lu SC, Ticku MK, Zakhari S. Emerging role of epigenetics in the actions of alcohol. *Alcohol Clin Exp Res* 2008; **32**: 1525-1534 [PMID: 18616668 DOI: 10.1111/j.1530-0277.2008.00729.x]
83 **Shukla SD**, Pruett SB, Szabo G, Arteel GE. Binge ethanol and liver: new molecular developments. *Alcohol Clin Exp Res* 2013; **37**: 550-557 [PMID: 23347137 DOI: 10.1111/acer.12011]
84 **Stepanova M**, Rafiq N, Younossi ZM. Components of metabolic syndrome are independent predictors of mortality in patients with chronic liver disease: a population-based study. *Gut* 2010; **59**: 1410-1415 [PMID: 20660697 DOI: 10.1136/gut.2010.213553]
85 **Corrao G**, Lepore AR, Torchio P, Valenti M, Galatola G, D'Amicis A, Aricó S, di Orio F. The effect of drinking coffee and smoking cigarettes on the risk of cirrhosis associated with alcohol consumption. A case-control study. Provincial Group for the Study of Chronic Liver Disease. *Eur J Epidemiol* 1994; **10**: 657-664 [PMID: 7672043]
86 **Pessione F**, Ramond MJ, Peters L, Pham BN, Batel P, Rueff B, Valla DC. Five-year survival predictive factors in patients with excessive alcohol intake and cirrhosis. Effect of alcoholic hepatitis, smoking and abstinence. *Liver Int* 2003; **23**: 45-53 [PMID: 12640727]
87 **DiMartini A**, Javed L, Russell S, Dew MA, Fitzgerald MG, Jain A, Fung J. Tobacco use following liver transplantation for alcoholic liver disease: an underestimated problem. *Liver Transpl* 2005; **11**: 679-683 [PMID: 15915490 DOI: 10.1002/lt.20385]
88 **El-Zayadi AR**. Heavy smoking and liver. *World J Gastroenterol* 2006; **12**: 6098-6101 [PMID: 17036378]
89 **Yu MW**, Lien JP, Liaw YF, Chen CJ. Effects of multiple risk factors for hepatocellular carcinoma on formation of aflatoxin B1-DNA adducts. *Cancer Epidemiol Biomarkers Prev* 1996; **5**: 613-619 [PMID: 8824363]
90 **Alebiosu CO**. An update on 'progression promoters' in renal diseases. *J Natl Med Assoc* 2003; **95**: 30-42 [PMID: 12656448]
91 **Malfertheiner P**, Schütte K. Smoking--a trigger for chronic inflammation and cancer development in the pancreas. *Am J Gastroenterol* 2006; **101**: 160-162 [PMID: 16405549 DOI: 10.1111/j.1572-0241.2006.00402.x]
92 **Agarwal R**. Smoking, oxidative stress and inflammation: impact on resting energy expenditure in diabetic nephropathy. *BMC Nephrol* 2005; **6**: 13 [PMID: 16303055 DOI: 10.1186/1471-2369-6-13]
93 **Liu PY**, Li YH, Chan SH, Lin LJ, Wu HL, Shi GY, Chen JH. Genotype-phenotype association of matrix metalloproteinase-3 polymorphism and its synergistic effect with smoking on the occurrence of acute coronary syndrome. *Am J Cardiol* 2006; **98**: 1012-1017 [PMID: 17027562 DOI: 10.1016/j.amjcard.2006.05.017]
94 **Taal MW**. Slowing the progression of adult chronic kidney disease: therapeutic advances. *Drugs* 2004; **64**: 2273-2289 [PMID: 15456327]
95 **Johnson DW**. Evidence-based guide to slowing the progression of early renal insufficiency. *Intern Med J* 2004; **34**: 50-57 [PMID: 14748914]
96 **Shi JP**, Fan JG, Wu R, Gao XQ, Zhang L, Wang H. [Prevalence and risk factors of hepatic steatosis in patients with chronic hepatitis B]. *Zhonghua Ganzangbing Zazhi* 2008; **16**: 519-523 [PMID: 18647531]
97 **Anan A**, Baskin-Bey ES, Isomoto H, Mott JL, Bronk SF, Albrecht JH, Gores GJ. Proteasome inhibition attenuates hepatic injury in the bile duct-ligated mouse. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**: G709-G716 [PMID: 16798723 DOI: 10.1152/ajpgi.00126.2006]
98 **Bagnardi V**, Blangiardo M, La Vecchia C, Corrao G. A meta-analysis of alcohol drinking and cancer risk. *Br J Cancer* 2001; **85**: 1700-1705 [PMID: 11742491 DOI: 10.1054/bjoc.2001.2140]
99 **Haas SL**, Ye W, Löhr JM. Alcohol consumption and digestive tract cancer. *Curr Opin Clin Nutr Metab Care* 2012; **15**: 457-467 [PMID: 22797570 DOI: 10.1097/MCO.0b013e3283566699]
100 **Tramacere I**, Negri E, Pelucchi C, Bagnardi V, Rota M, Scotti L, Islami F, Corrao G, La Vecchia C, Boffetta P. A meta-analysis on alcohol drinking and gastric cancer risk. *Ann Oncol* 2012; **23**: 28-36 [PMID: 21536659 DOI: 10.1093/annonc/mdr135]
101 **Seitz HK**, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* 2007; **7**: 599-612 [PMID: 17646865 DOI: 10.1038/nrc2191]
102 **Homann N**, Kärkkäinen P, Koivisto T, Nosova T, Jokelainen K, Salaspuro M. Effects of acetaldehyde on cell regeneration and differentiation of the upper gastrointestinal tract mucosa. *J Natl Cancer Inst* 1997; **89**: 1692-1697 [PMID: 9390538]
103 **Kim JS**, Shukla SD. Acute in vivo effect of ethanol (binge drinking) on histone H3 modifications in rat tissues. *Alcohol Alcohol* 2006; **41**: 126-132 [PMID: 16314425 DOI: 10.1093/alcalc/agh248]
104 **Jung SH**, Gombojav B, Park EC, Nam CM, Ohrr H, Won JU. Population based study of the association between binge drinking and mortality from cancer of oropharynx and esophagus in Korean men: the Kangwha cohort study. *Asian Pac J Cancer Prev* 2014; **15**: 3675-3679 [PMID: 24870776]
105 **Gupta S**, Wang F, Holly EA, Bracci PM. Risk of pancreatic cancer by alcohol dose, duration, and pattern of consumption, including binge drinking: a population-based study. *Cancer Causes Control* 2010; **21**: 1047-1059 [PMID: 20349126 DOI: 10.1007/s10552-010-9533-6]
106 **Mansoori AA**, Jain SK. Molecular Links between Alcohol and Tobacco Induced DNA Damage, Gene Polymorphisms and Pathophysiological Consequences: A Systematic Review of Hepatic Carcinogenesis. *Asian Pac J Cancer Prev* 2015; **16**: 4803-4812 [PMID: 26163595]
107 **Nixon K**, Crews FT. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. *J Neurochem* 2002; **83**: 1087-1093 [PMID: 12437579]
108 **Herrera DG**, Yague AG, Johnsen-Soriano S, Bosch-Morell F, Collado-Morente L, Muriach M, Romero FJ, Garcia-Verdugo JM. Selective impairment of hippocampal neurogenesis by chronic alcoholism: protective effects of an antioxidant. *Proc Natl Acad Sci USA* 2003; **100**: 7919-7924 [PMID: 12792022 DOI: 10.1073/pnas.1230907100]
109 **Crews F**, He J, Hodge C. Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav* 2007; **86**: 189-199 [PMID: 17222895 DOI: 10.1016/j.pbb.2006.12.001]
110 **Sowell ER**, Delis D, Stiles J, Jernigan TL. Improved memory functioning and frontal lobe maturation between childhood and adolescence: a structural MRI study. *J Int Neuropsychol Soc* 2001; **7**: 312-322 [PMID: 11311032]
111 **García-Moreno LM**, Expósito J, Sanhueza C, Angulo MT. [Prefrontal activity and weekend alcoholism in the young]. *Adicciones* 2008; **20**: 271-279 [PMID: 18813773]
112 **Brown SA**, Tapert SF, Granholm E, Delis DC. Neurocognitive functioning of adolescents: effects of protracted alcohol use. *Alcohol Clin Exp Res* 2000; **24**: 164-171 [PMID: 10698367]
113 **Schweinsburg AD**, Schweinsburg BC, Cheung EH, Brown GG, Brown SA, Tapert SF. fMRI response to spatial working memory in adolescents with comorbid marijuana and alcohol use disorders. *Drug Alcohol Depend* 2005; **79**: 201-210 [PMID: 16002029 DOI: 10.1016/j.drugalcdep.2005.01.009]
114 **Doallo S**, Cadaveira F, Corral M, Mota N, López-Caneda E,

Holguín SR. Larger mid-dorsolateral prefrontal gray matter volume in young binge drinkers revealed by voxel-based morphometry. *PLoS One* 2014; **9**: e96380 [PMID: 24789323 DOI: 10.1371/journal.pone.0096380]

115 **Mota N**, Parada M, Crego A, Doallo S, Caamaño-Isorna F, Rodríguez Holguín S, Cadaveira F, Corral M. Binge drinking trajectory and neuropsychological functioning among university students: a longitudinal study. *Drug Alcohol Depend* 2013; **133**: 108-114 [PMID: 23791027 DOI: 10.1016/j.drugalcdep.2013.05.024]

116 **Rourke SB**, Grant I. The neurobehavioral correlates of alcoholism. In: Grant I, Adams K, editors. Neuropsychological assessment of neuropsychiatric and neuromedical disorders. New York: Oxford University Press, 2009

117 **Hartley DE**, Elsabagh S, File SE. Binge drinking and sex: effects on mood and cognitive function in healthy young volunteers. *Pharmacol Biochem Behav* 2004; **78**: 611-619 [PMID: 15251270 DOI: 10.1016/j.pbb.2004.04.027]

118 **Heffernan T**, Clark R, Bartholomew J, Ling J, Stephens R. Does binge drinking in teenagers affect their everyday prospective memory? *Drug Alcohol Depend* 2010; **109**: 73-78 [PMID: 20071106 DOI: 10.1016/j.drugalcdep.2009.12.013]

119 **Heffernan TM**, Bartholomew J. Does excessive alcohol use in teenagers affect their everyday prospective memory? *J Adolesc Health* 2006; **39**: 138-140 [PMID: 16781978 DOI: 10.1016/j.jadohealth.2005.10.010]

120 **Rehm J**, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J. Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet* 2009; **373**: 2223-2233 [PMID: 19560604 DOI: 10.1016/S0140-6736(09)60746-7]

121 **Klatsky AL**, Armstrong MA, Friedman GD. Risk of cardiovascular mortality in alcohol drinkers, ex-drinkers and nondrinkers. *Am J Cardiol* 1990; **66**: 1237-1242 [PMID: 2239729]

122 **McElduff P**, Dobson AJ. How much alcohol and how often? Population based case-control study of alcohol consumption and risk of a major coronary event. *BMJ* 1997; **314**: 1159-1164 [PMID: 9146388]

123 **Mukamal KJ**, Maclure M, Muller JE, Mittleman MA. Binge drinking and mortality after acute myocardial infarction. *Circulation* 2005; **112**: 3839-3845 [PMID: 16365208 DOI: 10.1161/CIRCULATIONAHA.105.574749]

124 **Liu W**, Redmond EM, Morrow D, Cullen JP. Differential effects of daily-moderate versus weekend-binge alcohol consumption on atherosclerotic plaque development in mice. *Atherosclerosis* 2011; **219**: 448-454 [PMID: 21930274 DOI: 10.1016/j.atherosclerosis.2011.08.034]

125 **Marques-Vidal P**, Arveiler D, Evans A, Amouyel P, Ferrières J, Ducimetière P. Different alcohol drinking and blood pressure relationships in France and Northern Ireland: The PRIME Study. *Hypertension* 2001; **38**: 1361-1366 [PMID: 11751718]

126 **Sundell L**, Salomaa V, Vartiainen E, Poikolainen K, Laatikainen T. Increased stroke risk is related to a binge-drinking habit. *Stroke* 2008; **39**: 3179-3184 [PMID: 18832741 DOI: 10.1161/STROKEAHA.108.520817]

127 **Wannamethee G**, Shaper AG. Alcohol and sudden cardiac death. *Br Heart J* 1992; **68**: 443-448 [PMID: 1467026]

128 **Kauhanen J**, Kaplan GA, Goldberg DE, Salonen R, Salonen JT. Pattern of alcohol drinking and progression of atherosclerosis. *Arterioscler Thromb Vasc Biol* 1999; **19**: 3001-3006 [PMID: 10591681]

129 **Deanfield JE**, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 2007; **115**: 1285-1295 [PMID: 17353456 DOI: 10.1161/CIRCULATIONAHA.106.652859]

130 **Ras RT**, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. *Int J Cardiol* 2013; **168**: 344-351 [PMID: 23041097 DOI: 10.1016/j.ijcard.2012.09.047]

131 **Goslawski M**, Piano MR, Bian JT, Church EC, Szczurek M, Phillips SA. Binge drinking impairs vascular function in young adults. *J Am Coll Cardiol* 2013; **62**: 201-207 [PMID: 23623907 DOI: 10.1016/j.jacc.2013.03.049]

132 **Martyn C**. Hebdomadal rhythms of the heart. *BMJ* 2000; **321**: 1542-1543 [PMID: 11124165]

133 **Chenet L**, McKee M, Leon D, Shkolnikov V, Vassin S. Alcohol and cardiovascular mortality in Moscow; new evidence of a causal association. *J Epidemiol Community Health* 1998; **52**: 772-774 [PMID: 10396517]

134 **Evans C**, Chalmers J, Capewell S, Redpath A, Finlayson A, Boyd J, Pell J, McMurray J, Macintyre K, Graham L. "I don't like Mondays"-day of the week of coronary heart disease deaths in Scotland: study of routinely collected data. *BMJ* 2000; **320**: 218-219 [PMID: 10642230]

135 **Chenet L**, Britton A. Weekend binge drinking may be linked to Monday peaks in cardiovascular deaths. *BMJ* 2001; **322**: 998 [PMID: 11339228]

136 **Aroor AR**, Shukla SD. Binge ethanol intake in chronically exposed rat liver decreases LDL-receptor and increases angiotensinogen gene expression. *World J Hepatol* 2011; **3**: 250-255 [PMID: 21969878 DOI: 10.4254/wjh.v3.i9.250]

137 **Apte MV**, Pirola RC, Wilson JS. Mechanisms of alcoholic pancreatitis. *J Gastroenterol Hepatol* 2010; **25**: 1816-1826 [PMID: 21091991 DOI: 10.1111/j.1440-1746.2010.06445.x]

138 **Razvodovsky YE**. Alcohol poisoning and duodenum ulcer mortality in Russia, 1965-2005. *Adicciones* 2007; **19**: 423-431 [PMID: 18173104]

139 **Walter M**, Scholler G, Moyzes D, Hildebrandt M, Neuhaus R, Danzer G, Klapp BF. Psychosocial prediction of abstinence from ethanol in alcoholic recipients following liver transplantation. *Transplant Proc* 2002; **34**: 1239-1241 [PMID: 12072327]

140 **Moreau R**, Lebrec D. Acute renal failure in patients with cirrhosis: perspectives in the age of MELD. *Hepatology* 2003; **37**: 233-243 [PMID: 12540770 DOI: 10.1053/jhep.2003.50084]

141 **World Health Organization**. Global status report on alcohol and health. 2011

142 **Babor TF**. Public health science and the global strategy on alcohol. *Bull World Health Organ* 2010; **88**: 643 [PMID: 20865064 DOI: 10.2471/BLT.10.081729]

143 **Anderson P**, Chisholm D, Fuhr DC. Effectiveness and cost-effectiveness of policies and programmes to reduce the harm caused by alcohol. *Lancet* 2009; **373**: 2234-2246 [PMID: 19560605 DOI: 10.1016/S0140-6736(09)60744-3]

144 **Anderson P**, Baumberg B. Alcohol in Europe. London: Institute of Alcohol Studies, 2006

145 **Chisholm D**, Rehm J, Van Ommeren M, Monteiro M. Reducing the global burden of hazardous alcohol use: a comparative cost-effectiveness analysis. *J Stud Alcohol* 2004; **65**: 782-793 [PMID: 15700517]

146 **Ames SC**, Werch CE, Ames GE, Lange LJ, Schroeder DR, Hanson AC, Patten CA. Integrated smoking cessation and binge drinking intervention for young adults: a pilot investigation. *Ann Behav Med* 2010; **40**: 343-349 [PMID: 20730517 DOI: 10.1007/s12160-010-9222-4]

147 **Burton SM**, Tiffany ST. The effect of alcohol consumption on craving to smoke. *Addiction* 1997; **92**: 15-26 [PMID: 9060194]

148 **King AC**, Epstein AM. Alcohol dose-dependent increases in smoking urge in light smokers. *Alcohol Clin Exp Res* 2005; **29**: 547-552 [PMID: 15834219]

149 **Hoffman JH**, Welte JW, Barnes GM. Co-occurrence of alcohol and cigarette use among adolescents. *Addict Behav* 2001; **26**: 63-78 [PMID: 11196293]

150 **Slutske WS**. Alcohol use disorders among US college students and their non-college-attending peers. *Arch Gen Psychiatry* 2005; **62**: 321-327 [PMID: 15753245 DOI: 10.1001/archpsyc.62.3.321]

151 **Hanewinkel R**, Sargent JD, Poelen EA, Scholte R, Florek E, Sweeting H, Hunt K, Karlsdottir S, Jonsson SH, Mathis F, Faggiano F, Morgenstern M. Alcohol consumption in movies and adolescent binge drinking in 6 European countries. *Pediatrics* 2012; **129**: 709-720 [PMID: 22392174 DOI: 10.1542/peds.2011-2809]

152 **Lee K**, Lim HT, Hwang SS, Chae DW, Park SM. Socio-economic disparities in behavioural risk factors for cancer and use of cancer screening services in Korean adults aged 30 years and older: the Third Korean National Health and Nutrition Examination Survey, 2005 (KNHANES III). *Public Health* 2010; **124**: 698-704 [PMID: 20888016 DOI: 10.1016/j.puhe.2010.07.004]

P- Reviewer: Albuquerque A, de F Higuera-de la Tijera M, Peltec A
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.i27.2716

World J Hepatol 2015 November 28; 7(27): 2716-2728
ISSN 1948-5182 (online)

REVIEW

Hepatic manifestations of non-steroidal inflammatory bowel disease therapy

Robert Hirten, Keith Sultan, Ashby Thomas, David E Bernstein

Robert Hirten, Keith Sultan, Ashby Thomas, David E Bernstein, Division of Gastroenterology, North Shore University Hospital-Long Island Jewish Medical Center, Manhasset, NY 11030, United States

David E Bernstein, Division of Hepatology, North Shore University Hospital-Long Island Jewish Medical Center, Manhasset, NY 11030, United States

Author contributions: Hirten R, Sultan K and Bernstein DE contributed to conception and design; Hirten R, Sultan K and Thomas A contributed to drafting of the article; Hirten R, Sultan K, Thomas A and Bernstein DE contributed to critical revision of the article for important intellectual content; Hirten R, Sultan K, Thomas A and Bernstein DE contributed to final approval of the article.

Conflict-of-interest statement: The authors declare that no conflicts of interest exist.

Correspondence to: Dr. David E Bernstein, MD, Chief of Division of Hepatology, North Shore University Hospital-Long Island Jewish Medical Center, 300 Community Drive, Manhasset, NY 11030, United States. dbernste@nshs.edu
Telephone: +1-516-5624664
Fax: +1-516-5621688

Received: June 28, 2015
Peer-review started: June 29, 2015
First decision: July 28, 2015
Revised: October 25, 2015
Accepted: November 13, 2015
Article in press: November 17, 2015

Published online: November 28, 2015

Abstract

Inflammatory bowel disease (IBD) is composed of Crohn's disease and ulcerative colitis and is manifested by both bowel-related and extraintestinal manifestations. Recently the number of therapeutic options available to treat IBD has dramatically increased, with each new medication having its own mechanism of action and side effect profile. A complete understanding of the hepatotoxicity of these medications is important in order to distinguish these complications from the hepatic manifestations of IBD. This review seeks to evaluate the hepatobiliary complications of non-steroid based IBD medications and aide providers in the recognition and management of these side-effects.

Key words: Hepatotoxicity; Adverse drug reactions; Drug induced liver injury; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis

Core tip: Recently the number of medical therapies for inflammatory bowel disease (IBD) has greatly increased. Each medication has its own mechanism of action and side effect profile. This review article discusses the hepatic side effects of medications used to treat IBD enabling physicians to better recognize and manage these complications.

Hirten R, Sultan K, Thomas A, Bernstein DE. Hepatic manifestations of non-steroidal inflammatory bowel disease therapy. *World J Hepatol* 2015; 7(27): 2716-2728 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i27/2716.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i27.2716

INTRODUCTION

Inflammatory bowel disease (IBD) is primarily composed of Crohn's disease (CD) and ulcerative colitis (UC). The North American incidence is 19.2 cases per 100000 person years and 20.2 cases per 100000 person years for UC and CD, respectively[1]. Through a combination of genetic and environmental factors, IBD appears to be caused by an inappropriate and overactive response of the body's immune system directed primarily at the gastrointestinal tract. Though most disease manifestations are bowel-related, multiple extraintestinal manifestations including dermatologic (pyoderma gangrenosum, erythema nodosum), ophthalmic (uveitis and episcleritis), joint (large and small joint arthritis and sacroiliitis) as well hepatobiliary complications may occur. Additionally, biliary and hepatic manifestations are common, with up to 29% of IBD patients developing abnormal liver tests[2].

Recently the number of therapeutic options available to treat IBD has increased dramatically. Current guidelines emphasize a move away from short term corticosteroid based treatment and towards IBD targeted medications proven to either induce and/or maintain clinical remission. Each new class of medication has its own mechanism of action and side effect profile. While multiple reviews have evaluated the hepatobiliary manifestations of IBD, few have focused in detail on the hepatic side effects of these medications. A complete understanding of the prevalence, characteristics and management of the hepatoxicity of these medications is important to distinguish these effects from IBD itself, limit their toxicity and maximize their therapeutic benefit. This review evaluates the hepatobiliary complications, mainly drug induced liver injury (DILI), of currently available non-steroid based IBD therapies.

SULFASALAZINE AND 5-AMINOSALICYLATES

Sulfasalazine is a pro-drug composed of 5-aminosalicylic acid (5-ASA) linked to sulfapyridine by an azo bond, and has been used in the treatment of IBD for over 70 years[3]. The primary efficacy of sulfasalazine in IBD is through the 5-ASA moiety and although the exact mechanism of action is not known, 5-ASA has been shown to have an array of anti-inflammatory properties including effects on reactive oxygen species, nuclear factor kappa B, and cytokines[4-6]. Though mainly demonstrating benefit for UC, it is commonly used for CD. Common side effects include headache, nausea, dyspepsia, or allergic reactions, and are generally attributed to the sulfa component. The overall incidence of liver toxicity has been estimated to be between 0.4%-2.9%, depending upon the study group and underlying disease[7,8]. Though most liver test abnormalities are minor and reversible with drug discontinuation, previous studies have described severe hepatotoxicity to sulfasalazine due to a systemic hypersensitivity reaction characterized by high fevers, generalized lymphadenopathy, a maculopapular rash, elevated liver enzymes, eosinophilia and immune complexes[9]. Granulomatous hepatitis has been described in patients on sulfasalazine presenting with an elevated alkaline phosphatase and bilirubin along with the presence of granulomas on liver biopsy. This has been described in patients without CD and appears to be medication rather than disease related[10-12]. Also reported are rare cases of fatal fulminant hepatic necrosis from drug rash with eosinophilia and systemic symptoms, a devastating complication with an estimated mortality of 10% if not appropriately diagnosed and treated with high dose corticosteroids[13-20].

Due to adverse effects of the sulfapyridine moiety, 5-ASA formulations without sulfa, the mesalamine derivatives, were developed in the 1970s, and are now more widely used than sulfasalazine. A systematic review of the short-term adverse effects of mesalamine show that this class of medication is generally well tolerated, with rates of adverse events similar to placebo controls[21]. The most common side effects associated with mesalamine include flatulence, nausea, headache, dyspepsia, and diarrhea. Despite the absence of the sulfa moiety and limited systemic absorption of mesalamine, a randomized trial comparing mesalamine to sulfasalazine showed rates of DILI to be similar between the two medications, with 2.6% of patients taking mesalamine experiencing liver injury[8]. One case report described possible drug-induced autoimmune hepatitis in a patient with CD treated with mesalamine. This patient developed chronic hepatitis with evidence of fibrosis on liver biopsy, as well as an elevated anti-nuclear antibody and anti-smooth muscle antibody, which resolved with discontinuation of the medication[22]. Other case reports have linked mesalamine to systemic hypersensitivity reactions similar to sulfasalazine as well as to the development of hepatocellular cholestasis[23,24].

When signs of liver injury develop secondary to sulfasalazine or mesalamine, the dosage should be decreased or the medication stopped completely depending upon the severity of the liver test elevation and the patient's condition. While no formal guidelines exist specifically for sulfasalazine or mesalamine, cases suspicious for DILI should be evaluated and managed according to currently accepted guidelines[25].

AZATHIOPRINE AND 6-MERCAPTOPURINE

The thiopurine immunomodulators azathioprine (AZA) and its principle metabolite, 6-mercaptopurine (6-MP), have shown efficacy in the maintenance of steroid induced remission for both CD and UC[26]. AZA is converted to its active metabolite 6-MP through non-enzymatic reactions by compounds such as glutathione. Intracellular metabolism of 6-MP is dictated by the activity of several enzymatic pathways resulting in both the

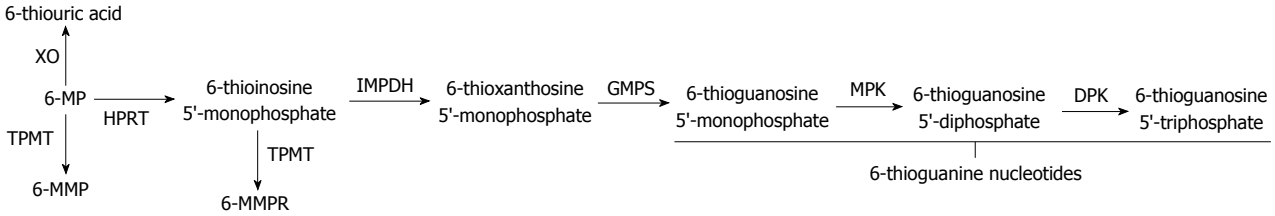

Figure 1 Azathioprine/6-mercaptopurine metabolism. Azathioprine is converted to 6-MP *via* nonenzymatic reactions. IMPDH and GMPS generate active 6-thioguanine nucleotides. TPMT results in the production of hepatotoxic 6-MMP and 6-MMPR metabolites. 6-MP: 6-mercaptopurine; TPMT: Thiopurine methyltransferase; XO: Xanthine oxidase; HPRT: Hypoxanthineguanine-phosphoribosyl-transferase; 6-MMP: 6-methylmercaptopurine; 6-MMPR: 6-methylmercaptopurine ribonucleotide; IMPDH: Inosine monophosphate dehydrogenase; GMPS: Guanosine monophosphate synthetase; MPK: Monophosphate kinase; DPK: Diphosphate kinase. Adapted from Cuffari C, Theoret Y, Latour S, Seidman G. 6-Mercaptopurine metabolism in Crohn's disease: Correlation with efficacy and toxicity. *Gut* 1996; 39: 401-406.

therapeutically active metabolite 6-thioguanine (6-TG), which is associated with myelosuppression, as well as the non-therapeutic metabolites 6-methylmercaptopurine (6-MMP) and 6-methylmercaptopurine ribonucleotide (6-MMPR) which have been associated with hepatotoxicity (Figure 1)[27].

AZA and 6-MP have been shown to result in both a hepatocellular and cholestatic hepatitis, with a number of studies evaluating the incidence of liver test abnormalities occurring in IBD patients treated with these medications. A large study of 3931 patients from the prospective Spanish nationwide database found that 4% of patients on thiopurines developed hepatotoxicity[28]. Another study that followed 786 patients with IBD, of whom 138 received either AZA or 6-MP, found that 7.1% of those treated per patient year developed elevated liver tests that were 1-2 times the upper limit of normal. Approximately 2.6% of those treated per patient year developed liver tests greater than twice the upper limit of normal[29]. A large systematic review of 3485 patients from 2007 evaluating the incidence of liver injury in patients with IBD exposed to AZA or 6-MP showed an overall prevalence of abnormal liver tests of 3.3%. When follow-up time was included, 2992 patients received treatment during a total of 6952 years of follow-up, with the average rate of drug-induced liver test elevation being 1.4% per patient year. These liver test abnormalities were generally noted to occur within the first few months of drug initiation[30].

The primary hepatic manifestations include hypersensitivity reactions, cholestasis, peliosis hepatitis, Disse space fibrosis, veno-occlusive disease and nodular regenerative hyperplasia (NRH)[31]. In hypersensitivity syndromes, symptoms usually develop within 2-3 wk of initiation with an elevated bilirubin and alkaline phosphatase, as well as with moderate elevations of aminotransferases. At the histologic level, parenchymal cell necrosis is noted[30,31]. NRH, peliosis hepatitis, fibrosis, and veno-occlusive disease have been shown to be dose-dependent injuries and likely secondary to damage to the endothelial cells lining the sinusoids and terminal hepatic venules[30,32]. This is often noted between 3 mo and 3 years of initiating treatment[30].

Thiopurine methyltransferase (TPMT) activity plays an important role in determining AZA and 6-MP metabolism and the development of side effects. Approximately 0.3% of the population has low or absent activity, 11% of the population has intermediate activity as they are heterozygote's, and 89% of the population possess the wild type with normal activity[33]. In general, 6-TG levels greater than 230-260 pmol/8 × 10^8 erythrocytes are associated with clinical efficacy, while levels over 450 pmol/8 × 10^8 erythrocytes are associated with bone marrow toxicity. Though high levels of 6-TG can also result in liver damage, particularly the development of NRH, most liver toxicity is believed to be related to 6-MMP and 6-MMPR, particularly at levels over 5700 pmol/8 × 10^8 erythrocytes[27,30,34].

Though no commercial testing is available for high TPMT activity, this appears to occur in up to 15% of the population with a preferential formation of the hepatotoxic 6-MMP and 6-MMPR metabolites[35]. Despite this association, routine monitoring for 6-MMP and 6-MMPR is not widely recommended. In patients who develop abnormal liver tests associated with the overproduction of 6-MMP and 6-MMPR the thiopurine may be stopped, the dose reduced or allopurinol may be used to inhibit xanthine oxidase. While an examination of the metabolic pathways involved would suggest this approach to result in an increase of both 6-TG and 6-MMP/6-MMPR levels, in fact, the effect is one of preferentially increased 6-TG over 6-MMP/6-MMPR. Typically AZA or 6-MP dosing is cut to one third or half the prior dose when used in conjunction with allopurinol, and the patient's blood work monitored closely.

As no formal guidelines exist, and most cases of liver toxicity occur within the first few months of therapy, we recommend following the protocol outlined by the United States Food and Drug Administration (FDA) (http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205919s000lbl.pdf), which suggests checking serum transaminase levels, alkaline phosphatase, and bilirubin levels weekly for the first month[30]. Following this initial therapy we agree with repeat testing monthly for the next 3 mo, then every three months thereafter, following along with the currently recommended monitoring of white blood cell counts. This sequence should be repeated with any dose increase. It is common for patients to have a slight elevation of liver enzymes with normalization on repeat testing. However, if elevations

become persistent or marked, the dose of AZA or 6-MP may be reduced in half with continued close monitoring, or by finer dose adjustments guided by metabolite levels. With normalization, careful resumption of the previous dose can be reattempted[30]. In one prospective study that evaluated this method, slightly less than half of patients were able to resume their previous dosage[36]. Patients whose liver tests fail to normalize should have the medication discontinued. If severe cholestatic jaundice occurs, the medication should be withdrawn with continued close monitoring of the liver tests for improvement. Patients should consider undergoing liver biopsy if liver tests remain persistently elevated after medication withdrawal[30].

METHOTREXATE

Methotrexate (MTX) was discovered in the 1940's and was first used to treat leukemia in 1948. It is a structural analogue of folate which competitively inhibits the enzyme dihydrofolate reductase and thereby the production of the intracellular metabolite folinic acid and the synthesis of purines and pyrimidines. Clinically, it has demonstrated effectiveness in the treatment of CD but not UC[37,38]. Generally, it is given intramuscularly at a dose of 25 mg weekly in combination with another medication used to induce remission, such as steroids or a biologic agent. Once a clinical response is observed, patients are switched to subcutaneous or intramuscular injections at a dose of 15 mg weekly. Multiple side effects have been reported including infections, pneumonitis, bone marrow suppression and liver test abnormalities.

There is extensive literature relating to MTX's effects on the liver with significant variability in reports of liver tests abnormalities and histologic changes on biopsy. While the mechanism resulting in hepatotoxicity is not fully understood, it is likely related to MTX induced folate deficiency, as supplementation has been shown to reduce hepatic adverse events[39,40]. Much of the evidence relating to its heptotoxicity comes from its use in rheumatoid arthritis and psoriatic arthritis, where it has been shown to have a variety of effects on the liver ranging from hepatic steatosis to fibrosis. The CORRONA database is a large cohort of patients which followed 2104 patients with rheumatoid arthritis and psoriatic arthritis exposed to methotrexate. Overall, approximately 22% of patients exposed to MTX with rheumatoid arthritis developed an elevated aspartate aminotransferase (AST)/alanine aminotransferase (ALT), while 35% of patients with psoriatic arthritis exposed to MTX had an AST/ALT > 1 × the upper limit of normal[41].

Similar results were found in patients with IBD on chronic low dose MTX. A retrospective study evaluating 87 subjects with a mean duration of therapy of 81 wk and an average dose of 1813 mg found that 24% of patients developed elevated liver tests. Of these patients, 44% had underlying risk factors for liver disease. While 23% of patients in the study had abnormal liver tests at baseline, 45% had normalization while on MTX, while 45% experienced a worsening of their liver tests[42]. A meta-analysis evaluating 12 studies of 457 children with IBD exposed to MTX found that 10.2% of patients developed abnormal liver biochemistry with 6.4% of patients requiring dose reduction and 4.5% of patients requiring discontinuation of the medication[43].

Beyond elevated liver tests, long term MTX use may lead to the development of fibrosis and cirrhosis. The histologic feature of methotrexate-induced liver toxicity tends to resemble nonalcoholic steatohepatitis[44]. Earlier studies reported up to a 50% chance of developing fibrosis, though there is concern that this may be an overestimation secondary to confounding factors[45-47]. A more recent meta-analysis of 15 studies examined the relationship between long term low-dose MTX and biopsy evidence of liver fibrosis. It showed that patients have a 6.7% chance of progressing at least one histologic grade of fibrosis for every gram of MTX taken and that the rate of progression of liver disease was associated with the cumulative dose of the medication. In addition, it revealed a 5.0% chance of having advanced pathologic changes on biopsy[48].

In general, measures to prevent or identify hepatotoxic effects should be employed prior to chronic use and risk factors for the development of hepatoxicity identified. These risk factors, created by the American Academy of Dermatology, allow for stratification and guidance in how aggressive liver enzymes should be monitored and whether a patient should be considered for liver biopsy[44]. Patients should be ruled out for chronic viral infections such as hepatitis B and hepatitis C, and educated to avoid hepatotoxic agents. In addition, all patients should undergo periodic monitoring of their liver tests. While there are no formal recommendations for laboratory monitoring by gastrointestinal societies, the American College of Rheumatology recommends monitoring liver tests every 2-4 wk for the first 3 mo, then every 8-12 wk for the next 3 mo. After 6 mo of therapy liver tests should be checked every 12 wk[49].

With regards to liver biopsy, the most recent set of American College of Gastroenterology guidelines recommend consideration of a biopsy prior to initiation of MTX in patients with elevated liver tests at baseline, patients with one or more risk factor for hepatotoxicity and in patients who are suspected to have chronic liver disease[26]. Given the lack of biopsy data in patients with CD, current guidelines defer to the American Rheumatology Associations guidelines which recommend a liver biopsy for patients who develop elevations in AST in 5 of 9 blood samples in a 12-mo period, 6 of 12 samples if performed monthly, or if there is a decrease in serum albumin below the normal range[26,50]. The role for current noninvasive technologies such as transient or MRI elastography, or serum non-invasive markers of fibrosis remains unclear.

ANTI TUMOR NECROSIS FACTOR-ALPHA

Tumor necrosis factor-alpha (TNF-α) is a pro-inflamma-

tory cytokine that plays a role in the immunopathology of IBD[51]. Infliximab (REMICADE®) is a chimeric monoclonal anti-TNF-α antibody which was first approved to treat CD in 1998 and UC in 2005[52]. Its most common side effects are headache, rash and cough, while infusion reactions have been seen in 5% of patients[53]. In addition, through its immunosuppressive properties, it has been associated with an increased risk of infection based on large cohort studies[54].

Infliximab has been associated with several forms of DILI. A large, prospective cohort study found that 6.7% of patients treated with infliximab experienced hepatocellular cytolytic injury, with mild elevations in AST/ALT > 1 × the upper limit of normal, but discontinuation rates were not significant compared to those who did not have liver enzyme elevations[55]. In another retrospective cohort study, 6% of patients, the vast majority who were on low-dose infliximab, developed mild idiopathic liver enzyme elevations. In up to 50% of these patients, other possible causes (medication, alcohol, metabolic, viral) were identified. The mean time to ALT elevation was 29 wk, and resolution occurred after a median of 17 wk in 82% of patients despite continuation of therapy[56]. In this study, a small subset of patients were found to have positive autoimmune markers, and underwent liver biopsies which demonstrated features of autoimmune hepatitis, requiring cessation of the initial anti-TNF-α treatment.

Although infliximab has been used in several studies to treat refractory autoimmune hepatitis, autoimmune hepatitis due to infliximab is becoming an increasingly recognized form of hepatocellular injury[57-59]. In a review of 34 cases of DILI secondary to TNF-α antagonists, 16/26 patients treated with infliximab had autoimmune antibodies or liver biopsies suggestive of autoimmune hepatitis, with interface hepatitis, piecemeal necrosis, and portal lymphocytic inflammation with plasma cells[60]. Patients with autoimmune features had a latency period of 16 wk before liver injury was apparent and higher peak ALT values. In the reported cases, patients often recovered with discontinuation of infliximab and corticosteroid therapy.

Cholestatic liver injury has been described in several case reports of patients treated with infliximab. Patients developed jaundice after infliximab infusions, with markedly elevated alkaline phosphatase and bilirubin. One patient who underwent liver biopsy demonstrated bland cholestasis. Cholestatic liver injury resolved in most cases within 6-8 wk with supportive therapy and withdrawal of infliximab[61,62]. In a severe case of acute cholestatic hepatitis after eight months of treatment with infliximab for rheumatoid arthritis, one patient progressed to liver failure requiring liver transplant. This patient's liver biopsy showed ductal proliferation with collapse and enucleation of the hepatocytes[63].

Apart from DILI, infliximab has also been shown to cause reactivation of hepatitis B, in patients with a positive surface antigen (HBsAg) and patients with anti-core antibodies (HBcAb) without HBsAg. In a systematic review of HBsAg-positive patients, 2/14 patients treated with infliximab experienced hepatitis B reactivation, characterized by jaundice with elevated liver enzymes and a positive HBV DNA[64]. The outcomes for patients with reactivation range from recovery with lamivudine treatment to fatal fulminant hepatitis[65]. Patients with HBsAg-positive or anti-HBc positive serology are considered to be at moderate risk (1%-10%) for reactivation (Table 1)[66]. Given the poor outcomes associated with hepatitis B and the availability of prophylactic antivirals, both the AGA and AASLD recommend screening for hepatitis B with HBsAg and anti-HBcAb prior to initiating therapy[66,67]. If a positive result is obtained, HBV DNA should be checked before initiating prophylaxis. Antiviral prophylaxis has been associated with an 87% relative risk reduction of reactivation and an 84% relative risk reduction of HBV-associated hepatitis flares. Tenofovir or entecavir is recommended for long-term antiviral prophylaxis given high resistance rates with lamivudine. In contrast to the experience with hepatitis B, hepatitis C has not been shown to be affected by infliximab usage, and screening for hepatitis C is not currently recommended.

Adalimumab (HUMIRA®) is a fully human monoclonal anti-TNF-α antibody, and was FDA-approved for use in CD in 2007 and UC in 2012. It has also been associated with DILI, though less seldom than infliximab. In a population-based study from Iceland, the absolute risk of DILI with adalimumab was reported to be 1/270 patients, compared to 1/120 infliximab users[68]. Idiosyncratic liver enzyme elevations can be seen with adalimumab, albeit at a lower incidence than infliximab. In a study of 1753 IBD patients treated with anti-TNF therapy, 33% of patients were initiated on adalimumab; however, only 6% of the 102 patients with elevated liver enzymes were adalimumab users (three times the number on infliximab)[56]. Resolution of mild liver enzyme elevations generally occurred despite continued use of adalimumab.

Two cases of drug-induced autoimmune hepatitis have been reported in patients being treated with adalimumab, including one patient with CD[69,70]. Liver enzyme elevations and autoantibodies were seen after 10-12 wk of treatment. Liver biopsies in both patients showed typical morphologic features of autoimmune hepatitis. Both patients recovered with cessation of the medication and initiation of corticosteroids. Interestingly, two cases of patients who developed autoimmune hepatitis with infliximab with subsequent treatment with adalimumab have also been described in the literature. In both cases, autoimmune hepatitis resolved after infliximab was discontinued, with no evidence of recurrence after adalimumab was initiated, suggesting that DILI from one anti-TNF-α treatment does not preclude therapy with another drug in the same class[71,72].

One case of severe cholestatic injury due to adalimumab has been reported[73]. A female patient with CD being treated with adalimumab developed severe jaundice after seven months. Bilirubin and alkaline phosphatase

Table 1 Risk of hepatitis B viral DNA associated with medication use and hepatitis B status

Risk group	Medication	Hepatitis B virus status
High risk (> 10%)	Corticosteroids > 4 wk (moderate-high dose)	HBsAg+/HBcAb+
Moderate risk (1%-10%)	TNF-α inhibitors	HBsAg+/HBcAb+ (1%-10%)
	Ustekinumab Vedolizumab Natalizumab	HBsAg-/HBcAb+ (1%)
	Corticosteroids > 4 wk	HBsAg+/HBcAb+ (1%-10%) (low dose) HBsAg-/HBcAb+ (1%) (moderate-high dose)
Low-risk (< 1%)	Azathioprine 6-mercaptopurine Methotrexate	HBsAg+/HBcAb+ HBsAg-/HBcAb+

Adapted from American Gastroenterological Association Institute Technical Review on Prevention and Treatment of Hepatitis B Virus Reactivation During Immunosuppressive Drug Therapy[66]. Corticosteroids (prednisone equivalent): Low dose: < 10 mg; Moderate dose: 10-20 mg; High dose: > 20 mg. HBsAg: Hepatitis B surface antigen; HBcAb: Anti-hepatitis B core antibody; TNF-α: Tumor necrosis factor-alpha.

were elevated, and liver biopsy demonstrated cannalicular cholestasis associated with large intracannicular bile thrombi throughout the parenchyma without other significant inflammation. Spontaneous recovery was seen 5 wk after discontinuation of adalimumab.

As with infliximab, adalimumab has been implicated in hepatitis B reactivation, including fatal hepatic failure in a patient with clinically resolved hepatitis B infection[74,75]. Consequently, hepatitis B screening and prophylaxis is recommended prior to starting patients on adalimumab. Certolizumab and golimumab are the remaining anti-TNF-α treatments which have been FDA-approved for use in CD and UC, respectively. Due to limited use, data on hepatoxicity is scarce, though the same recommendations for hepatitis B screening apply.

In addition to direct drug-related injury or injury by viral reactivation, there is evidence of an increased risk of malignancy affecting the liver in patients on TNF-α treatment. Nineteen cases of hepatosplenic T-cell lymphoma (HSTCL) have been associated with infliximab use[76]. This rare but aggressive malignancy is an extranodal form of non-Hodgkin's lymphoma that predominantly affects young males, and often has a poor prognosis. Presenting symptoms include hepatomegaly, splenomegaly, systemic B-type symptoms, elevated liver enzymes and signs of hepatitis. The exact causal relationship between infliximab and HSTCL is unclear, as patients were also treated with azathioprine, 6-mercaptopurine, or steroids. More long-term studies are needed to delineate the relationship between infliximab and HSTCL.

ANTI-INTEGRIN THERAPIES

The use of anti-integrin therapy for moderate to severe UC or CD that is refractory to anti-TNF agents or immunomodulators has increased with the approval of vedolizumab in May 2014. Integrins modulate the adhesion of leukocytes to endothelial cells and are responsible for a key step in the inflammatory cascade whereby activated leukocytes anchor to the endothelial wall prior to transmigration. Two anti-integrin therapies are currently approved to treat IBD, natalizumab and vedolizumab, while a third, ertolizumab, is currently in phase 3 testing.

Natalizumab (TYSABRI®) is a monoclonal antibody directed to the alpha-4 integrin. Initially used for multiple sclerosis, it was subsequently approved to induce and maintain clinical remission in patients with moderate to severe active CD in patients who failed conventional therapies and anti-TNF-α agents[77,78]. Its use has been significantly limited in CD due to morbidity and mortality in postmarketing cases of progressive multifocal leukencephalopathy that developed secondary to the JC virus. Initial trials examining natalizumab's efficacy in treating IBD did not mention hepatotoxicity[77,79-82]. Generally elevation of aminotransferases have occurred in approximately 5% of patients on natalizumab compared with 3%-4% of controls[83,84]. In the post marketing setting, clinically significant liver injury, including liver failure, has been reported with at least 59 cases of hepatic injury reported to the FDA's Adverse Event Reporting System. In a report of 6 cases of liver injury, there was a significant hepatocellular component with ALT's reaching 2212 IU/L in one patient. Serum bilirubins ranged from 4.5-16 g/dL and occurred as early as 6 d after treatment. In addition, there were varying degrees of autoantibodies that were positive[85]. The etiology of the liver injury that occurs is unclear but is likely immunologically mediated.

Vedolizumab (ENTYVIO®) and ertolizumab are newer anti-integrin therapies with less data relating to their hepatotoxic effects. Vedolizumab is a monoclonal antibody to the α4β7 integrin that modulates lymphocyte migration into the gut. In the phase 3 trials examining its efficacy, three patients developed elevated transaminases with or without bilirubin elevation after 2-5 doses of vedolizumab. One further case was reported in the open labeled trial. Some patients developed autoantibodies and were treated with systemic steroids[86-90]. Currently, screening for hepatitis B prior to treatment is not routinely recommended.

Ertolizumab is a monoclonal antibody that binds the β7 subunit of α4β7 and αEβ7 integrins. Phase 1 and phase 2 studies in UC have been performed while phase 3 studies are underway. No reported adverse events related to the liver have been reported[91,92].

USTEKINUMAB

Ustekinumab (STELARA®) is a human IgG_{1k} monoclonal antibody that targets interleukin (IL)-12 and IL-23 activity by blocking its receptors[93]. It was approved in 2009 for use in moderate to severe psoriasis and psoriatic arthritis. While it has demonstrated benefit in

patients with CD, it currently does not have FDA approval for this indication. In the two published trials evaluating its efficacy in CD, there were two hepatobiliary disorders reported as biliary colic and cirrhosis, which did not differ significantly in number from the control group[94,95]. Elevated liver enzymes were a relatively rare occurrence, occurring in up to 1.4% of patients through 64 wk of follow up in one study. In patients treated for up to 5 years with ustekinumab for moderate-severe psoriasis, hepatobiliary disorders were rare, representing only 0.2 events per 100 patient-years for up to 5 years of follow up[96-98].

IL-12 plays an important role in controlling hepatitis B viral infections through its facilitation of type 1 T helper lymphocytes and by inhibiting HBV replication through interferon-gamma production[99-101]. Given its mechanism of action, reactivation rates between 1%-10% would be expected (Table 1)[66]. There is therefore a theoretical risk of reactivation of hepatitis B with the use of ustekinumab. One retrospective study in patients with psoriasis reported 11 patients who where hepatitis B surface antigen positive and surface antibody negative who were started on ustekinumab. Out of the 7 patients in whom prophylaxis was not given, two experienced reactivation. Of the 4 patients in whom prophylaxis was initiated, no cases of reactivation were observed[101]. Assuming a 5% rate of reactivation of hepatitis B in patients exposed to ustekinamab, prophylaxis would result in 44 fewer reactivation events per 1000 patients treated and should be considered[66].

CYCLOSPORINE

Cyclosporine prevents the activation of T-lymphocytes by inhibiting the production and release of IL-Ⅱ. It is used to induce remission in refractory UC and serve as a bridge to medications such as AZA or 6-MP for maintenance therapy. Traditionally given as an intravenous infusion at a rate of 4 mg/kg, the lower 2 mg/kg dose has been shown to be equally effective with fewer reported side effects[102-104]. Over 80% of steroid refractory patients will respond to therapy and in many cases, cyclosporine provides an alternative to colectomy[105]. While side effects such as nephrotoxicity, hypertension and infections are more prevalent, hepatotoxicity is rare.

Animal studies evaluating its effects on the liver reveal increased bile acid and bilirubin concentrations when given at high doses, with a corresponding reduction of the biliary secretion of bile acids, cholesterol and phospholipids. Cholestasis was secondary to a decrease in bile acid dependent and independent bile flow fractions[106,107]. Case series that evaluated dosages ranging from 2-10 mg/kg per day found mild abnormalities in liver tests consistent with mild increases in alkaline phosphatase levels as well as slight increases in bilirubin and aminotransferases[108]. In addition, cyclosporine has been reported to increase the incidence of cholelithiasis[109]. Earlier studies evaluating cyclosporine use in post-renal transplant patients reported evidence of hepatotoxicity in 20%-82% of patients, characterized by transaminase elevations as well as elevations in bilirubin and alkaline phosphatase, which were dose responsive[110-112]. In IBD patients, the rate of liver tests abnormalities is closer to 1%-4% of patients, with a recent systematic review of the literature reporting only 11 cases of elevated liver tests in IBD patients treated with cyclosporine[113,114].

ANTIBIOTICS

Though their benefits are not well defined in prospective randomized trials, antimicrobials have long been used as either adjunctive therapy for IBD complications such as CD related abscesses, or as the primary therapy in pouchitis, an IBD like condition occurring in surgically constructed continent jejunal or "J-pouch" reservoirs following total colectomy for UC. Since current IBD models suggest that even normal bowel bacteria may play a role in initiating and propagating the aberrant immune response typical of IBD, the appeal of antibiotics is straight forward; that decreasing bacterial concentrations may lessen this immune stimulation and thus decrease the inflammatory response. Though there is some evidence of benefit to this approach in fistulizing peri-anal and colonic CD, a clearer benefit in UC is lacking. Currently, antibiotic therapy for UC is only recommended for end-stage cases of toxic megacolon, when bacteremia is common.

The two most commonly used antibiotics are metronidazole and ciprofloxacin, either individually or in combination. Metronidazole is a synthetic nitroimidazole derivative used to treat both anaerobic bacteria and protozoa, generally dosed orally at 10 to 20 mg/kg per day. Ornidazole, another nitroimidazole derivative not currently available in the United States, has also been studied for the treatment of CD. Though metronidazole is often given intravenously when utilized for inpatients with CD related abscesses, most clinical experience in CD is with oral use. While dose adjustment is recommended in patients with severe liver impairment, primary hepatotoxicity is extremely rare. Cases of DILI have been reported, typically presenting with a hepatocelluar injury pattern several weeks after the initiation of therapy[115,116]. Small prospective trials of metronidazole and ornidazole in CD have reported elevations of liver enzymes, that did not significantly differ from the placebo group[117,118].

Ciprofloxacin is a member of the fluoroquinolone class of antibiotics whose mechanism involves inhibition of DNA gyrase within sensitive bacteria, preventing the relaxation of supercoiled DNA and subsequent breakage of double stranded DNA. Minor elevations of serum AST and ALT related to ciprofloxacin use is quite common, affecting approximately 1% of users. A recent large study of a Veterans Administration population of 7862 patients with fluoroquinolone exposure matched against 45512 controls, each group excluding underlying liver disease, showed an increased risk of hepatotoxicity in exposed compared to unexposed patients, odds ratio

Table 2 Summary of medication side effects and management options

Medication	Prevalence of liver injury	Manifestation	Management[1]
Sulfasalazine	0.4%-2.9%	Mild liver test abnormalities	
		Severe systemic hypersensitivity	
		Granulomatous hepatitis	
		DRESS syndrome	
5-ASA	Approximately 2.6%	Mild liver test abnormalities	
		Drug induced autoimmune hepatitis	
		Systemic hypersensitivity reaction	
AZA/6-MP	1.4%-7.1%	Hypersensitivity reaction	Slight liver test elevation →
		Cholestasis	Monitor
		Peliosis hepatitis	
		Disse space fibrosis	Persistent elevation →
		Veno-occlussive disease	Consider stopping medication/reducing dose
		Nodular regenerative hyperplasia	Marked elevation →
		Hepatosplenic T-cell lymphoma	Stop medication
Methotrexate	Approximately 10.2%	Elevated liver tests	
		Fibrosis	
Anti-TNF-α agents	0.37%-6.7%	Hepatocellular injury	
		Cholestasis	
		Hepatosplenic T-cell lymphoma	
		Autoimmune hepatitis	
Anti-integrin therapy	< 5%	Hepatocellular injury	
		Cholestasis	
		Autoimmune hepatitis	
		Liver failure	
Ustekinumab	1.4%	Elevated liver tests	
Cyclosporine	1%-4%	Increased bile acids	
		Cholestasis	
		Hepatocellular injury	

[1]For general management of elevated liver tests. For specific recommendations see text. ASA: Aminosalicylic acid; AZA: Azathioprine; 6-MP: 6-Mercaptopurine; TNF: Tumor necrosis factor; DRESS: Drug reaction with eosinophilia and systemic symptoms.

(OR) = 1.20; 95%CI: 1.04-1.38[119]. Much rarer cases of severe hepatocellular, cholestatic or a mixed pattern of injury have been reported with fluoroquinolones, which are among the group of antibiotics most commonly associated with this complication. Clinical trials of ciprofloxacin for the treatment of CD have not shown any significant rates of liver injury, but given the small size of these studies it is not possible to determine whether there is any disease specific associated liver risk[120,121].

Finally, though CD is not widely considered to be caused by a specific infection, there is a possible association of CD with the organism Mycobacterium avium paratuberculosis (MAP), which has been found in the serum and tissue of some CD patients. Though there has been modest success with antimicrobial treatment regimens directed at MAP, it is unclear whether this success is related to its effect on MAP or a general alteration of the bowel flora. While isoniazid (INH) has an anti-mycobacterial effect, and has been used as part of MAP directed therapy, the common usage of INH in individuals with IBD is not as a direct therapy, but as a co-therapy to prevent reactivation of mycobacterium tuberculosis (TB) in individuals with suspected latent infection treated with anti-TNF-α therapy[122,123]. In the United States population the risk of latent TB has been estimated at 4%, with significant variations depending upon the region and socioeconomic status[124]. Current prescribing guidelines recommend testing for latent TB prior to initiation of biologic therapy with anti-TNF-α agents[125]. In patients at risk for latent TB, at least one month of INH therapy is suggested prior to the initiation of biologic therapy[126].

INH inhibits the synthesis of mycolic acid, an essential component of the bacterial cell wall, and is commonly associated with DILI. Mild elevations of serum transaminases occur in up to 20% of INH treated patients, and usually requires close monitoring without discontinuation of therapy. Traditionally more severe, and potentially fatal INH hepatitis has been directly associated with increasing patient age, ranging from a rate of 0.3% in individuals 20 to 34 years of age, and as high as 4.6% in those over age 65 years manifesting as an acute hepatocellular pattern. Though, more recent studies have suggested that rates may be as low as 0.1%[127,128]. Fatality rates once hepatitis develops have been reported as high as 10%, and are worse with increasing age, though overall fatality rates in those treated and monitored following current American Thoracic Society guidelines appear to be very low[129,130]. Current guidelines recommend routine monitoring of patients treated with INH prophylaxis who are at higher risk of underlying liver disease such as those with chronic viral hepatitis, recent pregnancy, and regular alcohol consumption. However, monitoring may be considered for anyone greater than 35 years of age. Suggested intervals of AST, ALT, and bilirubin testing are at baseline, then as often as every 4 wk while on

therapy. Discontinuation of therapy should be considered for an ALT > 5 × the upper limit of normal, or > 3 × the upper limit of normal with associated nausea, vomiting, abdominal pain, jaundice, or unexplained fatigue[131].

CONCLUSION

While both patients and physicians alike may look forward to a true "cure" for illness, the management of chronic medical conditions typically requires the use of medications, often for an indefinite length of time. This is certainly true for the management of IBD. The fact that IBD is itself an immune related disease, requiring immune suppressing or modifying therapies, for long periods of time, further increases the side effect potential of these treatments. IBD medications are prominent on the list of well described agents causing DILI, as well as other hepatobiliary manifestations, each with their own prevalence and management strategy (Table 2)[25]. While the data on newer therapies appears encouraging with regard to DILI specifically, further post marketing experience will be needed to determine the exact nature of this risk and appropriate prophylactic and management strategies.

REFERENCES

1 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

2 **Mendes FD**, Levy C, Enders FB, Loftus EV, Angulo P, Lindor KD. Abnormal hepatic biochemistries in patients with inflammatory bowel disease. *Am J Gastroenterol* 2007; **102**: 344-350 [PMID: 17100965 DOI: 10.1111/j.1572-0241.2006.00947.x]

3 **Svartz N**. Salazyoprin, a new sulfanilamide preparation: A. Therapeutic results in rheumatic polyarthritis. B. Therapeutic results in ulcerative colitis. C. Toxic manifestations in treatment with sulfanilamide preparation. *Acta Med Scand* 1942; **110**: 557-590

4 **Kaiser GC**, Yan F, Polk DB. Mesalamine blocks tumor necrosis factor growth inhibition and nuclear factor kappaB activation in mouse colonocytes. *Gastroenterology* 1999; **116**: 602-609 [PMID: 10029619 DOI: 10.1016/S0016-5085(99)70182-4]

5 **Verspaget HW**, Aparicio-Pagés MN, Verver S, Edelbroek PM, Hafkenscheid JC, Crama-Bohbouth GE, Peña AS, Weterman IT, Lamers CB. Influence of sulphasalazine and mesalazine on cellular and biochemical oxygen metabolite production. Effect of in vivo administration and an in vitro analysis. *Scand J Gastroenterol* 1991; **26**: 779-786 [PMID: 1680247 DOI: 10.3109/00365529108998599]

6 **Bantel H**, Berg C, Vieth M, Stolte M, Kruis W, Schulze-Osthoff K. Mesalazine inhibits activation of transcription factor NF-kappaB in inflamed mucosa of patients with ulcerative colitis. *Am J Gastroenterol* 2000; **95**: 3452-3457 [PMID: 11151876 DOI: 10.1111/j.1572-0241.2000.03360.x]

7 **Amos RS**, Pullar T, Bax DE, Situnayake D, Capell HA, McConkey B. Sulphasalazine for rheumatoid arthritis: toxicity in 774 patients monitored for one to 11 years. *Br Med J* (Clin Res Ed) 1986; **293**: 420-423 [PMID: 2874863 DOI: 10.1136/bmj.293.6544.420]

8 **Rachmilewitz D**. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. *BMJ* 1989; **298**: 82-86 [PMID: 2563951 DOI: 10.1136/bmj.298.6666.82]

9 **Boyer DL**, Li BU, Fyda JN, Friedman RA. Sulfasalazine-induced hepatotoxicity in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1989; **8**: 528-532 [PMID: 2566669 DOI: 10.1097/00005176-198905000-00018]

10 **Rafoth RJ**. Systemic granulomatous reaction to salicylazosulfapyridine (Azulfidine) in a patient with Crohn's disease. *Am J Dig Dis* 1974; **19**: 465-469 [PMID: 4151115 DOI: 10.1007/BF01255610]

11 **Namias A**, Bhalotra R, Donowitz M. Reversible sulfasalazine-induced granulomatous hepatitis. *J Clin Gastroenterol* 1981; **3**: 193-198 [PMID: 6113258 DOI: 10.1097/00004836-198106000-00017]

12 **Callen JP**, Soderstrom RM. Granulomatous hepatitis associated with salicylazosulfapyridine therapy. *South Med J* 1978; **71**: 1159-1160 [PMID: 28568 DOI: 10.1097/00007611-197809000-00031]

13 **Haines JD**. Hepatotoxicity after treatment with sulfasalazine. *Postgrad Med* 1986; **79**: 193-194, 197-198 [PMID: 2871549]

14 **Marinos G**, Riley J, Painter DM, McCaughan GW. Sulfasalazine-induced fulminant hepatic failure. *J Clin Gastroenterol* 1992; **14**: 132-135 [PMID: 1348259 DOI: 10.1097/00004836-199203000-00012]

15 **Ribe J**, Benkov KJ, Thung SN, Shen SC, LeLeiko NS. Fatal massive hepatic necrosis: a probable hypersensitivity reaction to sulfasalazine. *Am J Gastroenterol* 1986; **81**: 205-208 [PMID: 2869683]

16 **Bocquet H**, Bagot M, Roujeau JC. Drug-induced pseudolymphoma and drug hypersensitivity syndrome (Drug Rash with Eosinophilia and Systemic Symptoms: DRESS). *Semin Cutan Med Surg* 1996; **15**: 250-257 [PMID: 9069593 DOI: 10.1016/S1085-5629(96)80038-1]

17 **Aquino RT**, Vergueiro CS, Magliari ME, de Freitas TH. Sulfasalazine-induced DRESS syndrome (Drug Rash with Eosinophilia and Systemic Symptoms). *Sao Paulo Med J* 2008; **126**: 225-226 [PMID: 18853032 DOI: 10.1590/S1516-31802008000400006]

18 **Michel F**, Navellou JC, Ferraud D, Toussirot E, Wendling D. DRESS syndrome in a patient on sulfasalazine for rheumatoid arthritis. *Joint Bone Spine* 2005; **72**: 82-85 [PMID: 15681256 DOI: 10.1016/j.jbspin.2004.06.002]

19 **Piñana E**, Lei SH, Merino R, Melgosa M, De La Vega R, Gonzales-Obeso E, Ramírez E, Borobia A, Carcas A. DRESS-syndrome on sulfasalazine and naproxen treatment for juvenile idiopathic arthritis and reactivation of human herpevirus 6 in an 11-year-old Caucasian boy. *J Clin Pharm Ther* 2010; **35**: 365-370 [PMID: 20831538 DOI: 10.1111/j.1365-2710.2009.01081.x]

20 **Roujeau JC**. Clinical heterogeneity of drug hypersensitivity. *Toxicology* 2005; **209**: 123-129 [PMID: 15767024 DOI: 10.1016/j.tox.2004.12.022]

21 **Loftus EV**, Kane SV, Bjorkman D. Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 2004; **19**: 179-189 [PMID: 14723609 DOI: 10.1111/j.0269-2813.2004.01827.x]

22 **Deltenre P**, Berson A, Marcellin P, Degott C, Biour M, Pessayre D. Mesalazine (5-aminosalicylic acid) induced chronic hepatitis. *Gut* 1999; **44**: 886-888 [PMID: 10323894 DOI: 10.1136/gut.44.6.886]

23 **Hautekeete ML**, Bourgeois N, Potvin P, Duville L, Reynaert H, Devis G, Adler M, Klöppel G. Hypersensitivity with hepatotoxicity to mesalazine after hypersensitivity to sulfasalazine. *Gastroenterology* 1992; **103**: 1925-1927 [PMID: 1360436]

24 **Stoschus B**, Meybehm M, Spengler U, Scheurlen C, Sauerbruch T. Cholestasis associated with mesalazine therapy in a patient with Crohn's disease. *J Hepatol* 1997; **26**: 425-428 [PMID: 9059966 DOI: 10.1016/S0168-8278(97)80061-X]

25 **Chalasani NP**, Hayashi PH, Bonkovsky HL, Navarro VJ, Lee WM, Fontana RJ. ACG Clinical Guideline: the diagnosis and management of idiosyncratic drug-induced liver injury. *Am J Gastroenterol* 2014; **109**: 950-966; quiz 967 [PMID: 24935270 DOI: 10.1038/ajg.2014.131]

26 **Lichtenstein GR**, Hanauer SB, Sandborn WJ. Management of Crohn's disease in adults. *Am J Gastroenterol* 2009; **104**: 465-483;

quiz 464, 484 [PMID: 19174807 DOI: 10.1038/ajg.2008.168]
27 **Osterman MT**, Kundu R, Lichtenstein GR, Lewis JD. Association of 6-thioguanine nucleotide levels and inflammatory bowel disease activity: a meta-analysis. *Gastroenterology* 2006; **130**: 1047-1053 [PMID: 16618398 DOI: 10.1053/j.gastro.2006.01.046]
28 **Chaparro M**, Ordás I, Cabré E, Garcia-Sanchez V, Bastida G, Peñalva M, Gomollón F, García-Planella E, Merino O, Gutiérrez A, Esteve M, Márquez L, Garcia-Sepulcre M, Hinojosa J, Vera I, Muñoz F, Mendoza JL, Cabriada JL, Montoro MA, Barreiro-de Acosta M, Ceña G, Saro C, Aldeguer X, Barrio J, Maté J, Gisbert JP. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. *Inflamm Bowel Dis* 2013; **19**: 1404-1410 [PMID: 23665964 DOI: 10.1097/MIB.0b013e318281f28f]
29 **Gisbert JP**, Luna M, González-Lama Y, Pousa ID, Velasco M, Moreno-Otero R, Maté J. Liver injury in inflammatory bowel disease: long-term follow-up study of 786 patients. *Inflamm Bowel Dis* 2007; **13**: 1106-1114 [PMID: 17455203]
30 **Gisbert JP**, González-Lama Y, Maté J. Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review. *Am J Gastroenterol* 2007; **102**: 1518-1527 [PMID: 17391318 DOI: 10.1111/j.1572-0241.2007.01187.x]
31 **Romagnuolo J**, Sadowski DC, Lalor E, Jewell L, Thomson AB. Cholestatic hepatocellular injury with azathioprine: a case report and review of the mechanisms of hepatotoxicity. *Can J Gastroenterol* 1998; **12**: 479-483 [PMID: 9812167]
32 **Haboubi NY**, Ali HH, Whitwell HL, Ackrill P. Role of endothelial cell injury in the spectrum of azathioprine-induced liver disease after renal transplant: light microscopy and ultrastructural observations. *Am J Gastroenterol* 1988; **83**: 256-261 [PMID: 3278593]
33 **Weinshilboum RM**, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; **32**: 651-662 [PMID: 7191632]
34 **de Boer NK**, Mulder CJ, van Bodegraven AA. Nodular regenerative hyperplasia and thiopurines: the case for level-dependent toxicity. *Liver Transpl* 2005; **11**: 1300-1301 [PMID: 16184561 DOI: 10.1002/lt.20554]
35 **Schaeffeler E**, Fischer C, Brockmeier D, Wernet D, Moerike K, Eichelbaum M, Zanger UM, Schwab M. Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. *Pharmacogenetics* 2004; **14**: 407-417 [PMID: 15226673 DOI: 10.1097/01.fpc.0000114745.08559.db]
36 **Bastida G**, Nos P, Aguas M, Beltrán B, Rubín A, Dasí F, Ponce J. Incidence, risk factors and clinical course of thiopurine-induced liver injury in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2005; **22**: 775-782 [PMID: 16225485 DOI: 10.1111/j.1365-2036.2005.02636.x]
37 **Feagan BG**, Fedorak RN, Irvine EJ, Wild G, Sutherland L, Steinhart AH, Greenberg GR, Koval J, Wong CJ, Hopkins M, Hanauer SB, McDonald JW. A comparison of methotrexate with placebo for the maintenance of remission in Crohn's disease. North American Crohn's Study Group Investigators. *N Engl J Med* 2000; **342**: 1627-1632 [PMID: 10833208 DOI: 10.1056/NEJM200006013422202]
38 **Terdiman JP**, Gruss CB, Heidelbaugh JJ, Sultan S, Falck-Ytter YT. American Gastroenterological Association Institute guideline on the use of thiopurines, methotrexate, and anti-TNF-α biologic drugs for the induction and maintenance of remission in inflammatory Crohn's disease. *Gastroenterology* 2013; **145**: 1459-1463 [PMID: 24267474 DOI: 10.1053/j.gastro.2013.10.047]
39 **Prey S**, Paul C. Effect of folic or folinic acid supplementation on methotrexate-associated safety and efficacy in inflammatory disease: a systematic review. *Br J Dermatol* 2009; **160**: 622-628 [PMID: 18945303 DOI: 10.1111/j.1365-2133.2008.08876.x]
40 **van Ede AE**, Laan RF, Rood MJ, Huizinga TW, van de Laar MA, van Denderen CJ, Westgeest TA, Romme TC, de Rooij DJ, Jacobs MJ, de Boo TM, van der Wilt GJ, Severens JL, Hartman M, Krabbe PF, Dijkmans BA, Breedveld FC, van de Putte LB. Effect of folic or folinic acid supplementation on the toxicity and efficacy of methotrexate in rheumatoid arthritis: a forty-eight week, multicenter, randomized, double-blind, placebo-controlled study. *Arthritis Rheum* 2001; **44**: 1515-1524 [PMID: 11465701]
41 **Curtis JR**, Beukelman T, Onofrei A, Cassell S, Greenberg JD, Kavanaugh A, Reed G, Strand V, Kremer JM. Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. *Ann Rheum Dis* 2010; **69**: 43-47 [PMID: 19147616 DOI: 10.1136/ard.2008.101378]
42 **Fournier MR**, Klein J, Minuk GY, Bernstein CN. Changes in liver biochemistry during methotrexate use for inflammatory bowel disease. *Am J Gastroenterol* 2010; **105**: 1620-1626 [PMID: 20160715 DOI: 10.1038/ajg.2010.21]
43 **Valentino PL**, Church PC, Shah PS, Beyene J, Griffiths AM, Feldman BM, Kamath BM. Hepatotoxicity caused by methotrexate therapy in children with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2014; **20**: 47-59 [PMID: 24280876 DOI: 10.1097/01.MIB.0000436953.88522.3e]
44 **Kalb RE**, Strober B, Weinstein G, Lebwohl M. Methotrexate and psoriasis: 2009 National Psoriasis Foundation Consensus Conference. *J Am Acad Dermatol* 2009; **60**: 824-837 [PMID: 19389524 DOI: 10.1016/j.jaad.2008.11.906]
45 **Themido R**, Loureiro M, Pecegueiro M, Brandão M, Campos MC. Methotrexate hepatotoxicity in psoriatic patients submitted to long-term therapy. *Acta Derm Venereol* 1992; **72**: 361-364 [PMID: 1361285]
46 **Bath RK**, Brar NK, Forouhar FA, Wu GY. A review of methotrexate-associated hepatotoxicity. *J Dig Dis* 2014; **15**: 517-524 [PMID: 25139707 DOI: 10.1111/1751-2980.12184]
47 **MacDonald A**, Burden AD. Noninvasive monitoring for methotrexate hepatotoxicity. *Br J Dermatol* 2005; **152**: 405-408 [PMID: 15787807 DOI: 10.1111/j.1365-2133.2005.06605.x]
48 **Whiting-O'Keefe QE**, Fye KH, Sack KD. Methotrexate and histologic hepatic abnormalities: a meta-analysis. *Am J Med* 1991; **90**: 711-716 [PMID: 1828327 DOI: 10.1016/S0002-9343(05)80060-9]
49 **Saag KG**, Teng GG, Patkar NM, Anuntiyo J, Finney C, Curtis JR, Paulus HE, Mudano A, Pisu M, Elkins-Melton M, Outman R, Allison JJ, Suarez Almazor M, Bridges SL, Chatham WW, Hochberg M, MacLean C, Mikuls T, Moreland LW, O'Dell J, Turkiewicz AM, Furst DE. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. *Arthritis Rheum* 2008; **59**: 762-784 [PMID: 18512708 DOI: 10.1002/art.23721]
50 **Kremer JM**, Alarcón GS, Lightfoot RW, Willkens RF, Furst DE, Williams HJ, Dent PB, Weinblatt ME. Methotrexate for rheumatoid arthritis. Suggested guidelines for monitoring liver toxicity. American College of Rheumatology. *Arthritis Rheum* 1994; **37**: 316-328 [PMID: 8129787 DOI: 10.1002/art.1780370304]
51 **Van Deventer SJ**. Tumour necrosis factor and Crohn's disease. *Gut* 1997; **40**: 443-448 [PMID: 9176068 DOI: 10.1136/gut.40.4.443]
52 **van Hogezand RA**, Verspaget HW. The future role of anti-tumour necrosis factor-alpha products in the treatment of Crohn's disease. *Drugs* 1998; **56**: 299-305 [PMID: 9777308 DOI: 10.2165/00003495-199856030-00001]
53 **Cheifetz A**, Smedley M, Martin S, Reiter M, Leone G, Mayer L, Plevy S. The incidence and management of infusion reactions to infliximab: a large center experience. *Am J Gastroenterol* 2003; **98**: 1315-1324 [PMID: 12818276 DOI: 10.1111/j.1572-0241.2003.07457.x]
54 **Khanna R**, Feagan BG. Safety of infliximab for the treatment of inflammatory bowel disease: current understanding of the potential for serious adverse events. *Expert Opin Drug Saf* 2015; **14**: 987-997 [PMID: 25819509 DOI: 10.1517/14740338.2015.1029915]
55 **Sokolove J**, Strand V, Greenberg JD, Curtis JR, Kavanaugh A, Kremer JM, Anofrei A, Reed G, Calabrese L, Hooper M, Baumgartner S, Furst DE. Risk of elevated liver enzymes associated

with TNF inhibitor utilisation in patients with rheumatoid arthritis. *Ann Rheum Dis* 2010; **69**: 1612-1617 [PMID: 20448284 DOI: 10.1136/ard.2009.112136]

56 **Shelton E**, Chaudrey K, Sauk J, Khalili H, Masia R, Nguyen DD, Yajnik V, Ananthakrishnan AN. New onset idiosyncratic liver enzyme elevations with biological therapy in inflammatory bowel disease. *Aliment Pharmacol Ther* 2015; **41**: 972-979 [PMID: 25756190 DOI: 10.1111/apt.13159]

57 **Weiler-Normann C**, Schramm C, Quaas A, Wiegard C, Glaubke C, Pannicke N, Möller S, Lohse AW. Infliximab as a rescue treatment in difficult-to-treat autoimmune hepatitis. *J Hepatol* 2013; **58**: 529-534 [PMID: 23178709 DOI: 10.1016/j.jhep.2012.11.010]

58 **Fujii K**, Rokutanda R, Osugi Y, Koyama Y, Ota T. Adult-onset Still's disease complicated by autoimmune hepatitis: successful treatment with infliximab. *Intern Med* 2012; **51**: 1125-1128 [PMID: 22576401 DOI: 10.2169/internalmedicine.51.6824]

59 **Rajanayagam J**, Lewindon PJ. Infliximab as rescue therapy in paediatric autoimmune hepatitis. *J Hepatol* 2013; **59**: 908-909 [PMID: 23792030 DOI: 10.1016/j.jhep.2013.05.046]

60 **Ghabril M**, Bonkovsky HL, Kum C, Davern T, Hayashi PH, Kleiner DE, Serrano J, Rochon J, Fontana RJ, Bonacini M. Liver injury from tumor necrosis factor-α antagonists: analysis of thirty-four cases. *Clin Gastroenterol Hepatol* 2013; **11**: 558-564.e3 [PMID: 23333219 DOI: 10.1016/j.cgh.2012.12.025]

61 **Menghini VV**, Arora AS. Infliximab-associated reversible cholestatic liver disease. *Mayo Clin Proc* 2001; **76**: 84-86 [PMID: 11155419 DOI: 10.4065/76.1.84]

62 **Ierardi E**, Valle ND, Nacchiero MC, De Francesco V, Stoppino G, Panella C. Onset of liver damage after a single administration of infliximab in a patient with refractory ulcerative colitis. *Clin Drug Investig* 2006; **26**: 673-676 [PMID: 17163303 DOI: 10.2165/00044011-200626110-00008]

63 **Tobon GJ**, Cañas C, Jaller JJ, Restrepo JC, Anaya JM. Serious liver disease induced by infliximab. *Clin Rheumatol* 2007; **26**: 578-581 [PMID: 16547695 DOI: 10.1007/s10067-005-0169-y]

64 **Lee YH**, Bae SC, Song GG. Hepatitis B virus reactivation in HBsAg-positive patients with rheumatic diseases undergoing anti-tumor necrosis factor therapy or DMARDs. *Int J Rheum Dis* 2013; **16**: 527-531 [PMID: 24164839 DOI: 10.1111/1756-185X.12154]

65 **Manzano-Alonso ML**, Castellano-Tortajada G. Reactivation of hepatitis B virus infection after cytotoxic chemotherapy or immunosuppressive therapy. *World J Gastroenterol* 2011; **17**: 1531-1537 [PMID: 21472116 DOI: 10.3748/wjg.v17.i12.1531]

66 **Reddy KR**, Beavers KL, Hammond SP, Lim JK, Falck-Ytter YT. American Gastroenterological Association Institute guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. *Gastroenterology* 2015; **148**: 215-219; quiz e16-17 [PMID: 25447850 DOI: 10.1053/j.gastro.2014.10.039]

67 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]

68 **Björnsson ES**, Gunnarsson BI, Gröndal G, Jonasson JG, Einarsdottir R, Ludviksson BR, Gudbjörnsson B, Olafsson S. Risk of drug-induced liver injury from tumor necrosis factor antagonists. *Clin Gastroenterol Hepatol* 2015; **13**: 602-608 [PMID: 25131534 DOI: 10.1016/j.cgh.2014.07.062]

69 **Adar T**, Mizrahi M, Pappo O, Scheiman-Elazary A, Shibolet O. Adalimumab-induced autoimmune hepatitis. *J Clin Gastroenterol* 2010; **44**: e20-e22 [PMID: 19593165 DOI: 10.1097/MCG.0b013e3181a745e7]

70 **Grasland A**, Sterpu R, Boussoukaya S, Mahe I. Autoimmune hepatitis induced by adalimumab with successful switch to abatacept. *Eur J Clin Pharmacol* 2012; **68**: 895-898 [PMID: 22205272 DOI: 10.1007/s00228-011-1191-4]

71 **Cravo M**, Silva R, Serrano M. Autoimmune hepatitis induced by infliximab in a patient with Crohn's disease with no relapse after switching to adalimumab. *BioDrugs* 2010; **24** Suppl 1: 25-27 [PMID: 21175232 DOI: 10.2165/11586210-000000000-00000]

72 **Goldfeld DA**, Verna EC, Lefkowitch J, Swaminath A. Infliximab-induced autoimmune hepatitis with successful switch to adalimumab in a patient with Crohn's disease: the index case. *Dig Dis Sci* 2011; **56**: 3386-3388 [PMID: 21597977 DOI: 10.1007/s10620-011-1748-1]

73 **Kim E**, Bressler B, Schaeffer DF, Yoshida EM. Severe cholestasis due to adalimumab in a Crohn's disease patient. *World J Hepatol* 2013; **5**: 592-595 [PMID: 24179620 DOI: 10.4254/wjh.v5.i10.592]

74 **Matsumoto T**, Marusawa H, Dogaki M, Suginoshita Y, Inokuma T. Adalimumab-induced lethal hepatitis B virus reactivation in an HBsAg-negative patient with clinically resolved hepatitis B virus infection. *Liver Int* 2010; **30**: 1241-1242 [PMID: 20345703 DOI: 10.1111/j.1478-3231.2010.02238.x]

75 **Verhelst X**, Orlent H, Colle I, Geerts A, De Vos M, Van Vlierberghe H. Subfulminant hepatitis B during treatment with adalimumab in a patient with rheumatoid arthritis and chronic hepatitis B. *Eur J Gastroenterol Hepatol* 2010; **22**: 494-499 [PMID: 20306568 DOI: 10.1097/MEG.0b013e3283329d13]

76 **Thai A**, Prindiville T. Hepatosplenic T-cell lymphoma and inflammatory bowel disease. *J Crohns Colitis* 2010; **4**: 511-522 [PMID: 21122554 DOI: 10.1016/j.crohns.2010.05.006]

77 **Ghosh S**, Goldin E, Gordon FH, Malchow HA, Rask-Madsen J, Rutgeerts P, Vyhnálek P, Zádorová Z, Palmer T, Donoghue S. Natalizumab for active Crohn's disease. *N Engl J Med* 2003; **348**: 24-32 [PMID: 12510039 DOI: 10.1056/NEJMoa020732]

78 **Sandborn WJ**, Colombel JF, Enns R, Feagan BG, Hanauer SB, Lawrance IC, Panaccione R, Sanders M, Schreiber S, Targan S, van Deventer S, Goldblum R, Despain D, Hogge GS, Rutgeerts P. Natalizumab induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2005; **353**: 1912-1925 [PMID: 16267322 DOI: 10.1056/NEJMoa043335]

79 **Gordon FH**, Lai CW, Hamilton MI, Allison MC, Srivastava ED, Fouweather MG, Donoghue S, Greenlees C, Subhani J, Amlot PL, Pounder RE. A randomized placebo-controlled trial of a humanized monoclonal antibody to alpha4 integrin in active Crohn's disease. *Gastroenterology* 2001; **121**: 268-274 [PMID: 11487536 DOI: 10.1053/gast.2001.26260]

80 **Miller DH**, Khan OA, Sheremata WA, Blumhardt LD, Rice GP, Libonati MA, Willmer-Hulme AJ, Dalton CM, Miszkiel KA, O'Connor PW. A controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2003; **348**: 15-23 [PMID: 12510038 DOI: 10.1056/NEJMoa020696]

81 **Sands BE**, Kozarek R, Spainhour J, Barish CF, Becker S, Goldberg L, Katz S, Goldblum R, Harrigan R, Hilton D, Hanauer SB. Safety and tolerability of concurrent natalizumab treatment for patients with Crohn's disease not in remission while receiving infliximab. *Inflamm Bowel Dis* 2007; **13**: 2-11 [PMID: 17206633 DOI: 10.1002/ibd.20014]

82 **Targan SR**, Feagan BG, Fedorak RN, Lashner BA, Panaccione R, Present DH, Spehlmann ME, Rutgeerts PJ, Tulassay Z, Volfova M, Wolf DC, Hernandez C, Bornstein J, Sandborn WJ. Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial. *Gastroenterology* 2007; **132**: 1672-1683 [PMID: 17484865 DOI: 10.1053/j.gastro.2007.03.024]

83 **Keeley KA**, Rivey MP, Allington DR. Natalizumab for the treatment of multiple sclerosis and Crohn's disease. *Ann Pharmacother* 2005; **39**: 1833-1843 [PMID: 16219898 DOI: 10.1345/aph.1G134]

84 **Polman CH**, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, Panzara MA, Sandrock AW. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006; **354**: 899-910 [PMID: 16510744 DOI: 10.1056/NEJMoa044397]

85 **Bezabeh S**, Flowers CM, Kortepeter C, Avigan M. Clinically significant liver injury in patients treated with natalizumab. *Aliment Pharmacol Ther* 2010; **31**: 1028-1035 [PMID: 20163378]

86 **Feagan BG**, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, Van Assche G, Axler J, Kim HJ, Danese S, Fox I, Milch C, Sankoh S, Wyant T, Xu J, Parikh A. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2013; **369**: 699-710 [PMID: 23964932 DOI: 10.1056/NEJMoa1215734]

87 **Sandborn WJ**, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, Lukas M, Fedorak RN, Lee S, Bressler B, Fox I, Rosario M, Sankoh S, Xu J, Stephens K, Milch C, Parikh A. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2013; **369**: 711-721 [PMID: 23964933 DOI: 10.1056/NEJMoa1215739]

88 **Sands BE**, Feagan BG, Rutgeerts P, Colombel JF, Sandborn WJ, Sy R, D'Haens G, Ben-Horin S, Xu J, Rosario M, Fox I, Parikh A, Milch C, Hanauer S. Effects of vedolizumab induction therapy for patients with Crohn's disease in whom tumor necrosis factor antagonist treatment failed. *Gastroenterology* 2014; **147**: 618-627. e3 [PMID: 24859203 DOI: 10.1053/j.gastro.2014.05.008]

89 **Parikh A**, Leach T, Wyant T, Scholz C, Sankoh S, Mould DR, Ponich T, Fox I, Feagan BG. Vedolizumab for the treatment of active ulcerative colitis: a randomized controlled phase 2 dose-ranging study. *Inflamm Bowel Dis* 2012; **18**: 1470-1479 [PMID: 22147460 DOI: 10.1002/ibd.21896]

90 **Feagan BG**, Greenberg GR, Wild G, Fedorak RN, Paré P, McDonald JW, Cohen A, Bitton A, Baker J, Dubé R, Landau SB, Vandervoort MK, Parikh A. Treatment of active Crohn's disease with MLN0002, a humanized antibody to the alpha4beta7 integrin. *Clin Gastroenterol Hepatol* 2008; **6**: 1370-1377 [PMID: 18829392 DOI: 10.1016/j.cgh.2008.06.007]

91 **Vermeire S**, O'Byrne S, Keir M, Williams M, Lu TT, Mansfield JC, Lamb CA, Feagan BG, Panes J, Salas A, Baumgart DC, Schreiber S, Dotan I, Sandborn WJ, Tew GW, Luca D, Tang MT, Diehl L, Eastham-Anderson J, De Hertogh G, Perrier C, Egen JG, Kirby JA, van Assche G, Rutgeerts P. Etrolizumab as induction therapy for ulcerative colitis: a randomised, controlled, phase 2 trial. *Lancet* 2014; **384**: 309-318 [PMID: 24814090 DOI: 10.1016/S0140-6736(14)60661-9]

92 **Rutgeerts PJ**, Fedorak RN, Hommes DW, Sturm A, Baumgart DC, Bressler B, Schreiber S, Mansfield JC, Williams M, Tang M, Visich J, Wei X, Keir M, Luca D, Danilenko D, Egen J, O'Byrne S. A randomised phase I study of etrolizumab (rhuMAb β7) in moderate to severe ulcerative colitis. *Gut* 2013; **62**: 1122-1130 [PMID: 22717454 DOI: 10.1136/gutjnl-2011-301769]

93 **Benson JM**, Peritt D, Scallon BJ, Heavner GA, Shealy DJ, Giles-Komar JM, Mascelli MA. Discovery and mechanism of ustekinumab: a human monoclonal antibody targeting interleukin-12 and interleukin-23 for treatment of immune-mediated disorders. *MAbs* 2011; **3**: 535-545 [PMID: 22123062 DOI: 10.4161/mabs.3.6.17815]

94 **Sandborn WJ**, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, Sands BE, Hanauer SB, Targan S, Rutgeerts P, Ghosh S, de Villiers WJ, Panaccione R, Greenberg G, Schreiber S, Lichtiger S, Feagan BG. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med* 2012; **367**: 1519-1528 [PMID: 23075178 DOI: 10.1056/NEJMoa1203572]

95 **Sandborn WJ**, Feagan BG, Fedorak RN, Scherl E, Fleisher MR, Katz S, Johanns J, Blank M, Rutgeerts P. A randomized trial of Ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease. *Gastroenterology* 2008; **135**: 1130-1141 [PMID: 18706417 DOI: 10.1053/j.gastro.2008.07.014]

96 **Kimball AB**, Papp KA, Wasfi Y, Chan D, Bissonnette R, Sofen H, Yeilding N, Li S, Szapary P, Gordon KB. Long-term efficacy of ustekinumab in patients with moderate-to-severe psoriasis treated for up to 5 years in the PHOENIX 1 study. *J Eur Acad Dermatol Venereol* 2013; **27**: 1535-1545 [PMID: 23279003 DOI: 10.1111/jdv.12046]

97 **Papp KA**, Langley RG, Lebwohl M, Krueger GG, Szapary P, Yeilding N, Guzzo C, Hsu MC, Wang Y, Li S, Dooley LT, Reich K. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* 2008; **371**: 1675-1684 [PMID: 18486740 DOI: 10.1016/S0140-6736(08)60726-6]

98 **Papp KA**, Griffiths CE, Gordon K, Lebwohl M, Szapary PO, Wasfi Y, Chan D, Hsu MC, Ho V, Ghislain PD, Strober B, Reich K. Long-term safety of ustekinumab in patients with moderate-to-severe psoriasis: final results from 5 years of follow-up. *Br J Dermatol* 2013; **168**: 844-854 [PMID: 23301632 DOI: 10.1111/bjd.12214]

99 **Zeuzem S**, Carreño V. Interleukin-12 in the treatment of chronic hepatitis B and C. *Antiviral Res* 2001; **52**: 181-188 [PMID: 11672828 DOI: 10.1016/S0166-3542(01)00183-8]

100 **Cavanaugh VJ**, Guidotti LG, Chisari FV. Interleukin-12 inhibits hepatitis B virus replication in transgenic mice. *J Virol* 1997; **71**: 3236-3243 [PMID: 9060687]

101 **Chiu HY**, Chen CH, Wu MS, Cheng YP, Tsai TF. The safety profile of ustekinumab in the treatment of patients with psoriasis and concurrent hepatitis B or C. *Br J Dermatol* 2013; **169**: 1295-1303 [PMID: 23746170 DOI: 10.1111/bjd.12461]

102 **Kornbluth A**, Present DH, Lichtiger S, Hanauer S. Cyclosporin for severe ulcerative colitis: a user's guide. *Am J Gastroenterol* 1997; **92**: 1424-1428 [PMID: 9317057]

103 **Van Assche G**, D'Haens G, Noman M, Vermeire S, Hiele M, Asnong K, Arts J, D'Hoore A, Penninckx F, Rutgeerts P. Randomized, double-blind comparison of 4 mg/kg versus 2 mg/kg intravenous cyclosporine in severe ulcerative colitis. *Gastroenterology* 2003; **125**: 1025-1031 [PMID: 14517785 DOI: 10.1016/S0016-5085(03)01214-9]

104 **Rayner CK**, McCormack G, Emmanuel AV, Kamm MA. Long-term results of low-dose intravenous ciclosporin for acute severe ulcerative colitis. *Aliment Pharmacol Ther* 2003; **18**: 303-308 [PMID: 12895214 DOI: 10.1046/j.1365-2036.2003.01618.x]

105 **Lichtiger S**, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994; **330**: 1841-1845 [PMID: 8196726 DOI: 10.1056/NEJM199406303302601]

106 **Galán AI**, Fernández E, Morán D, Muñoz ME, Jiménez R. Cyclosporine A hepatotoxicity: effect of prolonged treatment with cyclosporine on biliary lipid secretion in the rat. *Clin Exp Pharmacol Physiol* 1995; **22**: 260-265 [PMID: 7671438 DOI: 10.1111/j.1440-1681.1995.tb01991.x]

107 **Stone BG**, Udani M, Sanghvi A, Warty V, Plocki K, Bedetti CD, Van Thiel DH. Cyclosporin A-induced cholestasis. The mechanism in a rat model. *Gastroenterology* 1987; **93**: 344-351 [PMID: 3596172]

108 **Kassianides C**, Nussenblatt R, Palestine AG, Mellow SD, Hoofnagle JH. Liver injury from cyclosporine A. *Dig Dis Sci* 1990; **35**: 693-697 [PMID: 2344802 DOI: 10.1007/BF01540169]

109 **van Petersen AS**, van der Pijl HW, Ringers J, Lemkes HH, de Fijter HW, Masclee AA. Gallstone formation after pancreas and/or kidney transplantation: an analysis of risk factors. *Clin Transplant* 2007; **21**: 651-658 [PMID: 17845641 DOI: 10.1111/j.1399-0012.2007.00704.x]

110 **Kahan BD**, Flechner SM, Lorber MI, Golden D, Conley S, Van Buren CT. Complications of cyclosporine-prednisone immunosuppression in 402 renal allograft recipients exclusively followed at a single center for from one to five years. *Transplantation* 1987; **43**: 197-204 [PMID: 3544376 DOI: 10.1097/00007890-198702000-00007]

111 **Lorber MI**, Van Buren CT, Flechner SM, Williams C, Kahan BD. Hepatobiliary and pancreatic complications of cyclosporine therapy in 466 renal transplant recipients. *Transplantation* 1987; **43**: 35-40 [PMID: 3541320 DOI: 10.1097/00007890-198701000-00009]

112 **Klintmalm GB**, Iwatsuki S, Starzl TE. Cyclosporin A hepatotoxicity in 66 renal allograft recipients. *Transplantation* 1981; **32**: 488-489 [PMID: 7041349]

113 **Rogler G**. Gastrointestinal and liver adverse effects of drugs used for treating IBD. *Best Pract Res Clin Gastroenterol* 2010; **24**: 157-165 [PMID: 20227029 DOI: 10.1016/j.bpg.2009.10.011]

114 **García-López S**, Gomollón-García F, Pérez-Gisbert J. Cyclosporine in the treatment of severe attack of ulcerative colitis: a systematic review. *Gastroenterol Hepatol* 2005; **28**: 607-614 [PMID: 16373009 DOI: 10.1016/S0210-5705(05)71523-5]

115 **Kancherla D**, Gajendran M, Vallabhaneni P, Vipperla K. Metronidazole induced liver injury: a rare immune mediated drug reaction. *Case Rep Gastrointest Med* 2013; **2013**: 568193 [PMID:

24455335]
116 **Tabak F**, Ozaras R, Erzin Y, Celik AF, Ozbay G, Senturk H. Ornidazole-induced liver damage: report of three cases and review of the literature. *Liver Int* 2003; **23**: 351-354 [PMID: 14708896 DOI: 10.1034/j.1478-3231.2003.00860.x]
117 **Rutgeerts P**, Van Assche G, Vermeire S, D'Haens G, Baert F, Noman M, Aerden I, De Hertogh G, Geboes K, Hiele M, D'Hoore A, Penninckx F. Ornidazole for prophylaxis of postoperative Crohn's disease recurrence: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* 2005; **128**: 856-861 [PMID: 15825069 DOI: 10.1053/j.gastro.2005.01.010]
118 **Rutgeerts P**, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, Kerremans R. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. *Gastroenterology* 1995; **108**: 1617-1621 [PMID: 7768364 DOI: 10.1016/0016-5085(95)90121-3]
119 **Alshammari TM**, Larrat EP, Morrill HJ, Caffrey AR, Quilliam BJ, LaPlante KL. Risk of hepatotoxicity associated with fluoroquinolones: a national case-control safety study. *Am J Health Syst Pharm* 2014; **71**: 37-43 [PMID: 24352180 DOI: 10.2146/ajhp130165]
120 **Arnold GL**, Beaves MR, Pryjdun VO, Mook WJ. Preliminary study of ciprofloxacin in active Crohn's disease. *Inflamm Bowel Dis* 2002; **8**: 10-15 [PMID: 11837933 DOI: 10.1097/00054725-200201000-00002]
121 **Colombel JF**, Lémann M, Cassagnou M, Bouhnik Y, Duclos B, Dupas JL, Notteghem B, Mary JY. A controlled trial comparing ciprofloxacin with mesalazine for the treatment of active Crohn's disease. Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Digestives (GETAID). *Am J Gastroenterol* 1999; **94**: 674-678 [PMID: 10086650 DOI: 10.1111/j.1572-0241.1999.935_q.x]
122 **Swift GL**, Srivastava ED, Stone R, Pullan RD, Newcombe RG, Rhodes J, Wilkinson S, Rhodes P, Roberts G, Lawrie BW. Controlled trial of anti-tuberculous chemotherapy for two years in Crohn's disease. *Gut* 1994; **35**: 363-368 [PMID: 8150348 DOI: 10.1136/gut.35.3.363]
123 **Keane J**, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001; **345**: 1098-1104 [PMID: 11596589 DOI: 10.1056/NEJMoa011110]
124 **Bennett DE**, Courval JM, Onorato I, Agerton T, Gibson JD, Lambert L, McQuillan GM, Lewis B, Navin TR, Castro KG. Prevalence of tuberculosis infection in the United States population: the national health and nutrition examination survey, 1999-2000. *Am J Respir Crit Care Med* 2008; **177**: 348-355 [PMID: 17989346 DOI: 10.1164/rccm.200701-057OC]
125 **Centers for Disease Control and Prevention (CDC)**. Tuberculosis associated with blocking agents against tumor necrosis factor-alpha--California, 2002-2003. *MMWR Morb Mortal Wkly Rep* 2004; **53**: 683-686 [PMID: 15295313]
126 **Doherty SD**, Van Voorhees A, Lebwohl MG, Korman NJ, Young MS, Hsu S. National Psoriasis Foundation consensus statement on screening for latent tuberculosis infection in patients with psoriasis treated with systemic and biologic agents. *J Am Acad Dermatol* 2008; **59**: 209-217 [PMID: 18485527 DOI: 10.1016/j.jaad.2008.03.023]
127 **Kopanoff DE**, Snider DE, Caras GJ. Isoniazid-related hepatitis: a U.S. Public Health Service cooperative surveillance study. *Am Rev Respir Dis* 1978; **117**: 991-1001 [PMID: 666111]
128 **Nolan CM**, Goldberg SV, Buskin SE. Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. *JAMA* 1999; **281**: 1014-1018 [PMID: 10086436 DOI: 10.1001/jama.281.11.1014]
129 **Black M**, Mitchell JR, Zimmerman HJ, Ishak KG, Epler GR. Isoniazid-associated hepatitis in 114 patients. *Gastroenterology* 1975; **69**: 289-302 [PMID: 1150039]
130 **Salpeter SR**. Fatal isoniazid-induced hepatitis. Its risk during chemoprophylaxis. *West J Med* 1993; **159**: 560-564 [PMID: 8279152]
131 **Saukkonen JJ**, Cohn DL, Jasmer RM, Schenker S, Jereb JA, Nolan CM, Peloquin CA, Gordin FM, Nunes D, Strader DB, Bernardo J, Venkataramanan R, Sterling TR. An official ATS statement: hepatotoxicity of antituberculosis therapy. *Am J Respir Crit Care Med* 2006; **174**: 935-952 [PMID: 17021358 DOI: 10.1164/rccm.200510-1666ST]

P- Reviewer: De Ponti F, Malnick SDH, Narciso-Schiavon JL, Triantafillidis JK, Xu R
S- Editor: Gong XM **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.i27.2729

World J Hepatol 2015 November 28; 7(27): 2729-2739
ISSN 1948-5182 (online)

REVIEW

Clinical significance of hepatitis B surface antigen mutants

Nicola Coppola, Lorenzo Onorato, Carmine Minichini, Giovanni Di Caprio, Mario Starace, Caterina Sagnelli, Evangelista Sagnelli

Nicola Coppola, Lorenzo Onorato, Carmine Minichini, Giovanni Di Caprio, Mario Starace, Evangelista Sagnelli, Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, 80131 Naples, Italy

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

Author contributions: Coppola N has contributed to conception of the paper and draft the article; Onorato L has analyzed the role of HBsAg mutants associated with HCC development; Minichini C has analyzed the HBV virology and HBsAg structure; Di Caprio G has analyzed the role of HBsAg mutants associated with immune escape; Starace M has analyzed the HBV virology and HBsAg structure; Sagnelli C has analyzed the role of HBsAg mutants associated with failed HBsAg detection; Sagnelli E has contributed to conception of the paper and draft the article.

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

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: +39-8-15666719
Fax: +39-8-15666013

Received: June 30, 2015
Peer-review started: June 30, 2015
First decision: September 18, 2015
Revised: September 27, 2015
Accepted: November 13, 2015
Article in press: November 17, 2015

Published online: November 28, 2015

Abstract

Hepatitis B virus (HBV) infection is a major public health problem in many countries, with nearly 300 million people worldwide carrying HBV chronic infection and over 1 million deaths per year due to cirrhosis and liver cancer. Several hepatitis B surface antigen (HBsAg) mutations have been described, most frequently due to a single amino acid substitution and seldom to a nucleotide deletion. The majority of mutations are located in the S region, but they have also been found in the pre-S1 and pre-S2 regions. Single amino acid substitutions in the major hydrophilic region of HBsAg, called the "a" determinant, have been associated with immune escape and the consequent failure of HBV vaccination and HBsAg detection, whereas deletions in the pre-S1 or pre-S2 regions have been associated with the development of hepatocellular carcinoma. This review article will focus on the HBsAg mutants and their biological and clinical implications.

Key words: Hepatitis B virus infection; Vaccine escape; Immune escape hepatocellular carcinoma; Hepatitis B surface antigen mutants

Core tip: Antibodies to the hepatitis B surface antigen (HBsAg) produced in response to hepatitis B virus infection or vaccination and those used in diagnostic assays to detect this antigen in serum are both directed against the "a" determinant region, common to all subtypes of the virus. Mutations occurring on the loops of the "a" determinant may be responsible for the lack of protection in immunized patients and in those individuals receiving hepatitis B immune globulin or for failed detection of HBsAg using commercial diagnostic

assays. There is growing evidence in the last decade of the association between HBsAg mutations and the development of hepatocellular carcinoma (HCC), suggesting that the pre-S1 or pre-S2 large deletions are those prevalently associated with the development of HCC. This review article will focus on the clinical impact of the various HBsAg mutants.

Coppola N, Onorato L, Minichini C, Di Caprio G, Starace M, Sagnelli C, Sagnelli E. Clinical significance of hepatitis B surface antigen mutants. *World J Hepatol* 2015; 7(27): 2729-2739 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i27/2729.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i27.2729

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem in most countries, with approximately 2 billion people worldwide showing exposure to the virus, nearly 300 million carrying HBV chronic infection and over 1 million deaths per year due to HBV-related end-stage liver disease, liver cirrhosis and liver cancer[1-5].

HBV is an enveloped Hepadnavirus with an incomplete double-stranded DNA genome of 3.2 Kb[6]. Eight genotypes, with a distinct geographical distribution, have been identified to date. Genotype A prevails in northwestern Europe and in the United States, genotypes B and C in Asia, genotype D in the Mediterranean basin, the Middle East, and India, genotype E in Western Africa, genotype F in South and Central America, genotype G in the United States and France, genotype H in Northern Latin America[7], genotype I in Laos, Vietnam, Eastern India[8,9] and North-Western China[10] and genotype J in Japan[11,12].

The worldwide prevalence of chronic HBV infection in the general population borders 5%, but it differs widely from one geographical area to another, from 0.1%-2.0% in the United States and Western Europe, from 2.0%-8.0% in Eastern Mediterranean countries and Japan, and from 5.0%-20.0% in South-Eastern Asia and sub-Saharan Africa[1,13].

Risk factors for HBV infection include transfusion of unscreened blood, renal dialysis, sexual promiscuity, sharing or re-using syringes among injection drug users, tattooing, piercing, working or residing in a health-care setting, living in a correctional facility and long-term household or intimate non-sexual contact with an HBsAg-positive individual. In highly endemic areas the majority of hepatitis B surface antigen (HBsAg) chronic carriers acquire HBV infection at birth or in the first decade of life, whereas in countries with a low endemicity HBV transmission occurs mostly in adulthood due to unprotected sexual contact, syringe sharing or parenteral exposure to contaminated medical equipment[7,14-17].

A vaccine against HBV became available in 1982, and ten years later the World Health Organization recommended universal vaccination of newborn babies with the HBsAg produced by yeast cells into which the genetic code for HBsAg had been inserted. The complete vaccination schedule induces protective antibody levels in more than 95% of infants, children and young adults.

The emergence of single or multiple amino acid substitutions in the HBsAg region has been found in infants born to HBsAg-positive mothers who underwent passive/active immunoprophylaxis at the birth, in HBsAg-positive liver transplant recipients treated with hyperimmune anti-HBs immune globulin and in patients who experienced loss of HBsAg after anti-HBV therapy.

This review article focuses on the impact of HBsAg mutants on vaccine escape, failure of diagnostic tests to detect HBsAg, and on the development of hepatocellular carcinoma (HCC).

HBV VIROLOGY

Human HBV is the prototype member of the Hepadnaviridae family, which includes a variety of avian and mammalian viruses sharing similar genomic organization, organ tropisms and a unique strategy of genome replication[14].

HBV is one of the smallest enveloped animal viruses with a diameter of 42 nmol/L consisting of an outer lipid envelope and an icosahedral nucleocapsid core composed of proteins. The nucleocapsid encloses the viral DNA and a DNA polymerase acting also as a reverse transcriptase[17]. The outer envelope contains the embedded proteins HBsAg, pre-S1 and pre-S2 involved in the viral binding of, and entry into susceptible cells. HBV is also called "Dane particle" after the name of the researcher who first observed it on electron microscopy together with filaments and 22 nmol/L spherical bodies in the serum of infected individuals[18]. HBV infects the hepatocytes, whereas the filaments and spherical bodies do not contain the viral DNA and do not infect the liver cells. These filaments and spherical bodies show the same HBsAg reactivity as the surface of HBV and are considered to be produced by HBsAg in excess during the life cycle of the virus[19].

The HBV genome consists of 3200 base pairs of partially double-stranded circular DNA containing four (P, C, S and X) overlapping open reading frames (ORF) with a nucleotide diversity of $\geqslant$ 8% in different genotypes[15,16]. The *P* gene codes for the viral polymerase/reverse transcriptase. It has four domains: A terminal domain, which serves as a protein primer for reverse transcription of pre-genomic viral RNA, a spacer region without no apparent function, the polymerase domain, which has reverse transcription activity, and the RNase H domain, responsible for the degradation of the RNA template during reverse transcription.

The core (*C*) gene codes for HBcAg, the major structural protein of the nucleocapsid. The preC/C ORF is transcribed into a precore/core fusion protein. During entry into the endoplasmic reticulum, 19 amino acids are cleaved from the N-terminal end of the precore protein by a signal peptidase. When transported into the

Golgi compartment, additional amino acids are removed from the C-terminal end by intra-Golgi proteases to form the HBe antigen. This antigen, which is secreted into the serum, is used as a marker of active HBV replication in clinical practice. The possibility that the circulating HBe antigen may suppress the immune response and favor HBV replication has been hypothesized[14] but never proven, and the biological function of this protein, if any, remains unknown. The preS/S ORF encodes the envelope proteins HBsAg, pre-S1 and pre-S2. The X gene codes for potent transactivating factors of viral and cellular genes (HBxAg), some of which possibly correlated to the development of HCC[20,21].

HBSAG STRUCTURE AND VARIANTS

The preS/S ORF encodes three different structurally related envelope proteins, termed the large (L), middle-sized (M) and small (S) protein, that is synthesized from alternative initiation codons. The three proteins share the same carboxy-terminus but have different amino terminal extensions. In particular, the S protein corresponding to the HBsAg consists of 226 amino acids (aa), the M protein contains an extra N-terminal extension of 55 aa, and the L protein has a further N-terminal sequence of 108-119 aa compared with the M protein[22]. The enhancer and basic core promoter regions of S region overlap with the *X* gene.

HBsAg is an envelope glycoprotein that is currently the primary element for diagnosis and target of immunoprophylaxis of HBV infection. The dominant epitopes of HBsAg, which are the targets of neutralizing B-cell responses, reside in the "a" determinant (aa 124-147) within the major hydrophilic region (MHR).

Several mutations in the S region have been described and those most frequently reported in the literature are listed in Table 1. In most cases, they were aa substitutions due to a single mutation, but nucleotide deletions have also been reported. The majority of mutations were located in the S region, but some mutations were also identified in the pre-S1 or pre-S2 regions. Mutations in the S region have been found in various HBV genotypes, while those in pre-S1 or pre-S2 have been frequently observed in patients with HBV genotype C (Table 1)[23-60].

CLINICAL SIGNIFICANCE OF HBsAg MUTANTS

Some HBsAg mutants have been associated with major biological or clinical events such as immune escape, failure to detect HBsAg and the development of HCC.

HBsAg mutants associated with immune escape

The MHR region, which is exposed to the outer surface of the virion, is situated between aa 99-169 of HBsAg. The antibodies produced after HBV vaccination and those used in diagnostic assays to detect serum HBsAg are both directed against this region and, specifically, to a cluster of B-cell epitopes, common to all subtypes of the virus, called "a" determinant and showing a two-loop structure of aa (124-147). Mutations may occur on both loops of the "a" determinant and may be responsible for a lack of protection and the occurrence of HBV infection in immunized patients (vaccine escape) or for failure to protect by the HBIG administered as a prophylactic measure or failure to detect HBsAg in diagnostic assays. Table 2 lists the studies suggesting the association of HBsAg mutants with vaccine escape or failed HBsAg detection.

In 1988 a follow-up Italian study[61] reported that children with a strong antibody response to HBV vaccine may still become infected with HBV. This observation was confirmed in other investigations and the conclusion on this point is that this phenomenon may involve nearly 2% of children born of HBsAg-positive mothers or with other HBsAg-positive household contacts[61,62]. More detailed analysis identified an association of vaccine escape with a point mutation from glycine to arginine at position 145 (G145R)[61]. This G145R mutation is the vaccine-escape mutant most frequently detected[62-68], stable over time[61,69] and horizontally transmissible[70,71].

He *et al*[72] studied 176 restaurant employees before and one year after the HBV vaccination was completed. Six (3.4%) of the 176 became HBV-DNA positive after vaccination and four (2.3%) of the six showed a point mutation within the "a" determinant (Gly-145-Ala, and Ile/Thr-126-Asn/Ser).

Ngui *et al*[73] tested 17 HBV-infected mother/infant pairs because the infants became HBV-infected despite careful passive-active HBV immunoprophylaxis. Complete concordance in the *S* gene sequence was identified in 15 mother/infant pairs, while in the remaining two pairs the sequence of the *S* gene differed: One infant harbored three nucleic acid changes (P120Q, F134Y and D144A) and the other was carrying the I126N substitution, mutations that may interfere with HBsAg/anti-HBs binding. Mismatches in the HBV *S* gene were also observed in 16 of 41 HBV-infected mother/infant pairs in Singapore, of whom the infants acquired HBV infection despite HBV passive/active immunization[74].

HBsAg mutants associated with failed HBsAg detection

In 1999, Coleman *et al*[75] demonstrated that three commercial assays did not detect serum HBsAg in patients showing mutations including G145R in the "a" determinant. Subsequently, Zhang *et al*[76] prepared a panel containing four dilutions of an HBsAg wild-type serum, three recombinant mutants (G145R, K141E, and T131I) and one negative sample. This panel was tested for HBsAg reactivity by the laboratories of 85 blood banks using different assays. HBsAg reactivity was detected only in 19.4% of the assays in the presence of the aa substitution G145R and in 20% in the presence of the T131I or K141E mutants.

Sticchi *et al*[77] found G145A HBsAg mutants in 8 (3.1%) of 256 HBsAg chronic carriers, alone in 5 and with other HBsAg mutations in 3 (T126I, T131A,

Table 1 Mutations reported in the hepatitis B surface antigen regions

Codon	Type mutation	Mutation	Phenotypic consequence	HBV genotype	Ref.
Wt[1] atg, Mt[1] acg, Wt[2/3] tac, Mt[2] tgc, Mt[3] cac, Wt[4] ttt, Mt[4] ttg	AAS	M197T[1], Y206C[2]/H[3], F220L[4]	Low serum HBV DNA	D	[23]
Wt[1] gga Mt[1] gca	AAS	G145A[1]	Immune escape[1] Lamivudine resistance[1]	A-B-C-D	[24,31,47]
	AAS	G145R	Immune escape	A, B-C-D	[28,35,36,40,41,45,47-50]
Wt tgc Mt tgg	AAS	C121W	Immune escape	A	[25]
Wr tgc, Mt tgg	AAS	C147W	Immune escape	D	[25,26]
Wt act, Mt att	AAS	T189I	Immune escape, reducing HBsAg detection signal	E-A	[27,28,33]
WT act, Mt aat	AAS	I126N	Immune escape	C	[29]
WtCaa, Mt cga	AAS	Q129R	Vaccine escape	B	[30,34]
Wt ttc, Mt tac	AAS	F161Y	Immune escape	C	[31,51]
Wt atg, Mt atc	AAS	M103I	Immune escape	D	[24,52]
Wt ttg, Mt tcg	AAS	L94S	HCC	D	[32]
WT gac, Mt gaa	AAS	D144E	Immune escape	D, C, A	[28,33,40,52]
Wt cct, Mt Tct	AAS	P127S	Immune escape	B, D	[33,34,39]
Wt act, Mt agt	AAS	T126S	Immune escape	B	[33,34,38]
WT act, MT atc	AAS	T126I	Immune escape	C, D	[28,34,53]
WT act, Mt agc	AAS	T143S	Immune escape	C, A	[34,53]
WTcca, Mt aca	AAS	P120T	Immune escape	B, D	[34,53]
Wt gca, Mt Gta	AAS	A184V	Immune escape	E	[27]
Wt gta, Mt gca	AAS	V184A	Immune escape	E	[27]
Wt Tcg, Mt acg	AAS	S143t	Immune escape	E	[25,27,53]
Wt tgt, Mt ttc	AAS	C76F	Immune escape	E	[27]
Wt cct, Mt act	AAS	P70T	Immune escape	E	[27]
Wt ata, Mt act	AAS	I82T	Immune escape	E	[27]
Wt att, Mt ctt	AAS	I110L	Immune escape	A, C	[25,53]
Wt tat, Mt ttt	AAS	Y134F	Immune escape	D	[25,55]
Wt agt, Mt aac	AAS	S207N	Immune escape	D	[25,56]
Wt tat, Mt cat	AAS	Y134H	Immune escape	D	[52]
Wt acg, Mt aat	AAS	T125N	Increased HBsAg reactivity in immunological diagnostic assays	D	[43]
Wt atg, Mt atc, WT aag, Mt agg	AAS	M103I-K122R	Immune escape	A-C-D	[24]
Wt tat, Mt tgt	AAS	Y100C	Immune escape	B-C	[45]
Wt ccc, Mt ctc, MtQ caa, MtS tcg, Mtt acc	AAS	P120L/Q/S/T	Immune escape	B-C	[45]
Wt tcg, Mt cta	AAS	S143L	Immune escape	D	[52]
Wt ctt, Mt cct	AAS	L127P	Immune escape	E	[27]
Wt cct, Mt ctt	AAS	P127L	Immune escape	A	[27]
Mutations in pre-S1 region					
Wt tct, Mt aca	AAS	S98T	Significant association with disease progression (LF, LC, HCC)	D	[57]
Wt aac, Mt act	AAS	N48T	Reduced HBsAg detection signals	C	[27]
Wt cag, Mt cct	AAS	Q82P	Reduced HBsAg detection signals	C	[27]
Wt acc, Mt aat	AAS	T97N	HBsAg not detected	C	[27]
Wt aat, Mt acc	AAS	N97T	HBsAg not detected	E	[27]
Wt cct, Mt cag	AAS	P93Q	HBsAg not detected	E, C	[27]
Deletion size (bp) 39	D	Region (nt) 3046-3084	Progression to advanced liver disease	C	[58]
Deletion size (bp) 108	D	Region (nt) 2959-3066	Progression to advanced liver disease	C	[58]
Deletion size (bp) 39	D	Region (nt) 3046-3084	Progression to advanced liver disease	C	[58]
Deletion size (bp) 108	D	Region 2959-3066	Progression to advanced liver disease	C	[58]
104th codon	AAS	Q104Stop	HCC development and immune escape	C	[59]
preS1 start	D	Not specified	HCC development and immune escape	C	[59]
Wt ucc, Mt gcc	AAS	S17A	Immune escape	C	[59]
Wt cct, Mt ctt	AAS	P32L	Immune escape	C	[59]
Wt tgg, Mt ctg, Mt agg	AAS	W43L/R	Immune escape	C	[59]
104th codon	AAS	Q104Stop	HCC development and immune escape	C	[59]
Mutations in pre-S2 region					
Not specified	AAS	preS2-W3Stop	Immune escape	C	[59]
From 8th codon to 23rd codon	D		Immune escape	C	[59]

AAS: Amino acid substitution; D: Deletion; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HBsAg: Hepatitis B surface antigen; LC: Liver cirrhosis; LF: Liver fibrosis.

Table 2 Studies on hepatitis B surface antigen mutations associated with immune escape and failure to detect hepatitis B surface antigen

Ref.	Country	No. of patients	Type of study	Patients with HBsAg mutation, *n* (%)	Clinical significance	HBsAg mutation
Sticchi *et al*[77]	Italy	256	Cross-sectional	8 (3.1)	Detection failure; vaccine escape	G145R, T126I
Luongo *et al*[79]	Italy	1	Case report	1	Vaccine escape	M125T, T127P Q129H
Lee *et al*[66]	South Korea	1	Case report	1	Vaccine escape	G145R, P120Q, I126T
Seddigh-Tonekaboni *et al*[68]	United Kingdom	4		2	Vaccine escape	P142S, G145R, G145A
Ngui *et al*[73]	England, Wales	17	Cross-sectional	2 (12)	Vaccine escape	P120Q, F134Y, D144A, I126N
Carman *et al*[62]	Multinational	32	Cross-sectional	1	Vaccine escape	G145R
Laoi *et al*[78]	Ireland	32	Cross-sectional	6 (18.5)	Vaccine escape, detection failure	G145A, F134L, D144E, S143L
Foy *et al*[80]	United States	1	Case report	1	Immune escape	D144E

HBsAg: Hepatitis B surface antigen.

C139Y, E/D144G, T126I, M133L, P120Q or T126I). In the three patients with a multiple mutation, HBsAg was undetectable by 3 of 5 routine assays used in this study.

HBsAg mutants associated with failure to detect HBsAg have also been observed in patients with acute hepatitis B[78-80]. Laoi *et al*[78] studied 32 consecutive patients with acute hepatitis B and found a single or multiple amino acid substitution in 6 (18.5%) isolates. The G145A substitution along with the F134L were responsible for failure to detect HBsAg in one of these 6 and the D144E and S143L in another two isolates, whereas the other mutants identified (R113Thr, Ser114-Pro, Thr118Val, Ala128Val) were of unclear significance.

HBsAg mutations associated with HCC development

The HBsAg mutations prevalently associated with the development of HCC are the large deletions involving the pre-S1 or pre-S2 regions[81]. These deletions naturally occur during the chronic phase of HBV infection and induce the synthesis of truncated variants of the large envelope protein, with important immunological and clinical consequences[82]. In fact, these variants present reduced antigenicity and, by altering the immune response, may favour the replicative activity of the virus[83]. In addition, the pre-S deletions decrease the expression of middle and small surface proteins, resulting in intracellular accumulation of viral particles that may induce stress in the endoplasmic reticulum, oxidative DNA damage and genomic instability, and possibly lead to a higher rate of neoplastic transformation[84]. The growing evidence on the association between HBsAg mutations and the development of HCC emerging in the last decade is shown in Table 3.

In 2003, Huy *et al*[85] conducted a multicenter cross-sectional study on 352 HBsAg-positive patients from 12 countries in five continents or subcontinents and demonstrated a higher prevalence of pre-S1 and/or pre-S2 deletions and pre-S2 start codon mutations in patients with HCC than in those without (35.7% *vs* 16.5%, $P < 0.05$). In accordance with this, a correlation between a pre-S deletion and the presence of liver cirrhosis or HCC was described in an observational Japanese study[86]. Also, in a cross-sectional Italian study, the prevalence of pre-S2 deletions or start codon mutations was much higher in the 19 patients with HBV-related HCC than in 91 HBV carriers without HCC (84.2% *vs* 43.9%, $P < 0.02$)[87]. In 2006, Chen *et al*[88] found a higher prevalence of pre-S deletions in 50 Taiwanese patients with HBV-related hepatocellular carcinoma than in 102 HBV-infected individuals without HCC (52.0% *vs* 29.4%, $P < 0.0001$). Similar data come from three studies performed in Taiwan[89], South Korea[90] and China[91], respectively. In addition, a South Korean study performed by Mun *et al*[92] demonstrated a correlation of both pre-S1 deletions and pre-S1 start codon mutations with the occurrence of HCC ($P = 0.027$ and $P = 0.048$, respectively); in this study the presence of pre-S2 deletions was also significantly associated with the development of liver cirrhosis ($P = 0.001$). The association of pre-S deletions and pre-S2 start codon mutations with the presence or the development of HCC was confirmed in other studies performed in southern Asia[93-95]. Moreover, a cross-sectional South Korean study on 119 HBsAg-positive patients[96] showed a higher prevalence of pre-S1 deletions in patients with HCC than in those without. In a case-control study[97] on 192 HBsAg-positive patients from Taiwan, the pre-S2, but not pre-S1 deletions, were associated with the occurrence of HCC, data endorsed by the results of a subsequent South Korean case-control study on 270 HBV-infected patients[98] that described a correlation between pre-S2 but not pre-S1 deletions or pre-S2 start codon mutations and HCC. The pre-S deletions were significantly associated with the development of HCC also in a study performed by Kao *et al*[99] on 168 HBV chronic patients from Taiwan.

A prospective study performed in South Korea investigated 195 patients with chronic HBV infection[100] and showed a higher incidence of HCC in those who

Table 3 Clinical significance of hepatitis B surface antigen mutations in chronic hepatitis B

Ref.	Country	No. of patients	Type of study	Patients with HBsAg mutation, *n* (%)	Clinical significance
Abe *et al*[103]	Japan	40	Case-control	27/30 (90) in HCC+ 0/10 (0) in HCC-	Correlation between preS1-S2 deletion and HCC ($P < 0.001$)
Bläckberg *et al*[106]	Sweden	35	Case-control	8/16 (50) in HCC+ 4/19 (21) in HCC-	No correlation between Pre-S2 mutations and HCC ($P > 0.05$)
Cao *et al*[94]	China	97	Case-control	34/47 (72.3) in HCC+ 13/50 (26) in HCC-	Correlation between pre-S deletion or pre-S2 start codon mutation and HCC ($P < 0.001$)
Chen *et al*[88]	Taiwan	152	Cross-sectional	26/50 (52.0) in HCC+ 30/102 (29.4) in HCC-	Correlation between pre-S deletion and HCC ($P < 0001$)
Chen *et al*[95]	Taiwan	240	Case-control	Pre-S deletion: 28/80 (35) in HCC+ *vs* 27/160 (16.9) in HCC-	Correlation between pre-S deletion ($P = 0.002$), W4P/R ($P = 0.021$) and M1V/I/A mutations ($P = 0.011$) and HCC
Choi *et al*[90]	South Korea	300	Cross-sectional	W4P/R: 10/80 (12.5) in HCC+ *vs* 7/160 (4.4) in HCC- M1V/I/A: 23/80 (28.8) in HCC+ *vs* 24/160 (15) in HCC- 31/72 (43.1) in HCC+	Correlation between pre-S deletion or pre-S2 start codon mutation and HCC ($P < 0.001$)
Fang *et al*[93]	China	66	Case-control	51/228 (22.4) in HCC- 15/33 (45.5) in HCC+	Correlation between pre-S deletion and HCC ($P < 0.01$)
Gao *et al*[91]	China	79	Cross-sectional	6/33 (18.2) in HCC- 10/26 (38.5) in HCC+	Correlation between pre-S deletion and HCC ($P = 0.001$)
Huang *et al*[104]	Taiwan	38	Case-control	3/53 (5.7) in HCC- 9/19 (47.4) in HCC+	Correlation between pre-S deletion and HCC ($P = 0.008$)
Hung *et al*[110]	Taiwan	313	Cross-sectional	1/19 (5.3) in HCC- 41/146 (40) in HCC+	Correlation between pre-S deletion and HCC ($P < 0.001$)
Huy *et al*[85]	12 countries	352	Cross-sectional	5/167 (3.0) in HCC- 17/49 (34.7) in HCC+ 50/303 (16.5) in HCC-	Correlation between pre-S1/S2 deletion and pre-S2 start codon mutations and HCC ($P < 0.05$)
Jang *et al*[96]	South Korea	119	Cross-sectional	17/48 (35.4) in HCC+ 13/71 (18.3) in HCC-	Correlation between pre-S deletion and HCC ($P < 0.05$)
Kao *et al*[99]	Taiwan	168	Case-control	56/112 (50.0) in HCC+ 4/56 (7.1) in HCC-	Correlation between pre-S deletion and HCC ($P < 0.001$)
Lee *et al*[98]	South Korea	270	Case-control	28/135 (18.5) in HCC+ 6/135 (4.4) in HCC-	Correlation between pre-S2 deletion and HCC ($P < 0.001$)
Lee *et al*[107]	South Korea	247	Cross-sectional	19/153 (12.4) in advanced liver disease (LC or HCC) 1/94 (1.1) in non-advanced liver disease	Correlation between W4P/R mutation and HCC or cirrhosis ($P < 0.05$)
Lin *et al*[89]	Taiwan	266	Cross-sectional	19/64 (29.7) in HCC+ 25/202 (12.4) in HCC-	Correlation between pre-S deletion and HCC ($P = 0.02$)
Mun *et al*[92]	South Korea	120	Cross-sectional	Pre-S1: 13/40 (32.5) in HCC+ *vs* 11/80 (13.7) in HCC- Pre-S1 start codon: 9/40 (22.5) in HCC+ *vs* 4/80 (5.0) in HCC- Pre-S2: 8/21 (38.1) in LC+ *vs* 4/59 (6.8) in LC-	Correlation between pre-S1 ($P = 0.027$) and pre-S1 start codon mutations ($P = 0.048$) and HCC. Correlation between pre-S2 deletions and cirrhosis ($P = 0.001$)
Qu *et al*[102]	China	193	Case-control	Pre-S deletion: 28/96 (29.2) *vs* 11/97 (11.3), Pre-S2 start codon: 17/96 (17.7) *vs* 7/97 (7.2), T31C: 23/96 (24.0) *vs* 37/97 (38.1), T53C: 36/96 (37.5) *vs* 23/97 (23.7), T766A: 13/96 (13.5) *vs* 14/97 (14.4) in HCC+ *vs* HCC-	Correlation between pre-S deletion ($P = 0.003$), pre-S2 start codon ($P = 0.027$), T31C ($P = 0.044$), T53C ($P = 0.027$) but not T766A mutation ($P = 0.966$) and HCC
Raimondo *et al*[87]	Italy	110	Cross-sectional	16/19 (84.2) in HCC 40/91 (43.9) in HCC-	Correlation between pre-S2 deletion or start codon mutation and HCC ($P < 0.02$)
Sinn *et al*[100]	South Korea	195	Cohort	13/24 (54.2) in HCC+ 31/171 (18.1) in HCC-	Correlation between pre-S mutation and HCC ($P < 0.001$)
Sugauchi *et al*[86]	Japan	160	Cross-sectional	20/58 (34.5) in advanced liver disease (LC or HCC) 17/102 (16.7) in non-advanced liver disease	Correlation between pre-S deletion and HCC or cirrhosis ($P < 0.05$)
Sung *et al*[108]	Hong Kong	26	Case-control	T31C: 6/16 (37.5) in HCC+ *vs* 0/10 (0.0) in HCC- T53C: 6/16 (37.5) in HCC+ *vs* 1/10 (10.0) in HCC-	Correlation between T31C and T53C mutations and HCC ($P < 0.05$)
Thongbai *et al*[105]	Thailand	154	Cross-sectional	24/65 (36.9) in HCC+ 34/89 (38.2) in HCC-	No correlation between pre-S1/S2/S deletion or start codon mutation and HCC ($P > 0.1$)
Yeung *et al*[97]	Taiwan	192	Case-control	28/96 (29.2) in HCC+ 14/96 (14.6) in HCC-	Correlation between pre-S deletion and HCC ($P = 0.015$)
Zhao *et al*[101]	China	317	Case-control	74/157 (47.1) in HCC+ 45/160 (28.1) in HCC-	Correlation between pre-S deletion and HCC ($P < 0.001$)

Zhu *et al*[109]	China	55	Case-control	4/20 (20.0) with Pre-S2 start codon, 5/20 (25.0) with T53C and 3/20 with T766A in HCC+ *vs* 0/20 in HCC-	Correlation between pre-S2 start codon (P = 0.014), T53C (P = 0.004) and T766A mutation (P = 0.043) and HCC

HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HBsAg: Hepatitis B surface antigen; LC: Liver cirrhosis.

tested positive for pre-S mutations. Two subsequent Chinese case-control studies on 317 and 193 HBsAg-positive patients, respectively, identified pre-S deletions[101,102] and pre-S2 start codon mutations[102] as independent predictors of HCC development. Abe *et al*[103] found a correlation between the presence of pre-S1 or pre-S2 deletions and the occurrence of HCC in a case-control study on 40 Asian children with chronic HBV infection, a finding confirmed in a retrospective study on 38 Taiwanese children[104] in which the presence of pre-S mutations was identified as an independent predictor of HCC development.

Instead, a cross-sectional study[105] enrolling 154 patients from Thailand failed to show an association between HCC and pre-S1 or pre-S2 deletions or start codon mutations. Likewise, a small case-control study showed no association between pre-S2 mutations and HCC in 35 patients from different countries[106].

The S region of the HBV genome may present point mutations that could alter HBsAg secretion. These point mutations were investigated by some Authors to identify a possible correlation between their presence and the development of HCC. Chen *et al*[95] found a correlation between the W4P/R mutation and the occurrence of HCC, an observation endorsed by the data from a cross-sectional study from South Korea[107] on 247 HBsAg-positive patients in which the prevalence of W4P/R mutants was higher in patients with cirrhosis or HCC than in those with a less severe liver illness. In addition, Qu *et al*[102] in a larger study confirmed the association of the T31C and T53C mutations with the occurrence of HCC previously demonstrated in a small cohort study[108] published in 2008. In Qu's study the T766A mutant and HCC were not associated, whereas a case-control study carried out by Zhu *et al*[109] on 55 HBV-infected Chinese patients showed a significant association between the pre-S2 start codon (P = 0.014), T53C (P = 0.004) and T766A (P = 0.043) mutations and the occurrence of HCC.

CONCLUSION

The association of single or multiple aa substitutions in the HBsAg region with failed protection in infants who received passive/active prophylaxis and in HBsAg-positive liver transplant patients undergoing continuous passive immunoprophylaxis should alert clinicians to the possible onset of acute hepatitis B or a reactivation of a previous HBV infection, respectively, in these cases.

Similarly, the possibility that some subjects resulting HBsAg-negative may harbor HBV infection because an aa substitution has made the presence of HBsAg undetectable with the commercially available assays should be taken into account by clinicians and healthcare personnel working in laboratories and blood banks.

Although several studies reported an association between HBsAg mutations and HCC, the data on this point are not conclusive because most of the studies were performed in south-eastern Asia, some of them were very small, most of them were cross-sectional and a few reported data contrasting with those from the majority of studies. A large worldwide study, planned on the basis of the data available, would almost certainly improve our knowledge on this topic.

REFERENCES

1 **Lavanchy D**. Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. *J Clin Virol* 2005; **34** Suppl 1: S1-S3 [PMID: 16461208 DOI: 10.1016/S1386-6532(05)00384-7]

2 **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]

3 **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]

4 **Coppola N**, Corvino AR, De Pascalis S, Signoriello G, Di Fiore E, Nienhaus A, Sagnelli E, Lamberti M. The long-term immunogenicity of recombinant hepatitis B virus (HBV) vaccine: contribution of universal HBV vaccination in Italy. *BMC Infect Dis* 2015; **15**: 149 [PMID: 25884719 DOI: 10.1186/s12879-015-0874-3]

5 **Borgia G**, Gentile I. Treating chronic hepatitis B: today and tomorrow. *Curr Med Chem* 2006; **13**: 2839-2855 [PMID: 17073632 DOI: 10.2174/092986706778521995]

6 **Cao GW**. Clinical relevance and public health significance of hepatitis B virus genomic variations. *World J Gastroenterol* 2009; **15**: 5761-5769 [PMID: 19998495]

7 **Arauz-Ruiz P**, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol* 2002; **83**: 2059-2073 [PMID: 12124470]

8 **Tran TT**, Trinh TN, Abe K. New complex recombinant genotype of hepatitis B virus identified in Vietnam. *J Virol* 2008; **82**: 5657-5663 [PMID: 18353958 DOI: 10.1128/jvi.02556-07]

9 **Olinger CM**, Jutavijittum P, Hübschen JM, Yousukh A, Samountry B, Thammavong T, Toriyama K, Muller CP. Possible new hepatitis B virus genotype, southeast Asia. *Emerg Infect Dis* 2008; **14**: 1777-1780 [PMID: 18976569 DOI: 10.3201/eid1411.080437]

10 **Yu H**, Yuan Q, Ge SX, Wang HY, Zhang YL, Chen QR, Zhang J, Chen PJ, Xia NS. Molecular and phylogenetic analyses suggest an additional hepatitis B virus genotype "I". *PLoS One* 2010; **5**: e9297 [PMID: 20174575 DOI: 10.1371/journal.pone.0009297]

11 **Zehender G**, Ebranati E, Gabanelli E, Sorrentino C, Lo Presti A, Tanzi E, Ciccozzi M, Galli M. Enigmatic origin of hepatitis B virus: an ancient travelling companion or a recent encounter? *World J Gastroenterol* 2014; **20**: 7622-7634 [PMID: 24976700 DOI: 10.3748/wjg.v20.i24.7622]

12 **Tatematsu K**, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M. A genetic variant of hepatitis B virus divergent from known

human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *J Virol* 2009; **83**: 10538-10547 [PMID: 19640977 DOI: 10.1128/jvi.00462-09]
13 **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]
14 **Ganem D**, Schneider R. Hepadnaviridae: the viruses and their replication. In: Knipe DM, Howley PM, editors. Fields Virology. Philadelphia: Lippincott-Raven, 2001: 2923-2970
15 **Sagnelli C**, Ciccozzi M, Pisaturo M, Lo Presti A, Cella E, Coppola N, Sagnelli E. The impact of viral molecular diversity on the clinical presentation and outcome of acute hepatitis B in Italy. *New Microbiol* 2015; **38**: 137-147 [PMID: 25915056]
16 **Coppola N**, Tonziello G, Colombatto P, Pisaturo M, Messina V, Moriconi F, Alessio L, Sagnelli C, Cavallone D, Brunetto M, Sagnelli E. Lamivudine-resistant HBV strain rtM204V/I in acute hepatitis B. *J Infect* 2013; **67**: 322-328 [PMID: 23796869]
17 **Coppola N**, Sagnelli C, Pisaturo M, Minichini C, Messina V, Alessio L, Starace M, Signoriello G, Gentile I, Filippini P, Sagnelli E. Clinical and virological characteristics associated with severe acute hepatitis B. *Clin Microbiol Infect* 2014; **20**: O991-O997 [PMID: 24930916 DOI: 10.1111/1469-0691.12720]
18 **Harrison T**. Desk Encyclopedia of General Virology. Boston: Academic Press, 2009: 455
19 **Howard CR**. The biology of hepadnaviruses. *J Gen Virol* 1986; **67** (Pt 7): 1215-1235 [PMID: 3014045 DOI: 10.1099/0022-1317-67-7-1215]
20 **Schaefer S**. Hepatitis B virus genotypes in Europe. *Hepatol Res* 2007; **37**: S20-S26 [PMID: 17627630 DOI: 10.1111/j.1872-034X.2007.00099.x]
21 **He B**, Fan Q, Yang F, Hu T, Qiu W, Feng Y, Li Z, Li Y, Zhang F, Guo H, Zou X, Tu C. Hepatitis virus in long-fingered bats, Myanmar. *Emerg Infect Dis* 2013; **19**: 638-640 [PMID: 23631923 DOI: 10.3201/eid1904.121655]
22 **Coppola N**, Loquercio G, Tonziello G, Azzaro R, Pisaturo M, Di Costanzo G, Starace M, Pasquale G, Cacciapuoti C, Petruzziello A. HBV transmission from an occult carrier with five mutations in the major hydrophilic region of HBsAg to an immunosuppressed plasma recipient. *J Clin Virol* 2013; **58**: 315-317 [PMID: 23856167 DOI: 10.1016/j.jcv.2013.06.020]
23 **Mirabelli C**, Surdo M, Van Hemert F, Lian Z, Salpini R, Cento V, Cortese MF, Aragri M, Pollicita M, Alteri C, Bertoli A, Berkhout B, Micheli V, Gubertini G, Santoro MM, Romano S, Visca M, Bernassola M, Longo R, De Sanctis GM, Trimoulet P, Fleury H, Marino N, Mazzotta F, Cappiello G, Spanò A, Sarrecchia C, Zhang JM, Andreoni M, Angelico M, Verheyen J, Perno CF, Svicher V. Specific mutations in the C-terminus domain of HBV surface antigen significantly correlate with low level of serum HBV-DNA in patients with chronic HBV infection. *J Infect* 2015; **70**: 288-298 [PMID: 25452041 DOI: 10.1016/j.jinf.2014.10.015]
24 **Martin CM**, Welge JA, Rouster SD, Shata MT, Sherman KE, Blackard JT. Mutations associated with occult hepatitis B virus infection result in decreased surface antigen expression in vitro. *J Viral Hepat* 2012; **19**: 716-723 [PMID: 22967103 DOI: 10.1111/j.1365-2893.2012.01595.x]
25 **Weinberger KM**, Bauer T, Böhm S, Jilg W. High genetic variability of the group-specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. *J Gen Virol* 2000; **81**: 1165-1174 [PMID: 10769057]
26 **Zheng X**, Weinberger KM, Gehrke R, Isogawa M, Hilken G, Kemper T, Xu Y, Yang D, Jilg W, Roggendorf M, Lu M. Mutant hepatitis B virus surface antigens (HBsAg) are immunogenic but may have a changed specificity. *Virology* 2004; **329**: 454-464 [PMID: 15518823 DOI: 10.1016/j.virol.2004.08.033]
27 **Olinger CM**, Weber B, Otegbayo JA, Ammerlaan W, van der Taelem-Brulé N, Muller CP. Hepatitis B virus genotype E surface antigen detection with different immunoassays and diagnostic impact of mutations in the preS/S gene. *Med Microbiol Immunol* 2007; **196**: 247-252 [PMID: 17503077 DOI: 10.1007/s00430-007-0050-5]
28 **Ly TD**, Servant-Delmas A, Bagot S, Gonzalo S, Férey MP, Ebel A, Dussaix E, Laperche S, Roque-Afonso AM. Sensitivities of four new commercial hepatitis B virus surface antigen (HBsAg) assays in detection of HBsAg mutant forms. *J Clin Microbiol* 2006; **44**: 2321-2326 [PMID: 16825343 DOI: 10.1128/JCM.00121-06]
29 **Hou J**, Wang Z, Cheng J, Lin Y, Lau GK, Sun J, Zhou F, Waters J, Karayiannis P, Luo K. Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. *Hepatology* 2001; **34**: 1027-1034 [PMID: 11679975 DOI: 10.1053/jhep.2001.28708]
30 **Yao QQ**, Dong XL, Wang XC, Ge SX, Hu AQ, Liu HY, Wang YA, Yuan Q, Zheng YJ. Hepatitis B virus surface antigen (HBsAg)-positive and HBsAg-negative hepatitis B virus infection among mother-teenager pairs 13 years after neonatal hepatitis B virus vaccination. *Clin Vaccine Immunol* 2013; **20**: 269-275 [PMID: 23254298 DOI: 10.1128/CVI.00539-12]
31 **Yong-Lin Y**, Qiang F, Ming-Shun Z, Jie C, Gui-Ming M, Zu-Hu H, Xu-Bing C. Hepatitis B surface antigen variants in voluntary blood donors in Nanjing, China. *Virol J* 2012; **9**: 82 [PMID: 22500577 DOI: 10.1186/1743-422X-9-82]
32 **Pollicino T**, Raffa G, Costantino L, Lisa A, Campello C, Squadrito G, Levrero M, Raimondo G. Molecular and functional analysis of occult hepatitis B virus isolates from patients with hepatocellular carcinoma. *Hepatology* 2007; **45**: 277-285 [PMID: 17256766 DOI: 10.1002/hep.21529]
33 **Abdelnabi Z**, Saleh N, Baraghithi S, Glebe D, Azzeh M. Sub-genotypes and mutations in the s and polymerase genes of hepatitis B virus carriers in the West Bank, palestine. *PLoS One* 2014; **9**: e113821 [PMID: 25503289 DOI: 10.1371/journal.pone.0113821]
34 **Darmawan E**, Turyadi KE, Nursanty NK, Thedja MD, Muljono DH. Seroepidemiology and occult hepatitis B virus infection in young adults in Banjarmasin, Indonesia. *J Med Virol* 2015; **87**: 199-207 [PMID: 25521058 DOI: 10.1002/jmv.24045]
35 **Romanò L**, Paladini S, Galli C, Raimondo G, Pollicino T, Zanetti AR. Hepatitis B vaccination. *Hum Vaccin Immunother* 2015; **11**: 53-57 [PMID: 25483515 DOI: 10.4161/hv.34306]
36 **Thakur V**, Kazim SN, Guptan RC, Hasnain SE, Bartholomeusz A, Malhotra V, Sarin SK. Transmission of G145R mutant of HBV to an unrelated contact. *J Med Virol* 2005; **76**: 40-46 [PMID: 15778957 DOI: 10.1002/jmv.20321]
37 **Norouzi M**, Ghorashi S, Abedi F, Nejatizadeh A, Ataei B, Malekzadeh R, Alavian S, Judaki M, Ghamari S, Namazi A, Rahimnia R, Khedive A, Jazayeri S. Identification of Hepatitis B Virus Surface Antigen (HBsAg) Genotypes and Variations in Chronic Carriers from Isfahan Province, Iran. *Iran J Public Health* 2012; **41**: 104-111 [PMID: 23113154]
38 **Komatsu H**, Inui A, Sogo T, Konishi Y, Tateno A, Fujisawa T. Hepatitis B surface gene 145 mutant as a minor population in hepatitis B virus carriers. *BMC Res Notes* 2012; **5**: 22 [PMID: 22233650 DOI: 10.1186/1756-0500-5-22]
39 **Mele A**, Tancredi F, Romanò L, Giuseppone A, Colucci M, Sangiuolo A, Lecce R, Adamo B, Tosti ME, Taliani G, Zanetti AR. Effectiveness of hepatitis B vaccination in babies born to hepatitis B surface antigen-positive mothers in Italy. *J Infect Dis* 2001; **184**: 905-908 [PMID: 11509998 DOI: 10.1086/323396]
40 **Torresi J**, Earnest-Silveira L, Deliyannis G, Edgtton K, Zhuang H, Locarnini SA, Fyfe J, Sozzi T, Jackson DC. Reduced antigenicity of the hepatitis B virus HBsAg protein arising as a consequence of sequence changes in the overlapping polymerase gene that are selected by lamivudine therapy. *Virology* 2002; **293**: 305-313 [PMID: 11886250 DOI: 10.1006/viro.2001.1246]
41 **Zuckerman JN**, Zuckerman AJ. Mutations of the surface protein of hepatitis B virus. *Antiviral Res* 2003; **60**: 75-78 [PMID: 14638401 DOI: 10.1016/j.antiviral.2003.08.013]
42 **Margeridon-Thermet S**, Shulman NS, Ahmed A, Shahriar R, Liu T, Wang C, Holmes SP, Babrzadeh F, Gharizadeh B, Hanczaruk B, Simen BB, Egholm M, Shafer RW. Ultra-deep pyrosequencing

of hepatitis B virus quasispecies from nucleoside and nucleotide reverse-transcriptase inhibitor (NRTI)-treated patients and NRTI-naive patients. *J Infect Dis* 2009; **199**: 1275-1285 [PMID: 19301976 DOI: 10.1086/597808]

43 **Araujo NM**, Vianna CO, Moraes MT, Gomes SA. Expression of Hepatitis B virus surface antigen (HBsAg) from genotypes A, D and F and influence of amino acid variations related or not to genotypes on HBsAg detection. *Braz J Infect Dis* 2009; **13**: 266-271 [PMID: 20231988 DOI: 10.1590/S1413-86702009000400005]

44 **Geretti AM**, Patel M, Sarfo FS, Chadwick D, Verheyen J, Fraune M, Garcia A, Phillips RO. Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana. *J Clin Microbiol* 2010; **48**: 3223-3230 [PMID: 20631103 DOI: 10.1128/JCM.02231-09]

45 **Dunford L**, Carr MJ, Dean J, Nguyen LT, Ta Thi TH, Nguyen BT, Connell J, Coughlan S, Nguyen HT, Hall WW, Thi LA. A multicentre molecular analysis of hepatitis B and blood-borne virus coinfections in Viet Nam. *PLoS One* 2012; **7**: e39027 [PMID: 22720022 DOI: 10.1371/journal.pone.0039027]

46 **Kuzin SN**, Zabotina EE, Zabelin NN, Kudriavtseva EN, Samokhvalov EI, Borisova OV, Manina TA, Lisitsina EV, Kuzina LE, Malyshev NA, Lavrov VF. [Heterogeneity of hepatitis B virus and diagnostic potential of modern test systems for the detection of HBsAg]. *Zh Mikrobiol Epidemiol Immunobiol* 2012; (**1**): 68-75 [PMID: 22442974]

47 **Lin YM**, Jow GM, Mu SC, Chen BF. Naturally occurring hepatitis B virus B-cell and T-cell epitope mutants in hepatitis B vaccinated children. *ScientificWorldJournal* 2013; **2013**: 571875 [PMID: 24379746 DOI: 10.1155/2013/571875]

48 **Bian T**, Yan H, Shen L, Wang F, Zhang S, Cao Y, Zhang S, Zhang Y, Bi S. Change in hepatitis B virus large surface antigen variant prevalence 13 years after implementation of a universal vaccination program in China. *J Virol* 2013; **87**: 12196-12206 [PMID: 24006443 DOI: 10.1128/JVI.02127-13]

49 **Magiorkinis E**, Paraskevis D, Pavlopoulou ID, Kantzanou M, Haida C, Hatzakis A, Boletis IN. Renal transplantation from hepatitis B surface antigen (HBsAg)-positive donors to HBsAg-negative recipients: a case of post-transplant fulminant hepatitis associated with an extensively mutated hepatitis B virus strain and review of the current literature. *Transpl Infect Dis* 2013; **15**: 393-399 [PMID: 23773581 DOI: 10.1111/tid.12094]

50 **Servant-Delmas A**, Mercier-Darty M, Ly TD, Wind F, Alloui C, Sureau C, Laperche S. Variable capacity of 13 hepatitis B virus surface antigen assays for the detection of HBsAg mutants in blood samples. *J Clin Virol* 2012; **53**: 338-345 [PMID: 22296790 DOI: 10.1016/j.jcv.2012.01.003]

51 **Wu C**, Shi H, Wang Y, Lu M, Xu Y, Chen X. A case of hepatitis B reactivation due to the hepatitis B virus escape mutant in a patient undergoing chemotherapy. *Virol Sin* 2012; **27**: 369-372 [PMID: 23180290 DOI: 10.1007/s12250-012-3284-3]

52 **Salpini R**, Colagrossi L, Bellocchi MC, Surdo M, Becker C, Alteri C, Aragri M, Ricciardi A, Armenia D, Pollicita M, Di Santo F, Carioti L, Louzoun Y, Mastroianni CM, Lichtner M, Paoloni M, Esposito M, D'Amore C, Marrone A, Marignani M, Sarrecchia C, Sarmati L, Andreoni M, Angelico M, Verheyen J, Perno CF, Svicher V. Hepatitis B surface antigen genetic elements critical for immune escape correlate with hepatitis B virus reactivation upon immunosuppression. *Hepatology* 2015; **61**: 823-833 [PMID: 25418031 DOI: 10.1002/hep.27604]

53 **Bahramali G**, Sadeghizadeh M, Amini-Bavil-Olyaee S, Alavian SM, Behzad-Behbahani A, Adeli A, Aghasadeghi MR, Amini S, Mahboudi F. Clinical, virologic and phylogenetic features of hepatitis B infection in Iranian patients. *World J Gastroenterol* 2008; **14**: 5448-5453 [PMID: 18803358 DOI: 10.3748/wjg.14.5448]

54 **Chamni N**, Louisirirotchanakul S, Oota S, Sakuldamrongpanish T, Saldanha J, Chongkolwatana V, Phikulsod S. Genetic characterization and genotyping of hepatitis B virus (HBV) isolates from donors with an occult HBV infection. *Vox Sang* 2014; **107**: 324-332 [PMID: 25040474 DOI: 10.1111/vox.12178]

55 **Youssef A**, Yano Y, El-Sayed Zaki M, Utsumi T, Hayashi Y. Characteristics of hepatitis viruses among Egyptian children with acute hepatitis. *Int J Oncol* 2013; **42**: 1459-1465 [PMID: 23404231 DOI: 10.3892/ijo.2013.1822]

56 **Hamkar R**, Aghakhani A, Soufian S, Banifazl M, Ghavami N, Nadri M, Sofian M, Ahmadi F, Razeghi E, Eslamifar A, Ramezani A. Surface gene mutations of hepatitis B virus among high-risk patients with occult hepatitis B virus infection. *Diagn Microbiol Infect Dis* 2010; **66**: 285-291 [PMID: 19903586 DOI: 10.1016/j.diagmicrobio.2009.10.006]

57 **Datta S**, Ghosh A, Dasgupta D, Ghosh A, Roychoudhury S, Roy G, Das S, Das K, Gupta S, Basu K, Basu A, Datta S, Chowdhury A, Banerjee S. Novel point and combo-mutations in the genome of hepatitis B virus-genotype D: characterization and impact on liver disease progression to hepatocellular carcinoma. *PLoS One* 2014; **9**: e110012 [PMID: 25333524 DOI: 10.1371/journal.pone.0110012]

58 **Yeung P**, Wong DK, Lai CL, Fung J, Seto WK, Yuen MF. Profile of pre-S deletions in the natural history of chronic hepatitis B infection. *J Med Virol* 2010; **82**: 1843-1849 [PMID: 20872710 DOI: 10.1002/jmv.21901]

59 **Kim H**, Lee SA, Kim DW, Lee SH, Kim BJ. Naturally occurring mutations in large surface genes related to occult infection of hepatitis B virus genotype C. *PLoS One* 2013; **8**: e54486 [PMID: 23349904 DOI: 10.1371/journal.pone.0054486]

60 **Zhang D**, Dong P, Zhang K, Deng L, Bach C, Chen W, Li F, Protzer U, Ding H, Zeng C. Whole genome HBV deletion profiles and the accumulation of preS deletion mutant during antiviral treatment. *BMC Microbiol* 2012; **12**: 307 [PMID: 23272650 DOI: 10.1186/1471-2180-12-307]

61 **Zanetti AR**, Tanzi E, Manzillo G, Maio G, Sbreglia C, Caporaso N, Thomas H, Zuckerman AJ. Hepatitis B variant in Europe. *Lancet* 1988; **2**: 1132-1133 [PMID: 2460710 DOI: 10.1016/S0140-6736(88)90541-7]

62 **Carman WF**, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; **336**: 325-329 [PMID: 1697396 DOI: 10.1016/0140-6736(90)91874-A]

63 **Hsu HY**, Chang MH, Liaw SH, Ni YH, Chen HL. Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. *Hepatology* 1999; **30**: 1312-1317 [PMID: 10534356 DOI: 10.1002/hep.510300511]

64 **François G**, Kew M, Van Damme P, Mphahlele MJ, Meheus A. Mutant hepatitis B viruses: a matter of academic interest only or a problem with far-reaching implications? *Vaccine* 2001; **19**: 3799-3815 [PMID: 11427251]

65 **Ireland JH**, O'Donnell B, Basuni AA, Kean JD, Wallace LA, Lau GK, Carman WF. Reactivity of 13 in vitro expressed hepatitis B surface antigen variants in 7 commercial diagnostic assays. *Hepatology* 2000; **31**: 1176-1182 [PMID: 10796895 DOI: 10.1053/he.2000.6407]

66 **Lee KM**, Kim YS, Ko YY, Yoo BM, Lee KJ, Kim JH, Hahm KB, Cho SW. Emergence of vaccine-induced escape mutant of hepatitis B virus with multiple surface gene mutations in a Korean child. *J Korean Med Sci* 2001; **16**: 359-362 [PMID: 11410701]

67 **Moerman B**, Moons V, Sommer H, Schmitt Y, Stetter M. Evaluation of sensitivity for wild type and mutant forms of hepatitis B surface antigen by four commercial HBsAg assays. *Clin Lab* 2004; **50**: 159-162 [PMID: 15074469]

68 **Seddigh-Tonekaboni S**, Lim WL, Young B, Hou JL, Waters J, Luo KX, Thomas HC, Karayiannis P. Hepatitis B surface antigen variants in vaccinees, blood donors and an interferon-treated patient. *J Viral Hepat* 2001; **8**: 154-158 [PMID: 11264736 DOI: 10.1046/j.1365-2893.2001.00275.x]

69 **Zuckerman AJ**, Zuckerman JN. Molecular epidemiology of hepatitis B virus mutants. *J Med Virol* 1999; **58**: 193-195 [PMID: 10447411]

70 **Oon CJ**, Chen WN, Goo KS, Goh KT. Intra-familial evidence of horizontal transmission of hepatitis B virus surface antigen mutant G145R. *J Infect* 2000; **41**: 260-264 [PMID: 11120616 DOI: 10.1053/jinf.2000.0751]

71 **Chakravarty R**, Neogi M, Roychowdhury S, Panda CK. Presence of hepatitis B surface antigen mutant G145R DNA in the peripheral blood leukocytes of the family members of an asymptomatic carrier and evidence of its horizontal transmission. *Virus Res* 2002; **90**: 133-141 [PMID: 12457969 DOI: 10.1016/S0168-1702(02)00147-8]

72 **He C**, Nomura F, Itoga S, Isobe K, Nakai T. Prevalence of vaccine-induced escape mutants of hepatitis B virus in the adult population in China: a prospective study in 176 restaurant employees. *J Gastroenterol Hepatol* 2001; **16**: 1373-1377 [PMID: 11851835 DOI: 10.1046/j.1440-1746.2001.02654.x]

73 **Ngui SL**, O'Connell S, Eglin RP, Heptonstall J, Teo CG. Low detection rate and maternal provenance of hepatitis B virus S gene mutants in cases of failed postnatal immunoprophylaxis in England and Wales. *J Infect Dis* 1997; **176**: 1360-1365 [PMID: 9359739 DOI: 10.1086/514133]

74 **Oon CJ**, Lim GK, Ye Z, Goh KT, Tan KL, Yo SL, Hopes E, Harrison TJ, Zuckerman AJ. Molecular epidemiology of hepatitis B virus vaccine variants in Singapore. *Vaccine* 1995; **13**: 699-702 [PMID: 7483783]

75 **Coleman PF**, Chen YC, Mushahwar IK. Immunoassay detection of hepatitis B surface antigen mutants. *J Med Virol* 1999; **59**: 19-24 [PMID: 10440803]

76 **Zhang R**, Wang L, Li J. Hepatitis B virus transfusion risk in China: proficiency testing for the detection of hepatitis B surface antigen. *Transfus Med* 2010; **20**: 322-328 [PMID: 20409073 DOI: 10.1111/j.1365-3148.2010.01007.x]

77 **Sticchi L**, Caligiuri P, Cacciani R, Alicino C, Bruzzone B. Epidemiology of HBV S-gene mutants in the Liguria Region, Italy: Implications for surveillance and detection of new escape variants. *Hum Vaccin Immunother* 2013; **9**: 568-571 [PMID: 23296324 DOI: 10.4161/hv.23236]

78 **Laoi BN**, Crowley B. Molecular characterization of hepatitis B virus (HBV) isolates, including identification of a novel recombinant, in patients with acute HBV infection attending an Irish hospital. *J Med Virol* 2008; **80**: 1554-1564 [PMID: 18649329 DOI: 10.1002/jmv.21273]

79 **Luongo M**, Critelli R, Grottola A, Gitto S, Bernabucci V, Bevini M, Vecchi C, Montagnani G, Villa E. Acute hepatitis B caused by a vaccine-escape HBV strain in vaccinated subject: sequence analysis and therapeutic strategy. *J Clin Virol* 2015; **62**: 89-91 [PMID: 25542480 DOI: 10.1016/j.jcv.2014.11.029]

80 **Foy MC**, Thio CL, Hwang HS, Saulynas M, Hamilton JP, Fine DM, Atta MG. False-negative hepatitis B virus (HBV) surface antigen in a vaccinated dialysis patient with a high level of HBV DNA in the United States. *Clin Vaccine Immunol* 2012; **19**: 820-822 [PMID: 22441395 DOI: 10.1128/CVI.05696-11]

81 **Kay A**, Zoulim F. Hepatitis B virus genetic variability and evolution. *Virus Res* 2007; **127**: 164-176 [PMID: 17383765 DOI: 10.1016/j.virusres.2007.02.021]

82 **Fan YF**, Lu CC, Chen WC, Yao WJ, Wang HC, Chang TT, Lei HY, Shiau AL, Su IJ. Prevalence and significance of hepatitis B virus (HBV) pre-S mutants in serum and liver at different replicative stages of chronic HBV infection. *Hepatology* 2001; **33**: 277-286 [PMID: 11124846 DOI: 10.1053/jhep.2001.21163]

83 **Chisari FV**, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; **13**: 29-60 [PMID: 7612225 DOI: 10.1146/annurev.iy.13.040195.000333]

84 **Wang HC**, Huang W, Lai MD, Su IJ. Hepatitis B virus pre-S mutants, endoplasmic reticulum stress and hepatocarcinogenesis. *Cancer Sci* 2006; **97**: 683-688 [PMID: 16863502 DOI: 10.1111/j.1349-7006.2006.00235.x]

85 **Huy TT**, Ushijima H, Win KM, Luengrojanakul P, Shrestha PK, Zhong ZH, Smirnov AV, Taltavull TC, Sata T, Abe K. High prevalence of hepatitis B virus pre-s mutant in countries where it is endemic and its relationship with genotype and chronicity. *J Clin Microbiol* 2003; **41**: 5449-5455 [PMID: 14662924 DOI: 10.1128/JCM.41.12.5449-5455.2003]

86 **Sugauchi F**, Ohno T, Orito E, Sakugawa H, Ichida T, Komatsu M, Kuramitsu T, Ueda R, Miyakawa Y, Mizokami M. Influence of hepatitis B virus genotypes on the development of preS deletions and advanced liver disease. *J Med Virol* 2003; **70**: 537-544 [PMID: 12794715 DOI: 10.1002/jmv.10428]

87 **Raimondo G**, Costantino L, Caccamo G, Pollicino T, Squadrito G, Cacciola I, Brancatelli S. Non-sequencing molecular approaches to identify preS2-defective hepatitis B virus variants proved to be associated with severe liver diseases. *J Hepatol* 2004; **40**: 515-519 [PMID: 15123368 DOI: 10.1016/j.jhep.2003.11.025]

88 **Chen BF**, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology* 2006; **130**: 1153-1168 [PMID: 16618410]

89 **Lin CL**, Liu CH, Chen W, Huang WL, Chen PJ, Lai MY, Chen DS, Kao JH. Association of pre-S deletion mutant of hepatitis B virus with risk of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; **22**: 1098-1103 [PMID: 17608857 DOI: 10.1111/j.1440-1746.2006.04515.x]

90 **Choi MS**, Kim DY, Lee DH, Lee JH, Koh KC, Paik SW, Rhee JC, Yoo BC. Clinical significance of pre-S mutations in patients with genotype C hepatitis B virus infection. *J Viral Hepat* 2007; **14**: 161-168 [PMID: 17305881 DOI: 10.1111/j.1365-2893.2006.00784.x]

91 **Gao ZY**, Li T, Wang J, Du JM, Li YJ, Li J, Lu FM, Zhuang H. Mutations in preS genes of genotype C hepatitis B virus in patients with chronic hepatitis B and hepatocellular carcinoma. *J Gastroenterol* 2007; **42**: 761-768 [PMID: 17876546 DOI: 10.1007/s00535-007-2085-1]

92 **Mun HS**, Lee SA, Jee Y, Kim H, Park JH, Song BC, Yoon JH, Kim YJ, Lee HS, Hyun JW, Hwang ES, Kook YH, Kim BJ. The prevalence of hepatitis B virus preS deletions occurring naturally in Korean patients infected chronically with genotype C. *J Med Virol* 2008; **80**: 1189-1194 [PMID: 18461612 DOI: 10.1002/jmv.21208]

93 **Fang ZL**, Sabin CA, Dong BQ, Wei SC, Chen QY, Fang KX, Yang JY, Huang J, Wang XY, Harrison TJ. Hepatitis B virus pre-S deletion mutations are a risk factor for hepatocellular carcinoma: a matched nested case-control study. *J Gen Virol* 2008; **89**: 2882-2890 [PMID: 18931087 DOI: 10.1099/vir.0.2008/002824-0]

94 **Cao Z**, Bai X, Guo X, Jin Y, Qian G, Tu H. High prevalence of hepatitis B virus pre-S mutation and its association with hepatocellular carcinoma in Qidong, China. *Arch Virol* 2008; **153**: 1807-1812 [PMID: 18726170 DOI: 10.1007/s00705-008-0176-9]

95 **Chen CH**, Changchien CS, Lee CM, Hung CH, Hu TH, Wang JH, Wang JC, Lu SN. Combined mutations in pre-s/surface and core promoter/precore regions of hepatitis B virus increase the risk of hepatocellular carcinoma: a case-control study. *J Infect Dis* 2008; **198**: 1634-1642 [PMID: 18939932 DOI: 10.1086/592990]

96 **Jang JS**, Kim HS, Kim HJ, Shin WG, Kim KH, Lee JH, Kim HY, Kim DJ, Lee MS, Park CK, Jeong BH, Kim YS, Jang MK. Association of concurrent hepatitis B surface antigen and antibody to hepatitis B surface antigen with hepatocellular carcinoma in chronic hepatitis B virus infection. *J Med Virol* 2009; **81**: 1531-1538 [PMID: 19623669 DOI: 10.1002/jmv.21577]

97 **Yeung P**, Wong DK, Lai CL, Fung J, Seto WK, Yuen MF. Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in chronic hepatitis B. *J Infect Dis* 2011; **203**: 646-654 [PMID: 21227916 DOI: 10.1093/infdis/jiq096]

98 **Lee MH**, Kim do Y, Kim JK, Chang HY, Kang SH, Ryu HJ, Ju HL, Kim SU, Lee JM, Park JY, Han KH, Chon CY, Ahn SH. Combination of preS deletions and A1762T/G1764A mutations in HBV subgenotype C2 increases the risk of developing HCC. *Intervirology* 2012; **55**: 296-302 [PMID: 21865669 DOI: 10.1159/000329941]

99 **Kao JH**, Liu CJ, Jow GM, Chen PJ, Chen DS, Chen BF. Fine mapping of hepatitis B virus pre-S deletion and its association with hepatocellular carcinoma. *Liver Int* 2012; **32**: 1373-1381 [PMID: 22676233 DOI: 10.1111/j.1478-3231.2012.02826.x]

100 **Sinn DH**, Choi MS, Gwak GY, Paik YH, Lee JH, Koh KC, Paik SW, Yoo BC. Pre-s mutation is a significant risk factor for hepatocellular carcinoma development: a long-term retrospective cohort study. *Dig Dis Sci* 2013; **58**: 751-758 [PMID: 23053886 DOI: 10.1007/s10620-012-2408-9]

101 **Zhao ZM**, Jin Y, Gan Y, Zhu Y, Chen TY, Wang JB, Sun Y, Cao ZG, Qian GS, Tu H. Novel approach to identifying the hepatitis B virus pre-S deletions associated with hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 13573-13581 [PMID: 25309088 DOI: 10.3748/wjg.v20.i37.13573]

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

103 **Abe K**, Thung SN, Wu HC, Tran TT, Le Hoang P, Truong KD, Inui A, Jang JJ, Su IJ. Pre-S2 deletion mutants of hepatitis B virus could have an important role in hepatocarcinogenesis in Asian children. *Cancer Sci* 2009; **100**: 2249-2254 [PMID: 19719772 DOI: 10.1111/j.1349-7006.2009.01309.x]

104 **Huang HP**, Hsu HY, Chen CL, Ni YH, Wang HY, Tsuei DJ, Chiang CL, Tsai YC, Chen HL, Chang MH. Pre-S2 deletions of hepatitis B virus and hepatocellular carcinoma in children. *Pediatr Res* 2010; **67**: 90-94 [PMID: 19816238 DOI: 10.1203/PDR.0b013e3181c1b0b7]

105 **Thongbai C**, Sa-nguanmoo P, Kranokpiruk P, Poovorawan K, Poovorawan Y, Tangkijvanich P. Hepatitis B virus genetic variation and TP53 R249S mutation in patients with hepatocellular carcinoma in Thailand. *Asian Pac J Cancer Prev* 2013; **14**: 3555-3559 [PMID: 23886144 DOI: 10.7314/APJCP.2013.14.6.3555]

106 **Bläckberg J**, Kidd-Ljunggren K. Mutations within the hepatitis B virus genome among chronic hepatitis B patients with hepatocellular carcinoma. *J Med Virol* 2003; **71**: 18-23 [PMID: 12858404 DOI: 10.1002/jmv.10458]

107 **Lee SA**, Kim KJ, Kim DW, Kim BJ. Male-specific W4P/R mutation in the pre-S1 region of hepatitis B virus, increasing the risk of progression of liver diseases in chronic patients. *J Clin Microbiol* 2013; **51**: 3928-3936 [PMID: 24025913 DOI: 10.1128/JCM.01505-13]

108 **Sung JJ**, Tsui SK, Tse CH, Ng EY, Leung KS, Lee KH, Mok TS, Bartholomeusz A, Au TC, Tsoi KK, Locarnini S, Chan HL. Genotype-specific genomic markers associated with primary hepatomas, based on complete genomic sequencing of hepatitis B virus. *J Virol* 2008; **82**: 3604-3611 [PMID: 18216102 DOI: 10.1128/JVI.01197-07]

109 **Zhu Y**, Jin Y, Guo X, Bai X, Chen T, Wang J, Qian G, Groopman JD, Gu J, Li J, Tu H. Comparison study on the complete sequence of hepatitis B virus identifies new mutations in core gene associated with hepatocellular carcinoma. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2623-2630 [PMID: 20699378 DOI: 10.1158/1055-9965.EPI-10-0469]

110 **Hung CH**, Chen CH, Lee CM, Hu TH, Lu SN, Wang JH, Huang CM. Role of viral genotypes and hepatitis B viral mutants in the risk of hepatocellular carcinoma associated with hepatitis B and C dual infection. *Intervirology* 2013; **56**: 316-324 [PMID: 23838434 DOI: 10.1159/000350738]

P- Reviewer: He JY, Horvat R **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.i27.2740

World J Hepatol 2015 November 28; 7(27): 2740-2748
ISSN 1948-5182 (online)

MINIREVIEWS

Cutaneous manifestations of hepatitis C in the era of new antiviral agents

Simone Garcovich, Matteo Garcovich, Rodolfo Capizzi, Antonio Gasbarrini, Maria Assunta Zocco

Simone Garcovich, Rodolfo Capizzi, Department of Dermatology, Policlinico A. Gemelli, Catholic University of Sacred Heart, 00168 Rome, Italy

Matteo Garcovich, Antonio Gasbarrini, Maria Assunta Zocco, UOC Internal Medicine and Gastroenterology, Policlinico A. Gemelli, Catholic University of Sacred Heart, 00168 Rome, Italy

Author contributions: Garcovich S contributed to the revision of the literature, to the drawing up the review and to the figures; Garcovich M contributed to the revision of the literature, the drawing up of the review and to the revision of the English; Capizzi R contributed to the revision of the review, to the revision of the literature and to the bibliography; Gasbarrini A contributed to the revision of the review, to the revision of the literature and to the bibliography; Zocco MA contributed to the revision of the review, to the revision of the English and to the final approval of the article to be published.

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

Correspondence to: Dr. Matteo Garcovich, UOC Internal Medicine and Gastroenterology, Policlinico A. Gemelli, Catholic University of Sacred Heart, Largo A. Gemelli, 8, 00168 Rome, Italy. matteogarcovich@yahoo.it
Telephone: +39-06-30156018
Fax: +39-06-35502775

Received: July 15, 2015
Peer-review started: July 24, 2015
First decision: September 22, 2015
Revised: October 23, 2015
Accepted: November 23, 2015

Article in press: November 25, 2015
Published online: November 28, 2015

Abstract

The association of chronic hepatitis C virus (HCV) infection with a wide spectrum of cutaneous manifestations has been widely reported in the literature, with varying strength of epidemiological association. Skin diseases which are certainly related with chronic HCV infection due to a strong epidemiological and pathogenetic association are mixed cryoglobulinemia, lichen planus and porphyria cutanea tarda. Chronic pruritus and necrolytic acral erythema are conditions that may share a possible association with HCV infection, while several immune-mediated inflammatory skin conditions, such as psoriasis, chronic urticaria and vitiligo, have been only anecdotally reported in the setting of chronic HCV infection. Traditional interferon-based treatment regimens for HCV infection are associated with substantial toxicity and a high-risk of immune-related adverse events, while the advent of new direct-acting antivirals with sustained virological response and improved tolerability will open the door for all-oral, interferon-free regimens. In the new era of these direct acting antivirals there will be hopefully a renewed interest in extra-hepatic manifestations of HCV infection. The aim of the present paper is to review the main cutaneous HCV-related disorders - mixed cryoglobulinemia, lichen planus, porphyria cutanea tarda and chronic pruritus - and to discuss the potential impact of new antiviral treatments on the course of these extra-hepatic manifestations of chronic HCV infection.

Key words: Chronic liver disease; Hepatitis C virus; Interferon-free agents; Extra-hepatic manifestation; Skin diseases; Mixed cryoglobulinemia; Lichen planus

Core tip: It is known that the association of hepatitis C virus (HCV) infection with a wide spectrum of cutaneous manifestations has been widely reported in the literature. In the new era of direct acting antivirals there will be hopefully a renewed interest in the diagnosis and treatment of extra-hepatic manifestations of HCV infection. The aim of the present paper is to review the main cutaneous HCV-related disorders and to discuss the potential impact of new antiviral treatments on the course of these extra-hepatic manifestations of chronic HCV infection in order to help all the clinicians dealing with patients undergoing antiviral treatment.

Garcovich S, Garcovich M, Capizzi R, Gasbarrini A, Zocco MA. Cutaneous manifestations of hepatitis C in the era of new antiviral agents. *World J Hepatol* 2015; 7(27): 2740-2748 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i27/2740.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i27.2740

INTRODUCTION

Hepatitis C virus (HCV) is known to induce both hepatic and extra-hepatic manifestations. Chronic HCV infection is now considered a systemic disease with multi-organ involvement. The association of chronic HCV infection with a wide spectrum of cutaneous manifestations has been widely reported in the literature, with varying strength of epidemiological association. In registry-based studies, about 17% of HCV patients present at least one skin manifestation, which can be directly or indirectly induced by chronic HCV infection[1]. In Table 1, the cutaneous extra-hepatic manifestations (cEHMs) are summarized according to the quality of epidemiological and pathogenetic relationship with chronic HCV-infection.

The recognition of cEHMs is important for the clinician for several reasons. First, to ensure early diagnosis by routine HCV-testing in patients with higher risk of infection than the general population; Second, some of these manifestations may improve after effective antiviral treatment without or in combination with skin-targeted therapy; Third, cEHMs may identify a difficult-to treat patient subset, requiring a tailored antiviral regimen as well as multidisciplinary care.

Skin disease which are certainly related with chronic HCV infection are mixed cryoglobulinemia (MC), lichen planus (LP) and porphyria cutanea tarda (PCT). In these disorders testing for HCV infection is recommended, in the light of the strong epidemiological and pathogenetic association. Psoriasis, chronic pruritus and necrolytic acral erythema are conditions sharing a possible association with HCV infection, although they lack definitive epidemiological and experimental evidence to support universal screening for HCV. Moreover, other immune-mediated inflammatory skin conditions, such as chronic urticaria and vitiligo, have been anecdotally reported in the setting of chronic HCV infection in retrospective studies and case-series. The complex interplay between the cutaneous immune system and the HCV-induced immune response in a genetically predisposed individual can induce or change the course of inflammatory skin conditions. For example, chronic HCV infection is a major infectious comorbidity complicating the therapeutic management of psoriasis, which is increasingly based on systemic immune-modulating agents, such as methotrexate, cyclosporine and TNF-alpha inhibitors.

Thus, the nature of HCV extra-hepatic manifestations requires a multidisciplinary approach, in order to ensure a correct diagnosis and optimize therapeutic interventions.

Traditional interferon (IFN)-based treatment regimens for HCV infection were associated with substantial toxicity and a high-risk of immune-related adverse events. These included a plethora of IFN-induced manifestations and the induction or worsening of immune-mediated, inflammatory skin conditions, such as psoriasis, eczema, lichenoid eruptions, pruritus and alopecia areata. IFN-related cutaneous adverse events have to be differentiated clinically from true, primary cEHMs of HCV infection.

In 2011, boceprevir and telaprevir were licensed for use in HCV as new first wave, first-generation direct-acting antivirals (DAAs), still to be administered in combination with IFN and ribavirin. To overcome this limit, in 2014 three new HCV DAAs (Sofosbuvir, Simeprevir and Daclatasvir) have been licensed worldwide for use as IFN-free, a major achievement in the field of hepatology[2]. For the first time in three decades the availability of new direct-acting antivirals with sustained virological response and improved tolerability will open the door for all-oral, IFN-free regimens. Nevertheless, these new treatment regimens have to be tested in real-life clinical scenarios, such as difficult to treat patient subsets with advanced fibrotic disease and/or substantial comorbidity. In the new era of these direct acting antivirals there will be hopefully a renewed interest in extra-hepatic manifestations of HCV infection.

The aim of the present paper is to review the main cutaneous HCV-related disorders - MC, LP, PCT and chronic pruritus - and to discuss the potential impact of new antiviral treatments on the course of these extra-hepatic manifestations of chronic HCV infection.

MIXED CRYOGLOBULINEMIA - CUTANEOUS MANIFESTATIONS

Skin is the most frequently involved organ of MC-related vascular inflammation in chronic HCV-infected patients and clinical manifestations include palpable purpura (21%) with or without livedo, Raynaud phenomenon (15%), pruritus (8%), urticaria (6%) and leg ulcers. Leucocytoclastic small vessel vasculitis is the main histological correlate of cutaneous lesions[3].

Systemic vasculitis involving the kidneys, heart and central nervous system characterizes the severe forms

Table 1 Classification of cutaneous extrahepatic manifestations of chronic hepatitis C virus infection

Cutaneous conditions with defined epidemiological and/or pathogenetic association
Mixed cryoglobulinemia
Lichen planus
Porphyria cutanea tarda
Cutaneous conditions with possible association
Pruritus (prurigo nodularis/lichen simplex cronicus)
Necrolytic acral erythema
Cutaneous conditions with anecdotal association
Psoriasis
Chronic urticaria
Vitiligo
Erythema multiforme
Erythema nodosum
Pyoderma gangrenosum

of MC disease, with increased risk of death due to end-organ complications, progression of liver disease and lymphoma. In severe MC disease, cutaneous lesions comprise hemorrhagic ulcers and skin necrosis, due to immune-complex deposition in small-to medium sized blood vessel with complement activation[4].

In patients with mild- to mode-rate symptomatic HCV-related MC, an optimal antiviral treatment is warranted in order to attain a sustained virological response and cutaneous improvement. Treatment goals in MC patients are clinical remission of MC-related organ manifestations, clearance of cryoglobulins, in addition to sustained virological responses, while limiting the use of immune-modulating agents with risk of liver toxicity. In the majority of MC patients, there is a good correlation of SVR and clinical response, with remission of end-organ manifestations and symptoms. Other useful laboratory response markers are clearance of cryoglobulins, complement levels (C3, C4, CH50 activity) and rheumatoid factor acitivity. On the other hand, patients who relapse after initial HCV antiviral treatment frequently develop a clinical relapse of MC disease[5].

Combination antiviral therapy with pegylated IFN/ribavirin yields a SVR in almost 52% of patients with symptomatic MC disease, as recently shown in a meta-analysis of ten clinical studies[6]. Triple antiviral therapy with Peg-IFN-alpha/ribavirin and a first-generation protease inhibitor (telaprevir or boceprevir) in severe HCV-associated MC vasculitis determined a complete clinical response of MC and SVR in 66.7% of treated patients of an open-label cohort study. In the same study, the improvement of clinical responses was at the costs of a higher rate of serious adverse events (46.6%), mostly haematological ones, in comparison to dual antiviral regimens[7]. Antiviral treatment with second-generation protease inhibitors could have a potential impact on the course of HCV-associated symptomatic MC, due to an improved tolerability profile and high efficacy. Preliminary clinical experience with sofosbuvir in dual or triple antiviral regimens for symptomatic MC highlighted the need for longer treatment durations (> 12 wk), especially in the background of advanced liver fibrosis, in order to obtain clearance of cryoglobulins and long-lasting clinical remission[8,9].

In severe MC vasculitis with advanced renal and liver involvement, the addition of immune-modulating agents is crucial for targeting the B-cell clonal autoimmune responses and clonal expansions in the blood and liver. Rituximab, an anti-CD20 monoclonal antibody, proved to be safe and superior to conventional immunosuppressive agents (glucocorticoids, azathioprine or cyclophosphamide) in the treatment of severe HCV-associated MC vasculitis[10]. Rituximab has been used successfully in monotherapy, or in combination or sequential treatment strategies with Peg-IFN-alpha/ribavirin, optimizing treatment outcomes in patients with severe MC disease[11,12]. Symptomatic moderate-to severe MC thus identifies a distinct subset of difficult-to treat HCV patients, who require a tailored treatment approach, combining new generation antivirals and immune-modulating agents. Furthermore, symptomatic MC patients are at increased risk of haematological adverse events during IFN-based regimens and could clearly profit from new all-oral treatment strategies. Considering the under-representation of these patients in registrative trials there is a need for large, prospective, multi-center studies, assessing the impact of new direct acting antivirals on the natural history of HCV-associated MC vasculitis syndrome.

LP

LP is an inflammatory disorder involving the skin and the oro-genital mucous membranes. It is the prototypical disease belonging to the spectrum of lichenoid tissue reactions (LTRs), which are characterized by an interface-dermatitis histological pattern, with a T-cell mediated autoimmune attack on basal keratinocytes. It can affect the skin and its appendages (hair and nails) as well the mucosal surfaces of the genitalia, oesophagus, urinary tract, high respiratory tract and the eyes, with organ-specific clinical phenotypes (Figure 1A). Oral lichen planus (OLP) (Figure 1B) is the most studied clinical phenotype of LP in the setting of chronic HCV infection, with the erosive form being the most severe and recurrent clinical variant[13].

The association between LP and chronic HCV infection has been described in several epidemiological studies, with conflicting results depending on study design and country-specific prevalence of HCV burden. In hyperendemic regions such as Egypt, Japan and Southern Europe the rate of association can be as high as 35%, whereas it can decline to 0.5% in the low-incidence countries of Northern Europe. Two recent meta-analysis of published studies support the association between chronic HCV infection and LP. Patients with LP have a five-fold greater risk of HCV-infection (OR 5.4; 95%CI: 3.5-8.3) compared to control subjects. Conversely, the odds ratio for a diagnosis of LP among patients with HCV is 2.5 (95%CI: 2.0-3.1)[14]. This association

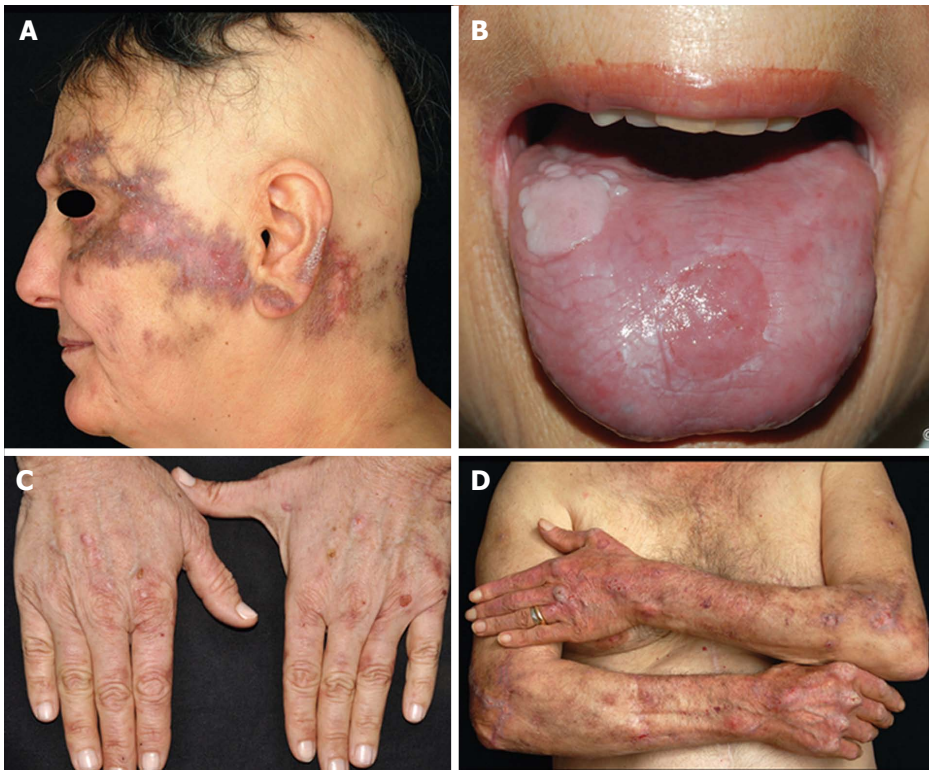

Figure 1 Cutaneous extra-hepatic manifestations of chronic hepatitis C virus infection. A: Cutaneous Lichen planus with inflammatory hyperpigmentation and cicatricial alopecia; B: Erosive oral lichen planus; C: Porphyria cutanea tarda, with typical involvement of sun-exposed acral skin; D: Chronic pruritus with secondary nodular scratch lesions, or prurigo nodularis.

proved to be not significant in the isolated cutaneous LP subtype. In summary, the variability in the association between HCV and LP can only be partially explained by a geographic effect and differences in study design, with genetic factors (HLA-DR-associations), age and IFN-based treatments also playing a role. Several authors agree to routinely check for HCV infection in patients with a clinically and histologically-confirmed diagnosis of LP disease[15,16].

The current pathogenetic hypothesis regard LP as a T-cell mediated autoimmune reaction to a viral protein or to a self-epitope shared by the virus and presented by basal keratinocytes, which are attacked by cytotoxic $CD8^+$ T cells. The immune dysregulation in LTRs is characterized by the subepithelial infiltration of $CD8^+$ cytotoxic T-cells, natural killer-cells, myeloid and plasmocytoid dendritic cells releasing Th1-cytokines, such as tumor necrosis factor (TNF)-alpha and IFN-gamma[17]. Type- Ⅰ IFNs (IFN-alpha and beta), produced by plasmocytoid dendritic cells, promote the epithelial expression of MxA protein and the recruitment of cytotoxic T-lymphocytes *via* CXCR-3/IP-10 CXCL-10 interactions. This cellular immune reaction pattern is common to many disease states, such as viral infections and cutaneous lupus erythematosus. Supposed trigger factors of lichenoid inflammation are viral antigens, cross-reactive autoantigens or xenobiotics (drugs, chemicals), which are presented by basal keratinocytes to immune cells[18]. Experimental data strongly suggest that HCV may be involved in the pathogenesis of OLP, inducing a cellular immune response directed against HCV antigens and basal cell epithelial damage. The presence of HCV-RNA in mucosal and cutaneous lesions has been demonstrated by polymerase chain reaction-based techniques in several studies, suggesting a tissue-compartmentalization of HCV, albeit with low levels of viral replication. Other studies did not replicate these findings[19,20].

Pilli *et al*[21] further demonstrated the presence of HCV-antigen specific CD4 and CD8 T-cells in the lesional cell-infiltrate of HCV^+ OLP patients, with higher frequency and IFN-gamma production than the clones in the peripheral blood. Whether the cytotoxic T-cell response may be directed against viral antigens presented by basal keratinocytes or simply induced by the local Th1-biased cytokine milieu is still under debate.

In HCV-infected patients, mucosal LP more frequently runs a chronic persistent course, with higher prevalence of erosive-ulcerative lesions and extensive disease, compared to non-infected LP patients. Treatment outcomes in patients with OLP associated with chronic HCV infection are often unsatisfactory compared to patients suffering from idiopathic disease. In addition, the evolution of oral lesions is often fluctuating, with repeated periods of relapse according to the degree of liver function decompensation, although some authors have found no correlation between the severity of LP disease and HCV-viral load or liver disease parameters[22,23]. IFN-based treatment regimens may negatively influence established LP or induce the onset

of lichenoid lesions, as type-Ⅰ IFNs are a major driver of lichenoid inflammation[24]. Accordingly, there is a relative contraindication for IFN-based antiviral treatment in patients with concomitant LP and chronic-active HCV infection.

In this patient subset, a new therapeutic strategy with IFN-free regimens (Sofosbuvir, Simeprevir and/or Daclatasvir according to genotype) should be taken into account, optimizing treatment outcomes for both liver and extra-hepatic involvement. As yet, there are no published reports on the impact of IFN-free regimens on the clinical course of LP.

In patients with stable liver disease and symptomatic muco-cutaneous LP, treatment should be aimed at suppressing lichenoid-tissue inflammation with immune-modulating agents, while reducing the risk of HCV replication or liver-toxicity. Treatment of LP is based on a step-wise approach with the use of topical and/or systemic immune-modulating agents, depending on disease's course (acute *vs* chronic), extent (localized *vs* multifocal disease) and impact on patient's quality of life[25]. Systemic treatment of LP should be restricted to severe mucosal or generalized-cutaneous disease, and includes different drugs, such as retinoids (acitretin), steroids and immunosuppressive agents (methotrexate, cyclosporine), albeit with a lack of high-quality evidence and clinical guidelines[26]. In HCV-infected patients, systemic treatment of LP needs to be tailored on a case-by case basis, with strict monitoring of liver-function and HCV-replication parameters. As systemic steroids carry a substantial risk of reactivating HVC replication, treatment with alternative systemic agents should be preferred. Cyclosporine A has been successfully used for the treatment of severe, mucosal LP and its safety in HCV-infected patients has been reported in the setting of transplantation and autoimmune diseases[27]. Moreover, in patients with chronic-active HCV infection and concomitant, severe LP the combination of new DAAs and cyclosporine A is possible, with a lower risk of drug interactions compared to first-generation protease-inhibitors[28].

PCT AND LIVER DISEASE

PCT comprise a group of diseases resulting from an inherited or acquired dysfunction of the uroporphyrinogen decarboxylase enzyme (UROD). PCT is the most frequently occurring type of non-acute porphyria. The acquired, sporadic form of PCT (type Ⅰ) occur in predisposed individuals with deficient activity of the enzyme in the liver, which is triggered by the exposure to liver toxins (hepatotoxic aromatic hydrocarbons), drugs (alcohol, estrogens), cigarette smoking, dyalisis and hepatopatic viruses, with HCV at the forefront[29].

The epidemiological association of PCT with HCV infection is strong, with increasing rates from Northern Europe, Australia and England (20%) to Southern Europe (70%-90%). When adjusting for geographical variation and variability in study designs the association between PCT and HCV remains significant, with a reported 50% mean HCV prevalence in PCT patients[30]. Screening for HCV infection and a comprehensive assessment of liver-function is therefore mandatory in the diagnostic workup of PCT. On the other hand, in patients with active HCV-related hepatic disease routine testing for porphyrin metabolism is not recommended, as there is only a 5% reported prevalence of preclinical or overt PCT in the HCV infected patient population[31]. Iron overload (hepatic siderosis) is a critical pathogenetic event, disrupting the enzymatic activity of UROD by inducing the formation of an intracellular inhibitor, probably derived from hydroxy-methylbilane and/or uroporphyrinogen.

HCV infection is an important trigger of PCT, interacting with other hepatotoxic and genetic susceptibility factors, usually preceding the clinical expression of PCT. HCV increases the production of reactive oxygen species, leading to hepatic down-regulation of hepcidin, the key regulator of iron absorption and metabolism[32]. This contributes to iron overload in conjunction with other factors, such as genetic predisposition, further decreasing the activity of UROD below the critical threshold for deranged porphyrin metabolism[33].

The clinical aspects of PCT are characterized by typical cutaneous lesions (vesicles, bullae, erosions and crusting) developing in sun-exposed areas, resulting in scarring, milia and mottled hypo-/hyper-pigmentation (Figure 1C). Other diagnostic clues are photosensitivity, skin fragility, facial hypertrichosis and late-stage sclerodermoid plaques. Laboratory investigations are necessary for diagnosis and differentiation with other cutaneous porphyrias, as previously reviewed. There are no significant differences in the clinical presentation of PCT between HCV positive and HCV-negative cases, but the former present more advanced histological and biochemical liver disease. Genetic studies have described in PCT patients the importance of the mutation profile in the *HFE*-gene, which is linked to hereditary hemochromatosis. Up to two-thirds of PCT patients carries a mutation of the *HFE* gene (H63D or C282Y mutations), and heterozygous carriers are at a higher risk of progression to severe liver disease and fibrosis. HFE-genotyping is clinically relevant since PCT patients with homozygosity for C282Y mutation do not respond to chloroquine and should be preferentially treated with phlebotomy[34]. In a Spanish study, the same mutation was significantly associated with HCV-negative PCT patients, which presented hepatic siderosis. The H63D mutation was reportedly more frequent in HCV^+ PCT patients, with milder defects in iron haemostasis[35].

Standard of care for PCT includes photoprotection, anti-malarial drugs (chloroquine) and phlebotomy, the latter to reduce hepatic iron stores. In the setting of chronic HCV infection, effective antiviral treatment may potentially improve clinical and laboratoristic manifestations of PCT, especially when preceded by therapeutic phlebotomy. Since iron is also involved in the progression of HCV-relate liver disease, depletion of iron stores by phlebotomy can improve both conditions. In a

clinical study with IFN-based regimens, the presence of PCT was independently associated with an insufficient virological response[36]. Consequently, effective management of PCT and iron-reduction should precede antiviral therapy, as phlebotomy proved beneficial for treatment outcomes of IFN-based regimens[37]. On the other hand, occurrence of new-onset PCT has been reported in some cases during IFN/ribavirin therapy[38]. Indeed, ribavirin is known to induce haemolytic anemia, which further aggravates liver iron excess and progression to clinically manifest PCT in predisposed individuals. For this reason, studies addressing the role of new triple of quadruple-antiviral drug regimens in PCT + HCV^+ patients in combination with iron-reduction therapy are warranted. In conclusion, PCT + HCV^+ patients should undergo a comprehensive assessment of iron metabolism, hereditary hemochromatosis, *HFE*-gene mutations and evaluate exposure to hepatotoxic trigger factors in order to optimize therapeutic management.

PSORIASIS

Psoriasis is a common, chronic, immune-mediated inflammatory disease which affects the skin (plaque psoriasis) and/or joints (psoriatic arthritis). As other immune-mediated disorders, such as rheumatoid arthritis and IBDs, psoriasis results from the complex interplay between genetic factors and environmental triggers and has been recently linked to the metabolic syndrome. Moderate-to severe psoriasis and psoriatic arthritis are associated with substantial systemic inflammation and both conditions are driven by the overproduction of Th1-Th17 cytokines [TNF-alpha, IFN-gamma, interleukin (IL)-17, IL-12/23][39]. Chronic HCV infection and related liver disease represent one of the many comorbidities affecting psoriasis patients, thus representing a challenge for its clinical management. The epidemiological association between psoriasis and chronic HCV infection has been described mainly by hospital-based clinical studies and observational studies in countries with a heterogeneous burden of HCV and psoriatic disease. An increased risk of HCV infection among patient with moderate-to severe psoriasis and psoriatic arthritis has been reported in Taiwan, Japan and Italy, independently of exposure to interferon-based antiviral regimens, which are a well-known trigger of psoriasis[40-43]. Other observational studies from Italy and the United States did not confirm these findings, probably due different study-designs and limited sample of psoriatic patients[44,45]. Recently, a large case-control study further supported the association between psoriasis and HCV infection, reporting a significant odds-ratio of 1.75 (95%CI: 1.37-2.25) in the multivariate analysis. In the same study there is a significant interaction with smoking, a risk factor common to both psoriasis and progression of HCV-related liver disease[46]. Interestingly, in two hospital-based studies Imafuku *et al*[47] described a distinct patient subset with psoriasis and associated chronic HCV infection. These HCV-infected patients present a *de-novo* onset of psoriasis in higher age, with a significantly lower BMI, compared to HCV negative psoriatic controls, while maintaining a positive correlation with diabetes and hypertension. This evidence supports the role of HCV infection as a trigger factor for psoriatic disease in genetically predisposed individuals[47]. A potential confounding factor in these studies is the increased monitoring and screening procedures related to moderate-to severe psoriasis, a condition increasingly treated with a wide array of systemic immunomodulating agents. This could account for an increased HCV detection rate in these patients, which are additionally exposed to a significant risk of nonalcoholic fatty liver disease and to potential hepatotoxic drugs, such as methotrexate and retinoids. Thus, additional prospective studies are warranted in order to support the role of psoriasis as a true extrahepatic manifestation of chronic HCV infection and to evaluate the course of psoriatic disease after effective and sustained antiviral response. Screening for HBV and HCV infection is routinely performed before initiating systemic treatment of psoriasis, as a chronic active infection can have a clinical impact on treatment selection[48]. As yet there are no comprehensive and definitive recommendations addressing the issue of universal screening for HCV infection in the general psoriasis population. Treatment with immunomodulating drugs, such as TNF-alpha antagonists (etanercept, adalimumab, infliximab), has proved safe and effective in controlling psoriatic disease in HCV-positive patients, as reported in several case-series[49]. Furthermore, the risk of HCV reactivation in patients exposed to TNF-alpha inhibition was considered to be low, as only three cases of HCV-related liver disease occurred in a total of 216 exposed patients[50]. There is a need for long-term data on the safety of the new biologic agents, with TNF-alpha antagonists and IL-23/12 blockers, in the treatment of moderate-severe plaque psoriasis and psoriatic arthritis with concomitant HCV infection.

PRURITUS AND PRURITIC ERUPTIONS IN CHRONIC HCV INFECTION

Pruritus is a common symptom of hepatic diseases, mainly linked to chronic cholestatic disorders (primary biliary cirrhosis, primary sclerosing cholangitis), where it can run a chronic (> 6 wk duration) and refractory course. Although frequently overlooked by clinicians, in chronic HCV infection pruritus represent the most common extra-hepatic cutaneous manifestation, affecting up to 15% of patients in a large cohort study.

Proper clinical assessment of pruritus in HCV infection is important, as this can be an early, acute and transient symptom of a recent infection or a persistent manifestation of chronic HCV infection, possibly responding to effective antiviral treatment. Chronic pruritus (CP) is now defined as persisting more than 6 wk, involving primarily-diseased, non-inflamed or secondarily diseased

skin. CP is increasingly being recognized as the skin equivalent of pain, because it is associated with a significant impairment of quality of life and is frequently treatment-resistant[51]. In the setting of chronic HCV infection patients present mainly with two clinical pictures, either with generalized pruritus on apparently non-diseases (normal) skin, or pruritus associated with secondary scratch lesions (papules, nodules, excoriations, lichenification). Chronic scratch lesions are typical of prurigo nodularis (Figure 1D) and lichen simplex cronicus, both pruritic conditions significantly associated with an increased prevalence of HCV infection in case-control studies[52,53]. In the presence of primary, inflammatory skin changes, pruritus is related to a cutaneous disease, such as psoriasis, LP and urticaria, which are frequently observed in HCV infected patients. Despite the paucity of data on the clinical course reported in previous studies, pruritus associated with chronic HCV infection may recognize multiple pathogenetic factors[54]. Dry-skin (xerosis) and skin barrier defects, alterations of peripheral pruriception due to neuropeptide imbalance and concomitant cholestasis can all contribute to the genesis of chronic pruritus in HCV infected patients.

Recently the role of autotaxin (lysophospholipase D) and its product lysophosphatidic acid has been discovered in the pathogenesis of pruritus linked to cholestatic liver disease. Patient with cholestatic pruritus present high serum levels of lysophosphatidic acid, a potent neuronal activator, and increased autotaxin activity. Serum autotaxin activity showed a significant correlation to itch intensity, both diminishing after effective therapeutic interventions[55]. Furthermore, both autotaxin activity and lysophosphatidic acid are increased in chronic HCV infection, showing a strong correlation with liver cirrhosis stage, related complications and prognosis[56]. Further studies should address the role of autotaxin serum activity and lysophosphatidic acid as mediators linking HCV-related liver fibrosis and non-cholestatic pruritus.

During active antiviral treatment, new-onset of localized or generalized pruritus associated with primary skin lesions has to be carefully approached by the clinician, in order to differentiate dermatological adverse events from true extra-hepatic skin manifestations. Traditional treatment with IFN and ribavirin can induce pruritic skin eruption in 8%-10% of HCV-patients, as well as elicit pre-existent pruritic skin diseases, such as eczema and psoriasis[57]. First generation protease inhibitors, telaprevir and boceprevir, have also been increasingly associated (41%-61% rate) with adverse cutaneous drug reactions, most commonly grade 1-2 pruritic eczematous eruptions. Telaprevir-associated dermatitis occur more frequently (56% *vs* 34%) in comparison to IFN/ribavirin treated patients, and is constantly associated with pruritus (95% of cases) and secondary lesions (xerosis, excoriations and lichenification)[58]. In the case of progressive (grade 2) or severe-generalized (grade 3) pruritic skin eruption treatment telaprevir needs to be discontinued. The risk of severe cutaneous drug reactions, namely Stevens-Johnson Syndrome and Drug Reaction with Eosinophilia and Systemic Symptoms, has been reported in telaprevir-treated patients and early diagnostic signs (cutaneous pain, mucous membrane involvement, systemic inflammation) should be promptly detected[59]. Cutaneous adverse reactions represent an emerging issue of dual-and triple antiviral combination therapies and require effective recognition and management, in order to ensure adherence to antiviral treatment.

CONCLUSION

HCV infection is one of the main cause of chronic liver disease worldwide[60]. In the last decades clinical support for patients with chronic HCV infection has advanced rapidly due to the enhanced understanding of the patho-physiology of the disease and the improvements in therapy and prevention. Unfortunately, HCV infection is not limited to the hepatic involvement but it may lead to extra-hepatic diseases as well, in particular dermatological and mucocutaneous manifestations. In addition, dermatological adverse events are a potential concern during classic (IFN-based) and "new" (DAAs) anti-viral treatment. For this reason a throughout knowledge of cEHMs is mandatory for HCV-treating physicians, in order to deal with both hepatic and extra-hepatic diseases and to correctly address dermatological side effects during treatment. Skin disease which are certainly related with chronic HCV infection due to a strong epidemiological and pathogenetic association are MC, LP and PCT. Necrolytic acral erythema and CP are conditions that may share a possible association with HCV infection, while several immune-mediated inflammatory skin conditions, such as chronic urticaria and vitiligo, have been only anecdotally reported in the setting of chronic HCV infection. Psoriasis has been recently associated with chronic HCV-infection and notably both conditions share a common background of TNF-alpha based chronic systemic inflammation. Some of these conditions may complicate the clinical scenario of HCV infection, thus resulting in difficult-to treat patients, who may require a tailored antiviral regimen as well as multidisciplinary care. This is why the upcoming of new direct acting antivirals will help boost interest in extra-hepatic manifestations of HCV infection. Anyhow these new treatment regimens have to be tested in real-life clinical scenarios, such as patient subsets with advanced fibrotic disease and/or substantial comorbidity. It is possible but yet unproven that the more effective and rapid antiviral response observed with new IFN-free antiviral regimens will improve outcome in these clinical settings, especially when HCV infection is burdened by extra-hepatic manifestations.

REFERENCES

1 **Cacoub P**, Poynard T, Ghillani P, Charlotte F, Olivi M, Piette JC, Opolon P. Extrahepatic manifestations of chronic hepatitis C.

MULTIVIRC Group. Multidepartment Virus C. *Arthritis Rheum* 1999; **42**: 2204-2212 [PMID: 10524695]
2 **European Association for Study of Liver**. EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep.2015.03.025]
3 **Cacoub P**, Comarmond C, Domont F, Savey L, Saadoun D. Cryoglobulinemia Vasculitis. *Am J Med* 2015; **128**: 950-955 [PMID: 25837517 DOI: 10.1016/j.amjmed.2015.02.017]
4 **Ramos-Casals M**, Stone JH, Cid MC, Bosch X. The cryoglobulinaemias. *Lancet* 2012; **379**: 348-360 [PMID: 21868085 DOI: 10.1016/S0140-6736(11)60242-0]
5 **Terrier B**, Semoun O, Saadoun D, Sène D, Resche-Rigon M, Cacoub P. Prognostic factors in patients with hepatitis C virus infection and systemic vasculitis. *Arthritis Rheum* 2011; **63**: 1748-1757 [PMID: 21400476 DOI: 10.1002/art.30319]
6 **Fabrizi F**, Dixit V, Messa P. Antiviral therapy of symptomatic HCV-associated mixed cryoglobulinemia: meta-analysis of clinical studies. *J Med Virol* 2013; **85**: 1019-1027 [PMID: 23588727 DOI: 10.1002/jmv.23562]
7 **Saadoun D**, Resche Rigon M, Pol S, Thibault V, Blanc F, Pialoux G, Karras A, Bazin-Kara D, Cazorla C, Vittecoq D, Musset L, Peltier J, Decaux O, Ziza JM, Lambotte O, Cacoub P. PegIFNα/ribavirin/protease inhibitor combination in severe hepatitis C virus-associated mixed cryoglobulinemia vasculitis. *J Hepatol* 2015; **62**: 24-30 [PMID: 25135864 DOI: 10.1016/j.jhep.2014.08.015]
8 **Stine JG**, Cornella S, Shah NL. Treatment of chronic hepatitis C complicated by mixed cryoglobulinemia with new protease inhibitor, sofosbuvir. *Ann Rheum Dis* 2014; **73**: e64 [PMID: 25038237 DOI: 10.1136/annrheumdis-2014-206180]
9 **Cornella SL**, Stine JG, Kelly V, Caldwell SH, Shah NL. Persistence of mixed cryoglobulinemia despite cure of hepatitis C with new oral antiviral therapy including direct-acting antiviral sofosbuvir: A case series. *Postgrad Med* 2015; **127**: 413-417 [PMID: 25746436 DOI: 10.1080/00325481.2015.1021660]
10 **De Vita S**, Quartuccio L, Isola M, Mazzaro C, Scaini P, Lenzi M, Campanini M, Naclerio C, Tavoni A, Pietrogrande M, Ferri C, Mascia MT, Masolini P, Zabotti A, Maset M, Roccatello D, Zignego AL, Pioltelli P, Gabrielli A, Filippini D, Perrella O, Migliaresi S, Galli M, Bombardieri S, Monti G. A randomized controlled trial of rituximab for the treatment of severe cryoglobulinemic vasculitis. *Arthritis Rheum* 2012; **64**: 843-853 [PMID: 22147661 DOI: 10.1002/art.34331]
11 **Saadoun D**, Resche Rigon M, Sene D, Terrier B, Karras A, Perard L, Schoindre Y, Coppéré B, Blanc F, Musset L, Piette JC, Rosenzwajg M, Cacoub P. Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. *Blood* 2010; **116**: 326-334; quiz 504-505 [PMID: 20439619]
12 **Dammacco F**, Tucci FA, Lauletta G, Gatti P, De Re V, Conteduca V, Sansonno S, Russi S, Mariggiò MA, Chironna M, Sansonno D. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. *Blood* 2010; **116**: 343-353 [PMID: 20308602 DOI: 10.1182/blood-2009-10-245878]
13 **Le Cleach L**, Chosidow O. Clinical practice. Lichen planus. *N Engl J Med* 2012; **366**: 723-732 [PMID: 22356325 DOI: 10.1056/NEJMcp1103641]
14 **Shengyuan L**, Songpo Y, Wen W, Wenjing T, Haitao Z, Binyou W. Hepatitis C virus and lichen planus: a reciprocal association determined by a meta-analysis. *Arch Dermatol* 2009; **145**: 1040-1047 [PMID: 19770446 DOI: 10.1001/archdermatol.2009.200]
15 **Lodi G**, Pellicano R, Carrozzo M. Hepatitis C virus infection and lichen planus: a systematic review with meta-analysis. *Oral Dis* 2010; **16**: 601-612 [PMID: 20412447]
16 **Rübsam K**, Schroll A, Weisenseel P, Multhaup S, Ruzicka T, Prinz JC. Lichen planus and hepatitis virus infections: causal association? *J Dtsch Dermatol Ges* 2011; **9**: 464-468 [PMID: 21410879 DOI: 10.1111/j.1610-0387.2011.07619.x]
17 **Carbone T**, Nasorri F, Pennino D, Donnarumma M, Garcovich S, Eyerich K, Bergamo F, Cavani A. CD56 highCD16 - NK cell involvement in cutaneous lichen planus. *Eur J Dermatol* 2010; **20**: 724-730 [PMID: 20959273 DOI: 10.1684/ejd.2010.1096]
18 **Sontheimer RD**. Lichenoid tissue reaction/interface dermatitis: clinical and histological perspectives. *J Invest Dermatol* 2009; **129**: 1088-1099 [PMID: 19242512 DOI: 10.1038/sj.jid.2009.42]
19 **Nagao Y**, Sata M, Noguchi S, Seno'o T, Kinoshita M, Kameyama T, Ueno T. Detection of hepatitis C virus RNA in oral lichen planus and oral cancer tissues. *J Oral Pathol Med* 2000; **29**: 259-266 [PMID: 10890556 DOI: 10.1034/j.1600-0714.2000.290604.x]
20 **Carrozzo M**, Quadri R, Latorre P, Pentenero M, Paganin S, Bertolusso G, Gandolfo S, Negro F. Molecular evidence that the hepatitis C virus replicates in the oral mucosa. *J Hepatol* 2002; **37**: 364-369 [PMID: 12175632 DOI: 10.1016/S0168-8278(02)00183-6]
21 **Pilli M**, Penna A, Zerbini A, Vescovi P, Manfredi M, Negro F, Carrozzo M, Mori C, Giuberti T, Ferrari C, Missale G. Oral lichen planus pathogenesis: A role for the HCV-specific cellular immune response. *Hepatology* 2002; **36**: 1446-1452 [PMID: 12447871 DOI: 10.1002/hep.1840360622]
22 **el-Kabir M**, Scully C, Porter S, Porter K, Macnamara E. Liver function in UK patients with oral lichen planus. *Clin Exp Dermatol* 1993; **18**: 12-16 [PMID: 8440044 DOI: 10.1111/j.1365-2230.1993.tb00957.x]
23 **Tanei R**, Watanabe K, Nishiyama S. Clinical and histopathologic analysis of the relationship between lichen planus and chronic hepatitis C. *J Dermatol* 1995; **22**: 316-323 [PMID: 7545708]
24 **Dalekos GN**, Christodoulou D, Kistis KG, Zervou EK, Hatzis J, Tsianos EV. A prospective evaluation of dermatological side-effects during alpha-interferon therapy for chronic viral hepatitis. *Eur J Gastroenterol Hepatol* 1998; **10**: 933-939 [PMID: 9872615 DOI: 10.1097/00042737-199811000-00006]
25 **Manousaridis I**, Manousaridis K, Peitsch WK, Schneider SW. Individualizing treatment and choice of medication in lichen planus: a step by step approach. *J Dtsch Dermatol Ges* 2013; **11**: 981-991 [PMID: 23981330 DOI: 10.1111/ddg.12141]
26 **Cheng S**, Kirtschig G, Cooper S, Thornhill M, Leonardi-Bee J, Murphy R. Interventions for erosive lichen planus affecting mucosal sites. *Cochrane Database Syst Rev* 2012; **2**: CD008092 [PMID: 22336835 DOI: 10.1002/14651858.CD008092.pub2]
27 **Galeazzi M**, Bellisai F, Manganelli S, Morozzi G, Sebastiani GD. Cyclosporine A for the treatment of autoimmune disorders in HCV infected patients. *Autoimmun Rev* 2006; **5**: 493-498 [PMID: 16920576 DOI: 10.1016/j.autrev.2006.01.002]
28 **Tischer S**, Fontana RJ. Drug-drug interactions with oral anti-HCV agents and idiosyncratic hepatotoxicity in the liver transplant setting. *J Hepatol* 2014; **60**: 872-884 [PMID: 24280292 DOI: 10.1016/j.jhep.2013.11.013]
29 **Elder GH**. Update on enzyme and molecular defects in porphyria. *Photodermatol Photoimmunol Photomed* 1998; **14**: 66-69 [PMID: 9638727 DOI: 10.1111/j.1600-0781.1998.tb00014.x]
30 **Gisbert JP**, García-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in porphyria cutanea tarda: systematic review and meta-analysis. *J Hepatol* 2003; **39**: 620-627 [PMID: 12971974 DOI: 10.1016/S0168-8278(03)00346-5]
31 **Bonkovsky HL**, Poh-Fitzpatrick M, Pimstone N, Obando J, Di Bisceglie A, Tattrie C, Tortorelli K, LeClair P, Mercurio MG, Lambrecht RW. Porphyria cutanea tarda, hepatitis C, and HFE gene mutations in North America. *Hepatology* 1998; **27**: 1661-1669 [PMID: 9620340 DOI: 10.1002/hep.510270627]
32 **Nishina S**, Hino K, Korenaga M, Vecchi C, Pietrangelo A, Mizukami Y, Furutani T, Sakai A, Okuda M, Hidaka I, Okita K, Sakaida I. Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. *Gastroenterology* 2008; **134**: 226-238 [PMID: 18166355 DOI: 10.1053/j.gastro.2007.10.011]
33 **Wallace DF**, Subramaniam VN. Co-factors in liver disease: the role of HFE-related hereditary hemochromatosis and iron. *Biochim Biophys Acta* 2009; **1790**: 663-670 [PMID: 18848602 DOI: 10.1016/j.bbagen.2008.09.002]
34 **Teubner A**, Richter M, Schuppan D, Köstler E, Stölzel U. [Hepatitis C, hemochromatosis and porphyria cutanea tarda]. *Dtsch*

Med Wochenschr 2006; **131**: 691-695 [PMID: 16555178 DOI: 10.12659/AJCR.889955]

35 **Toll A**, Celis R, Ozalla MD, Bruguera M, Herrero C, Ercilla MG. The prevalence of HFE C282Y gene mutation is increased in Spanish patients with porphyria cutanea tarda without hepatitis C virus infection. *J Eur Acad Dermatol Venereol* 2006; **20**: 1201-1206 [PMID: 17062032 DOI: 10.1111/j.1468-3083.2006.01746.x]

36 **Fernández I**, Castellano G, de Salamanca RE, Colina F, Gómez de la Cámara A, Morán MJ, Muñoz R, Solís-Herruzo JA. Porphyria cutanea tarda as a predictor of poor response to interferon alfa therapy in chronic hepatitis C. *Scand J Gastroenterol* 2003; **38**: 314-319 [PMID: 12737448 DOI: 10.1080/00365520310000672]

37 **Desai TK**, Jamil LH, Balasubramaniam M, Koff R, Bonkovsky HL. Phlebotomy improves therapeutic response to interferon in patients with chronic hepatitis C: a meta-analysis of six prospective randomized controlled trials. *Dig Dis Sci* 2008; **53**: 815-822 [PMID: 17846887 DOI: 10.1007/s10620-007-9945-7]

38 **Azim J**, McCurdy H, Moseley RH. Porphyria cutanea tarda as a complication of therapy for chronic hepatitis C. *World J Gastroenterol* 2008; **14**: 5913-5915 [PMID: 18855993]

39 **Boehncke WH**, Schön MP. Psoriasis. *Lancet* 2015; **386**: 983-994 [PMID: 26025581 DOI: 10.1016/S0140-6736(14)61909-7]

40 **Tsai TF**, Wang TS, Hung ST, Tsai PI, Schenkel B, Zhang M, Tang CH. Epidemiology and comorbidities of psoriasis patients in a national database in Taiwan. *J Dermatol Sci* 2011; **63**: 40-46 [PMID: 21543188 DOI: 10.1016/j.jdermsci.2011.03.002]

41 **Yang YW**, Keller JJ, Lin HC. Medical comorbidity associated with psoriasis in adults: a population-based study. *Br J Dermatol* 2011; **165**: 1037-1043 [PMID: 21711339 DOI: 10.1111/j.1365-2133.2011.10494.x]

42 **Imafuku S**, Naito R, Nakayama J. Possible association of hepatitis C virus infection with late-onset psoriasis: a hospital-based observational study. *J Dermatol* 2013; **40**: 813-818 [PMID: 23961783 DOI: 10.1111/1346-8138.12240]

43 **Taglione E**, Vatteroni ML, Martini P, Galluzzo E, Lombardini F, Delle Sedie A, Bendinelli M, Pasero G, Bencivelli W, Riente L. Hepatitis C virus infection: prevalence in psoriasis and psoriatic arthritis. *J Rheumatol* 1999; **26**: 370-372 [PMID: 9972971]

44 **Kanada KN**, Schupp CW, Armstrong AW. Association between psoriasis and viral infections in the United States: focusing on hepatitis B, hepatitis C and human immunodeficiency virus. *J Eur Acad Dermatol Venereol* 2013; **27**: 1312-1316 [PMID: 22564047 DOI: 10.1111/j.1468-3083.2012.04563.x]

45 **Palazzi C**, Olivieri I, D'Amico E, D'Agostino L, Nicolucci A, Pennese E, Petricca A. Hepatitis C virus infection in psoriatic arthritis. *Arthritis Rheum* 2005; **53**: 223-225 [PMID: 15818717 DOI: 10.1002/art.21079]

46 **Cohen AD**, Weitzman D, Birkenfeld S, Dreiher J. Psoriasis associated with hepatitis C but not with hepatitis B. *Dermatology* 2010; **220**: 218-222 [PMID: 20185894 DOI: 10.1159/000286131]

47 **Imafuku S**, Nakayama J. Profile of patients with psoriasis associated with hepatitis C virus infection. *J Dermatol* 2013; **40**: 428-433 [PMID: 23414394]

48 **Frankel AJ**, Van Voorhees AS, Hsu S, Korman NJ, Lebwohl MG, Bebo BF, Gottlieb AB. Treatment of psoriasis in patients with hepatitis C: from the Medical Board of the National Psoriasis Foundation. *J Am Acad Dermatol* 2009; **61**: 1044-1055 [PMID: 19811848 DOI: 10.1016/j.jaad.2009.03.044]

49 **Zanni M**, Missale G, Santilli D, Di Nuzzo S. Etanercept in the treatment of psoriasis and psoriatic arthritis with concomitant hepatitis C virus infection: clinical and virological study in three patients. *Eur J Dermatol* 2011; **21**: 564-567 [PMID: 21543290 DOI: 10.1684/ejd.2011.1318]

50 **Pompili M**, Biolato M, Miele L, Grieco A. Tumor necrosis factor-α inhibitors and chronic hepatitis C: a comprehensive literature review. *World J Gastroenterol* 2013; **19**: 7867-7873 [PMID: 24307780 DOI: 10.3748/wjg.v19.i44.7867]

51 **Kini SP**, DeLong LK, Veledar E, McKenzie-Brown AM, Schaufele M, Chen SC. The impact of pruritus on quality of life: the skin equivalent of pain. *Arch Dermatol* 2011; **147**: 1153-1156 [PMID: 21680760 DOI: 10.1001/archdermatol.2011.178]

52 **Kanazawa K**, Yaoita H, Tsuda F, Murata K, Okamoto H. Association of prurigo with hepatitis C virus infection. *Arch Dermatol* 1995; **131**: 852-853 [PMID: 7611811 DOI: 10.1001/archderm.1995.01690190108028]

53 **Soylu S**, Gül U, Kiliç A. Cutaneous manifestations in patients positive for anti-hepatitis C virus antibodies. *Acta Derm Venereol* 2007; **87**: 49-53 [PMID: 17225016 DOI: 10.2340/00015555-0186]

54 **Dega H**, Francès C, Dupin N, Lebre C, Simantov A, Callot C, Laporte JL, Blot C, Opolon P, Poynard T, Chosidow O. [Pruritus and the hepatitis C virus. The MULTIVIRC Unit]. *Ann Dermatol Venereol* 1998; **125**: 9-12 [PMID: 9747198]

55 **Kremer AE**, van Dijk R, Leckie P, Schaap FG, Kuiper EM, Mettang T, Reiners KS, Raap U, van Buuren HR, van Erpecum KJ, Davies NA, Rust C, Engert A, Jalan R, Oude Elferink RP, Beuers U. Serum autotaxin is increased in pruritus of cholestasis, but not of other origin, and responds to therapeutic interventions. *Hepatology* 2012; **56**: 1391-1400 [PMID: 22473838 DOI: 10.1002/hep.25748]

56 **Watanabe N**, Ikeda H, Nakamura K, Ohkawa R, Kume Y, Aoki J, Hama K, Okudaira S, Tanaka M, Tomiya T, Yanase M, Tejima K, Nishikawa T, Arai M, Arai H, Omata M, Fujiwara K, Yatomi Y. Both plasma lysophosphatidic acid and serum autotaxin levels are increased in chronic hepatitis C. *J Clin Gastroenterol* 2007; **41**: 616-623 [PMID: 17577119 DOI: 10.1097/01.mcg.0000225642.90898.0e]

57 **Berk DR**, Mallory SB, Keeffe EB, Ahmed A. Dermatologic disorders associated with chronic hepatitis C: effect of interferon therapy. *Clin Gastroenterol Hepatol* 2007; **5**: 142-151 [PMID: 16919505 DOI: 10.1016/j.cgh.2006.06.010]

58 **Roujeau JC**, Mockenhaupt M, Tahan SR, Henshaw J, Martin EC, Harding M, van Baelen B, Bengtsson L, Singhal P, Kauffman RS, Stern RS. Telaprevir-related dermatitis. *JAMA Dermatol* 2013; **149**: 152-158 [PMID: 23560295 DOI: 10.1001/jamadermatol.2013.938]

59 **Cacoub P**, Bourlière M, Lübbe J, Dupin N, Buggisch P, Dusheiko G, Hézode C, Picard O, Pujol R, Segaert S, Thio B, Roujeau JC. Dermatological side effects of hepatitis C and its treatment: patient management in the era of direct-acting antivirals. *J Hepatol* 2012; **56**: 455-463 [PMID: 21884670 DOI: 10.1016/j.jhep.2011.08.006]

60 **Lavanchy D**. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]

P- Reviewer: Dang SS, He JY **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i27.2749

World J Hepatol 2015 November 28; 7(27): 2749-2756
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Vitamin E reduces liver stiffness in nonalcoholic fatty liver disease

Aiko Fukui, Naoto Kawabe, Senju Hashimoto, Michihito Murao, Takuji Nakano, Hiroaki Shimazaki, Toshiki Kan, Kazunori Nakaoka, Masashi Ohki, Yuka Takagawa, Tomoki Takamura, Hiroyuki Kamei, Kentaro Yoshioka

Aiko Fukui, Naoto Kawabe, Senju Hashimoto, Michihito Murao, Takuji Nakano, Hiroaki Shimazaki, Toshiki Kan, Kazunori Nakaoka, Masashi Ohki, Yuka Takagawa, Tomoki Takamura, Kentaro Yoshioka, Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, Aichi 470-1192, Japan

Aiko Fukui, Hiroyuki Kamei, Department of Clinical Pharmacy Practice and Health Care Management, Faculty of Pharmacy, Meijo University, Aichi 468-8503, Japan

Author contributions: Fukui A proposed the study; Kawabe N, Hashimoto S, Murao M, Nakano T, Shimazaki H, Kan T, Nakaoka M, Ohki M, Takagawa Y, Takamura T and Yoshioka K performed the research; Fukui A collected and analyzed the data; Fukui A, Kawabe N, Kamei H and Yoshioka K contributed to the design and interpretation of the study and to further drafts; Yoshioka K is the guarantor; all authors contributed equally to this work.

Institutional review board statement: This study conformed to the Japanese Good Clinical Practice and the Declaration of Helsinki, and was reviewed and approved by the Ethics Committee of the Fujita Health University Hospital (IRB number: 14-020).

Informed consent statement: Each patient gave a written informed consent.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Correspondence to: Kentaro Yoshioka, MD, Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, 1-98 Dengakugakubo, Kutsukake, Toyoake, Aichi 470-1192, Japan. kyoshiok@fujita-hu.ac.jp
Telephone: +81-562-932324
Fax: +81-562-938601

Received: July 3, 2015
Peer-review started: July 6, 2015
First decision: September 8, 2015
Revised: September 20, 2015
Accepted: November 10, 2015
Article in press: November 11, 2015
Published online: November 28, 2015

Abstract

AIM: To evaluate the efficacy of vitamin E treatment on liver stiffness in nonalcoholic fatty liver disease (NAFLD).

METHODS: Thirty-eight NAFLD patients were administered vitamin E for > 1 year. The doses of vitamin E were 150, 300, or 600 mg; three times per day after each meal. Responses were assessed by liver enzyme levels [aspartate aminotransferase (AST), alanine aminotranferease (ALT), and γ-glutamyl transpeptidase (γ-GTP)], noninvasive scoring systems of hepatic fibrosis-4 [FIB-4 index and aspartate aminotransferase-to-platelet index (APRI)], and liver stiffness [velocity of shear wave (Vs)] measured by acoustic radiation force impulse elastography. Vs measurements were performed at baseline and 12 mo after baseline. The patients were genotyped for the patatin-like phospholipase domain containing 3 (*PNPLA3*) polymorphisms and then divided into either the CC/CG or GG group to examine each group's responses to vitamin E treatment.

RESULTS: We found marked differences in the platelet count, serum albumin levels, alkaline phosphatase

levels, FIB-4 index, APRI, and Vs at baseline depending on the *PNPLA3* polymorphism. AST, ALT, and γ-GTP levels (all $P < 0.001$); FIB-4 index ($P = 0.035$); APRI ($P < 0.001$); and Vs ($P < 0.001$) significantly decreased from baseline to 12 mo in the analysis of all patients. In the subset analyses of *PNPLA3* genotypes, AST levels ($P = 0.011$), ALT levels ($P < 0.001$), γ-GTP levels ($P = 0.005$), APRI ($P = 0.036$), and Vs ($P = 0.029$) in genotype GG patients significantly improved, and AST and ALT levels (both $P < 0.001$), γ-GTP levels ($P = 0.003$), FIB-4 index ($P = 0.017$), and APRI ($P < 0.001$) in genotype CC/CG patients.

CONCLUSION: One year of vitamin E treatment improved noninvasive fibrosis scores and liver stiffness in NAFLD patients. The responses were similar between different *PNPLA3* genotypes.

Key words: Vitamin E; Acoustic radiation force impulse; Nonalcoholic fatty liver disease; Velocity of shear wave; Patatin-like phospholipase domain containing 3

Core tip: Responses to vitamin E treatment in non-alcoholic fatty liver disease patients were assessed by noninvasive scoring systems of hepatic fibrosis, and liver stiffness (velocity of shear wave) was measured by acoustic radiation force impulse elastography. Vitamin E treatment for 1 year improved not only liver enzyme levels but also noninvasive fibrosis scores and liver stiffness. Subsequently, the patients were divided into two groups according to patatin-like phospholipase domain containing 3 (*PNPLA3*) genotype (CC/CG or GG) to examine whether either group responded differently to the treatment. The responses were similar between different *PNPLA3* genotypes.

Fukui A, Kawabe N, Hashimoto S, Murao M, Nakano T, Shimazaki H, Kan T, Nakaoka K, Ohki M, Takagawa Y, Takamura T, Kamei H, Yoshioka K. Vitamin E reduces liver stiffness in nonalcoholic fatty liver disease. *World J Hepatol* 2015; 7(27): 2749-2756 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i27/2749.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i27.2749

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common liver disease[1], and nonalcoholic steatohepatitis (NASH) characterized by steatosis with necroinflammation and eventual fibrosis[2] can lead to end-stage cirrhosis and hepatocellular carcinoma[3].

Although there are no generally approved treatments for NASH, several treatment options have demonstrated efficacy in various clinical trials. Statins[4-7], insulin sensitizers such as thiazolidinediones[8,9], and metformin[10] are effective for the treatment of NAFLD[11]. Oxidative stress plays a central role in the transition from simple steatosis to NASH[12]. An effective therapeutic strategy is to target reduction in oxidative stress using, for example, administration of vitamin E. A recent trial has provided substantial evidence for the previously suggested efficacy of vitamin E in inducing histological improvement of NASH[13].

Evaluation of liver fibrosis is essential in chronic liver diseases because the prognosis of the diseases and the treatment decisions often depend on fibrosis. Liver biopsy is still considered the gold standard for liver fibrosis assessment, despite being an invasive method and not completely risk free[14]. In recent years, noninvasive methods, aimed at replacing liver biopsy, have been developed for the evaluation of liver fibrosis. Development of noninvasive tools will enable monitoring of the disease progression and response to therapy. Velocity of shear wave (Vs) measured by acoustic radiation force impulse (ARFI) has been reported to be a good method for assessing the stage of liver fibrosis[15,16]. Vs has been reported to be useful in diagnosing NAFLD[17-19].

The single-nucleotide polymorphism rs738409 (C > G) in the patatin-like phospholipase domain containing 3 (*PNPLA3*) was strongly associated with increased hepatic fat levels and with hepatic inflammation in NAFLD patients[20-23]. However, the effect of the *PNPLA3* polymorphisms on the response to treatment has not been reported.

The aims of this study were to evaluate the efficacy of vitamin E treatment for NAFLD by noninvasive methods and to assess the association between the treatment response and the *PNPLA3* polymorphism present.

MATERIALS AND METHODS

Patients

Vitamin E was administered for > 1 year to 38 patients with NAFLD as treatments for atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation from January 2011 to July 2015. The patients showed no improvement in aminotransferase levels following lifestyle modification such as dietary modification and exercise before beginning vitamin E treatment. Their clinical data were retrospectively studied (Table 1). The diagnosis of NAFLD was confirmed by liver biopsy in 10 patients, by ultrasonic examination in 23 patients, and by presence of cirrhosis with no obvious etiology and with metabolic risk factors such as obesity and metabolic syndrome in 5 patients[11]. None of the patients consumed > 40 g of alcohol per day. The patients who increased the dose of or started other medicines for NAFLD, such as pioglitazone, metformin, ursodeoxycholic acid, statins, ezetimibe, or angiotensin 2 receptor antagonist, during the study period were excluded from this study.

Vitamin E administration

The total doses of 150, 300 or 600 mg vitamin E were

Table 1 Clinical characteristics and laboratory values of the patients at baseline

	All patients	*PNPLA3* genotype		*P* value
		CC/CG	GG	*PNPLA3* CC/CG *vs* GG
No. of subjects	38	19	19	
Sex (male/female)	10/28	6/13	4/15	0.461
Age (yr)	62.0 ± 11.6	58.4 ± 11.9	65.6 ± 10.1	0.061
Height (cm)	156.6 ± 9.9	158.7 ± 11.3	154.4 ± 7.6	0.226
Body weight (kg)	68.2 ± 14.9	69.7 ± 16.1	66.7 ± 13.5	0.685
Body mass index (kg/m^2)	27.9 ± 4.7	27.7 ± 4.0	28.0 ± 5.3	0.988
Classification of NAFLD				
Fatty liver confirmed by an imaging examination	23	13	10	0.347
NASH confirmed by a liver biopsy	9	5	4	
Nonviral liver cirrhosis diagnosed clinically	5	1	4	
Burn-out NASH confirmed by a liver biopsy	1	0	1	
Child-Pugh grade of cirrhotic patients (A/B/C)	4/2/0	1/0/0	3/2/0	0.439
Concurrent diabetes mellitus (+/-)	15/23	5/14	10/9	0.097
Concurrent hepatocellular cancer (+/-)	0/38	0/19	0/19	-
Dosage of vitamin E (150 mg/300 mg/600 mg)	2/4/32	2/1/16	0/3/16	0.223
Concurrent medication				
Pioglitazone	3	2	1	0.547
Metformin	2	2	0	0.146
Ursodeoxycholic acid	27	14	13	0.721
HMG-CoA reductase inhibitor	13	6	7	0.732
Ezetimibe	7	6	1	0.036[a]
Angiotensin receptor 2 antagonist	9	6	3	0.252
Serum biochemical tests				
Platelet count (× 10^4/μL)	16.7 ± 7.5	19.3 ± 7.1	14.1 ± 6.9	0.046[a]
Prothrombin activity (%)	98.5 ± 18.7	104.2 ± 17.6	93.2 ± 18.2	0.061
Hemoglobin A1c (%)	6.38 ± 1.06	6.26 ± 0.97	6.49 ± 1.14	0.394
Total protein (g/dL)	7.42 ± 0.52	7.47 ± 0.49	7.36 ± 0.54	0.435
Serum albumin (g/dL)	4.27 ± 0.39	4.41 ± 0.36	4.14 ± 0.37	0.032[a]
Total bilirubin (mg/dL)	0.98 ± 0.50	0.84 ± 0.29	1.11 ± 0.61	0.172
AST (IU/L)	61.1 ± 29.9	58.2 ± 32.2	64.1 ± 27.2	0.191
ALT (IU/L)	68.5 ± 41.2	69.9 ± 36.0	67.1 ± 45.7	0.385
Alkaline phosphatase (IU/L)	312 ± 108	270 ± 85	355 ± 112	0.032[a]
γ-GTP (IU/L)	87.4 ± 70.0	72.6 ± 59.1	101.5 ± 76.4	0.142
Total cholesterol (mg/dL)	185 ± 37	194 ± 43	176 ± 28	0.172
Triglycerides (mg/dL)	151 ± 85	147 ± 99	156 ± 64	0.351
Cholinesterase (IU/L)	333 ± 124	353 ± 91	313 ± 147	0.172
Scoring systems of hepatic fibrosis				
FIB-4 index	3.80 ± 2.78	2.61 ± 1.74	4.98 ± 3.10	0.006[a]
APRI	1.50 ± 0.94	1.17 ± 0.75	1.84 ± 0.98	0.014[a]
Velocity of shear wave (m/s)	2.20 ± 0.91	1.81 ± 0.76	2.56 ± 0.89	0.010[a]

Data are presented as number of patients or means ± SD. Statistical analysis was performed by χ^2 test or Mann-Whitney *U* test. [a]$P < 0.05$. PNPLA3: Patatin-like phospholipase domain containing 3; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; APRI: Aspartate aminotransferase-to-platelet ratio index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; γ-GTP: γ-Glutamyltransferase.

orally given into 3 administrations per day after each meal for > 1 year: 150 mg for 2 patients, 300 mg for 4 patients, or 600 mg for 32 patients (100 mg is equivalent to 100 IU; Eisai Pharmaceutical Co., Tokyo, Japan). In the American Association for the Study of Liver Diseases practice guideline, vitamin E was recommended to be administered to nondiabetic adults with biopsy-proven NASH at a daily dose of 800 IU[11]. However, the dosage of vitamin E accepted by health insurance in Japan is 150-300 mg for atherosclerosis or diabetic retinopathy, and 300-600 mg for prevention of lipid peroxidation.

Laboratory data and noninvasive scoring systems

Laboratory data were collected at three time points: at baseline (beginning of vitamin E administration), 6 and 12 mo after baseline. Two noninvasive scoring systems of hepatic fibrosis, the fibrosis-4 (FIB-4) index and the aspartate aminotransferase-to-platelet index (APRI), were calculated from the measurements using the originally reported formulas[24,25]. The APRI formula was aspartate aminotransferase (AST) level (U/L)/AST (upper limit of normal; U/L)/platelet (10^9/L) × 100 and the FIB-4 score formula was age (years) × AST level (U/L)/platelet (10^9/L) × [alanine aminotranferease (ALT)]$^{1/2}$ (U/L). These noninvasive scoring systems were used at each of the time points.

Vs measurement by ARFI elastography

Vs measurement by ARFI elastography was performed at baseline and 12 mo after baseline using a Siemens ACUSON S2000 (Siemens Medical Systems Co., Ltd., Tokyo, Japan). The examination was performed on the right lobe of the liver. A measurement depth of 2-3 cm below the liver capsule was chosen. Ten successful

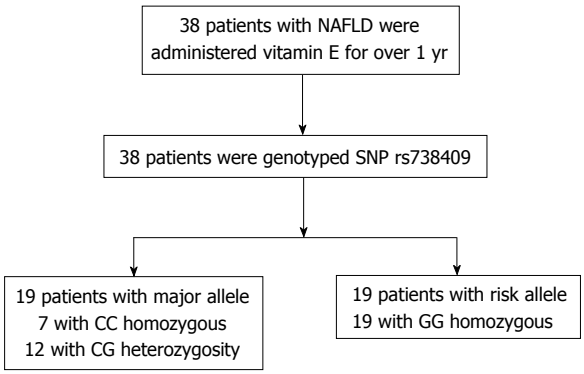

Figure 1 Characteristics of patients included in this study. The study comprised 38 patients with nonalcoholic fatty liver disease (NAFLD) who were administered vitamin E for > 1 year. The patients were genotyped SNP rs738409 and divided into two groups by genotype (CC/CG and GG) to examine the difference in the response to vitamin E.

acquisitions at different locations were performed on each patient, and the results are expressed as median values in m/s. Vs is considered to be proportional to the square root of tissue elasticity. Histological improvement was not examined because sequential liver biopsy was not performed.

PNPLA3 rs738409 genotyping

Genomic DNA was extracted from whole blood samples using QIA amp DNA Mini Kits (Qiagen, Tokyo, Japan), according to the manufacturer's protocol. The rs738409 *PNPLA3* SNP was genotyped using TaqMan predesigned SNP genotyping assays (Applied Biosystems, Tokyo, Japan), according to the manufacturer's protocol. The patients were divided into two groups by *PNPLA3* genotype (CC/CG *vs* GG) to examine the different responses to vitamin E in each group (Figure 1).

Statistical analysis

Differences in the two groups in terms of clinical characteristics and laboratory values at baseline were analyzed using either the χ^2 test or the Mann-Whitney *U* test. Differences between the laboratory values obtained at three time points were analyzed using the Freedman test. Differences between the laboratory values obtained at two time points were analyzed using Wilcoxon's signed-rank test with Bonferroni's correction. Differences were judged as significant if the *P* value was < 0.05 (two-tailed). All statistical analyses were conducted using SPSS software (SPSS Statistics Version 22; IBM Co., Armonk, NY).

RESULTS

Baseline characteristics

Seven obese patients included in this study exceeded their ideal body weight (body mass index was > 32 kg/m^2), and 39.5% of patients had type 2 diabetes (Table 1). Platelet counts and serum albumin levels in the GG group were significantly lower than those in the CC/CG group (P = 0.046, and P = 0.032, respectively). Alkaline phosphatase levels, FIB-4 index, APRI, and Vs in the GG group were significantly higher than those in the CC/CG group (P = 0.032, P = 0.006, P = 0.014, and P = 0.010, respectively).

Body weight change

Body weight in all patients did not change from baseline to 12 mo (68.2 ± 14.9 kg and 72.4 ± 16.8 kg, respectively). There were no patients who achieved > 7% weight loss during study periods.

Effect of vitamin E on serum AST, ALT, and γ-glutamyl transpeptidase levels

Serum AST, ALT and γ-glutamyl transpeptidase (γ-GTP) levels in all patients significantly decreased from baseline to 6 mo (P < 0.001, P < 0.001, and P = 0.019, respectively) and 12 mo (P < 0.001, P < 0.001, and P < 0.001, respectively). Those in the CC/CG group also significantly decreased from baseline to 6 mo (P = 0.004, P = 0.022, and P = 0.047, respectively) and 12 mo (P < 0.001, P < 0.001, and P = 0.003, respectively). Serum AST and ALT levels in the GG group significantly decreased from baseline to 6 mo (P = 0.045, and P = 0.004, respectively) and 12 mo (P = 0.011, and P < 0.001, respectively), and serum γ-GTP levels in the GG group significantly decreased from baseline to 12 mo (P = 0.005) (Figure 2).

Effect of vitamin E on the FIB-4 index

The FIB-4 index in all patients significantly decreased from baseline to 6 and 12 mo (P = 0.015 and P = 0.035, respectively). FIB-4 index in the CC/CG group also significantly decreased from baseline to 6 and 12 mo (P = 0.014 and P = 0.017, respectively). On the other hand, the FIB-4 index did not change in the GG group (Figure 3A).

Effect of vitamin E on APRI

APRI in all patients significantly decreased from baseline to 6 and 12 mo (P < 0.001 and P < 0.001, respectively). APRI in the CC/CG group significantly decreased from baseline to 6 and 12 mo (P = 0.004 and P < 0.001, respectively). APRI in the GG group also significantly decreased from baseline to 6 and 12 mo (P = 0.028 and P = 0.036, respectively; Figure 3B).

Effect of vitamin E on Vs

Vs in all patients decreased from baseline to 12 mo (P = 0.005). Vs in the GG group also decreased from baseline to 12 mo (P = 0.029), and Vs in the CC/CG group also tended to decrease; however, the decrease was not significant (Figure 4).

DISCUSSION

The present study showed that a 1-year treatment of vitamin E improved not only laboratory values but

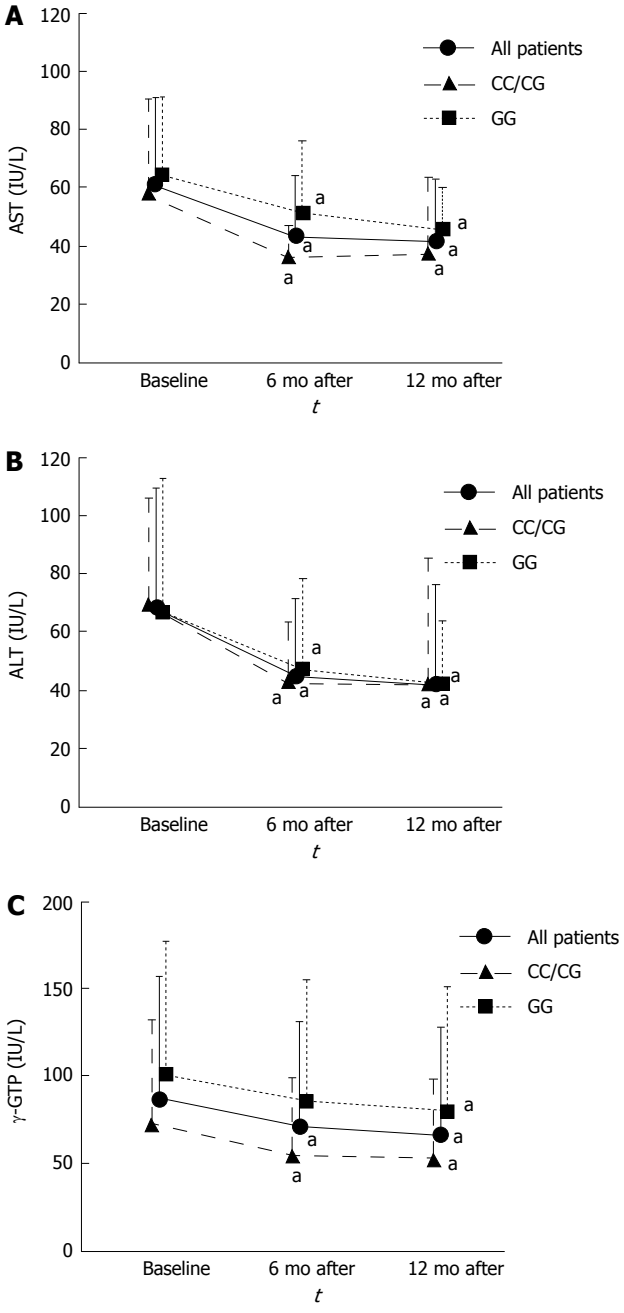

Figure 2 Effects of vitamin E treatment on liver enzyme levels. [a]Indicate a difference when the results are compared with baseline values (P < 0.05). A: Evolution of AST during the study period. Serum AST levels in all patients decreased from baseline to 6 and 12 mo (P < 0.001 and P < 0.001, respectively). Serum AST levels in the CC/CG group decreased from baseline to 6 and 12 mo (P = 0.004 and P < 0.001, respectively). Serum AST levels in the GG group also decreased from baseline to 6 and 12 mo (P = 0.045 and P = 0.011, respectively); B: Evolution of ALT during the study period. Serum ALT levels in all patients decreased from baseline to 6 and 12 mo (P < 0.001 and P < 0.001, respectively). Serum ALT levels in the CC/CG group decreased from baseline to 6 and 12 mo (P = 0.022 and P < 0.001, respectively). Serum ALT levels in the GG group also decreased from baseline to 6 and 12 mo (P = 0.004 and P < 0.001, respectively); C: Evolution of γ-GTP during the study period. Serum γ-GTP levels in all patients decreased from baseline to 6 and 12 mo (P = 0.019 and P < 0.001, respectively). Serum γ-GTP levels in the CC/CG group decreased from baseline to 6 and 12 mo (P = 0.047 and P = 0.003, respectively). Those in the GG group decreased from baseline to 12 mo (P = 0.005). AST: Aspartate aminotransferase; ALT: Alanine aminotranferease; γ-GTP: γ-glutamyl transpeptidase.

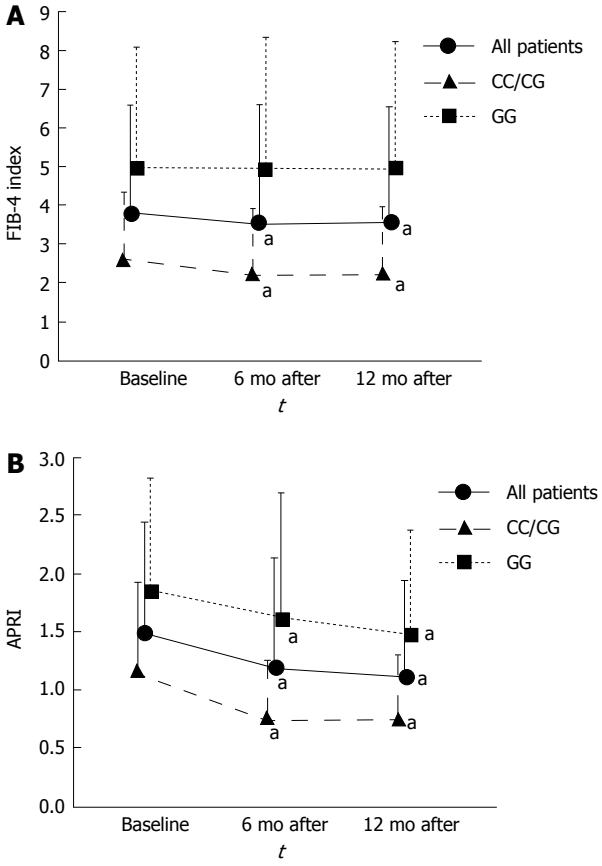

Figure 3 Effects of vitamin E treatment on results of noninvasive scoring systems of hepatic fibrosis. [a]Indicate a difference compared with baseline values (P < 0.05). A: Evolution of FIB-4 index during the study period. FIB-4 index in all patients decreased from baseline to 6 and 12 mo (P = 0.015 and P = 0.035, respectively). The FIB-4 index in the CC/CG group also decreased from baseline to 6 and 12 mo (P = 0.014 and P = 0.017, respectively); B: Evolution of APRI during the study period. APRI in all patients decreased from baseline to 6 and 12 mo (P < 0.001 and P < 0.001, respectively). APRI in the CC/CG group decreased from baseline to 6 and 12 mo (P = 0.004 and P < 0.001, respectively). APRI in the GG group also decreased from baseline to 6 and 12 mo (P = 0.028 and P = 0.036, respectively). FIB-4: Fibrosis-4; APRI: Aminotransferase-to-platelet index.

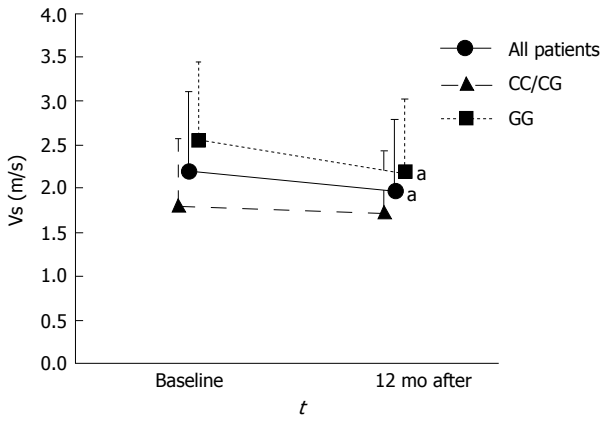

Figure 4 Evolution of velocity of shear wave during the study period. [a]Indicate a difference compared with baseline values (P < 0.05). Velocity of shear wave (Vs) in all patients decreased from baseline to 12 mo (P = 0.005). Vs in the GG group also decreased from baseline to 12 mo (P = 0.029), and that in the CC/CG group also tended to decrease (P = 0.083).

also the noninvasive scores related to hepatic fibrosis and liver stiffness in NAFLD patients. The treatment responses are similar between different *PNPLA3* genotypes.

In general, lifestyle modification should be the first line of treatment in patients with NAFLD, and it was reported that weight reduction greater than 7% achieved through lifestyle intervention improves aminotransferase levels and liver histology[26]. Because the weight of patients in the present study did not change during the study period, it was assumed that the outcomes were not affected by weight loss.

Recently, a large, multicenter randomized controlled trial was conducted by the NASH Clinical Research Network to evaluate the efficacy of vitamin E treatment for amelioration of NASH in adults [pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis (PIVENS)][13]. This trial reported that serum AST, ALT, and γ-GTP levels in the vitamin E group decreased compared with the placebo group and that the changes occurred in the first 24 wk and were sustained throughout the study period. In our study, the changes occurred 6 mo after baseline, which was consistent with the PIVENS study.

Liver biopsy is regarded as the gold standard in the assessment of patients with NAFLD/NASH. However, liver biopsy is an invasive procedure with potential complications, and sampling error can result in substantial misdiagnosis and staging inaccuracies. Recently, several indices have been developed for noninvasive tests that help to diagnose advanced liver disease. The FIB-4 index and APRI can easily be used at the bedside or in an outpatient setting because of the simple calculation of only a few laboratory values. In our study, the FIB-4 index and APRI markedly decreased from baseline to 6 and 12 mo. These findings may indicate that the administration of vitamin E improved liver fibrosis. However, since these scoring systems are calculated using ALT, the reduction of the score may be attributed to the reduction of hepatic inflammation.

In recent years, several studies have reported the usefulness of ARFI elastography for the assessment of liver stiffness and a positive correlation between Vs and biopsy-proven fibrosis stage in patients with NAFLD[17-19]. ARFI elastography has mainly been used in diagnosis[27] and there are no reports of it being used for assessment of the efficacy of vitamin E treatment in NAFLD patients. In our study, Vs markedly decreased from baseline to 12 mo. In the subset analysis of 32 patients without six patients with daily doses of 150 mg and 300 mg of vitamin E, Vs markedly decreased from baseline to 12 mo ($P = 0.004$). The reduction of Vs probably indicates a reduction in liver fibrosis. The PIVENS trial reported that the vitamin E group had a reduction in steatosis, lobular inflammation, and activity score, whereas fibrosis scores did not markedly improve[13]. The fact that ARFI revealed a reduction in liver stiffness in the present study despite no demonstration of a significant reduction in fibrosis by liver biopsies in the PIVENS trial may indicate that ARFI is more sensitive than liver biopsies for detecting the reduction of fibrosis. There may be possibility that the reduction of Vs is attributed to factors other than reduction of fibrosis.

Yoneda *et al*[17] reported that Vs differed between groups with different inflammatory activity in 54 patients. Fierbinteanu Braticevici *et al*[19] reported that Vs had a positive correlation with inflammation in 64 patients. On the other hand, Palmeri *et al*[18] reported that Vs was not associated with inflammation scores in 172 patients. Thus, the association between Vs and inflammation is still unclear, and further studies are required in this field.

The association between the *PNPLA3* polymorphisms with not only fatty liver and triglyceride content, but also with inflammation and fibrosis in NAFLD has been reported[22,28,29]. A meta-analysis reported that GG homozygous had a 3.24-fold greater risk of higher necroinflammatory scores and a 3.2-fold greater risk of developing fibrosis than CC homozygous[29]. In the present study, there were some differences at baseline depending on the *PNPLA3* polymorphism. The platelet counts and serum albumin levels were lower, and alkaline phosphatase levels, the FIB-4 index, APRI, and Vs were higher in GG patients than in CC/CG patients. Our results were consistent with previous reports.

In the subset analyses of *PNPLA3* genotypes, AST and ALT levels, APRI, and Vs in genotype GG patients and AST, ALT, and γ-GTP levels, FIB-4 index, and APRI in genotype CC/CG patients improved following vitamin E treatment. Vitamin E treatment for 1 year improved not only liver enzyme levels but also noninvasive fibrosis scores and liver stiffness in NAFLD patients. The responses are similar between different *PNPLA3* genotypes.

The most effective dosage of vitamin E is unclear. In the PIVENS trial, 800 IU of vitamin E was administered per day. However, a previous study reported that patients with vascular disease or diabetes mellitus who received long-term supplementation with vitamin E (400 IU/d) had a higher risk of heart failure and hospitalization for heart failure[30]. These results may suggest that we have to avoid vitamin E treatment for patients with vascular disease or diabetes mellitus. In the present study, no patients had heart failure during the observation period.

The present study had several limitations: (1) it was a retrospective study; (2) there was no control group; (3) the sample size was insufficient to provide significant differences in some indicators; and (4) sequential liver biopsy was not performed for observing histological improvement. The preliminary findings of the present study need further verification *via* a well-controlled, prospective study with a sufficiently large sample size to confirm the efficacy of vitamin E by noninvasive scoring systems of hepatic fibrosis and Vs and differences of response according to *PNPLA3* polymorphisms.

In conclusion, vitamin E treatment for 1 year improved not only laboratory values but also the noninvasive scores of hepatic fibrosis and liver stiffness in NAFLD patients. These responses were similar between different

PNPLA3 genotypes.

ACKNOWLEDGMENTS

The authors thank Dr. Hiroshi Takahashi of the Division of Medical Statistics, Fujita Health University for assisting with statistical analysis.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is a common liver disease, and eventual fibrosis can lead to end-stage cirrhosis and hepatocellular carcinoma. An effective therapeutic strategy is to target reduction in oxidative stress using, for example, administration of vitamin E. Liver biopsy is still considered the gold standard for liver fibrosis assessment, despite being an invasive method and not completely risk free. Velocity of shear wave (Vs) measured by acoustic radiation force impulse (ARFI) has been reported to be a good method for assessing the stage of liver fibrosis. The single-nucleotide polymorphism rs738409 (C > G) in the patatin-like phospholipase domain containing 3 (PNPLA3) was strongly associated with increased hepatic fat levels and with hepatic inflammation in NAFLD patients. In this study, the authors evaluated the efficacy of vitamin E treatment for NAFLD by noninvasive methods and to assess the association between the treatment response and the patatin-like phospholipase domain containing 3 (*PNPLA3*) polymorphism.

Research frontiers

Few prior reports showed that vitamin E improved serum aspartate aminotransferase (AST), alanine aminotranferease (ALT), and γ-glutamyl transpeptidase (γ-GTP) levels and reduced steatosis, lobular inflammation, and activity score, but did not markedly improve fibrosis scores in nonalcoholic steatohepatitis (NASH). The results of the authors' study contribute to non-invasive evaluation of the efficacy of vitamin E treatment for NAFLD/NASH.

Innovations and breakthroughs

The pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis trial reported that serum AST, ALT, and γ-GTP levels in the vitamin E group decreased compared with the placebo group, and that vitamin E group had a reduction in steatosis, lobular inflammation, and activity score, whereas fibrosis scores did not markedly improve. The present study showed that a 1-year treatment of vitamin E improved not only laboratory values but also the noninvasive scores related to hepatic fibrosis and liver stiffness in NAFLD patients, and that the treatment responses were similar between different *PNPLA3* genotypes.

Applications

This study suggests that liver stiffness is useful for monitoring the efficacy of vitamin E treatment for NAFLD/NASH. The patients with NAFLD/NASH can be evaluated the therapeutic effect of vitamin E using noninvasive tools without liver biopsy.

Terminology

ARFI: Vs measured by ARFI has been reported to be a good method for assessing the stage of liver fibrosis; *PNPLA3*: The single-nucleotide polymorphism rs738409 (C > G) in the *PNPLA3* was strongly associated with increased hepatic fat levels and with hepatic inflammation in NAFLD patients.

Peer-review

The authors investigated the effect of vitamin E on NAFLD. They suggested that vitamin E treatment for 1 year reduced stiffness in NAFLD patients and the responses were similar between different *PNPLA3* genotypes. The work was logically designed and nicely described.

REFERENCES

1 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

2 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]

3 **Bhala N**, Angulo P, van der Poorten D, Lee E, Hui JM, Saracco G, Adams LA, Charatcharoenwitthaya P, Topping JH, Bugianesi E, Day CP, George J. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. *Hepatology* 2011; **54**: 1208-1216 [PMID: 21688282 DOI: 10.1002/hep.24491]

4 **Lewis JH**, Mortensen ME, Zweig S, Fusco MJ, Medoff JR, Belder R. Efficacy and safety of high-dose pravastatin in hypercholesterolemic patients with well-compensated chronic liver disease: Results of a prospective, randomized, double-blind, placebo-controlled, multicenter trial. *Hepatology* 2007; **46**: 1453-1463 [PMID: 17668878 DOI: 10.1002/hep.21848]

5 **Foster T**, Budoff MJ, Saab S, Ahmadi N, Gordon C, Guerci AD. Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: the St Francis Heart Study randomized clinical trial. *Am J Gastroenterol* 2011; **106**: 71-77 [PMID: 20842109 DOI: 10.1038/ajg.2010.299]

6 **Ekstedt M**, Franzén LE, Mathiesen UL, Holmqvist M, Bodemar G, Kechagias S. Statins in non-alcoholic fatty liver disease and chronically elevated liver enzymes: a histopathological follow-up study. *J Hepatol* 2007; **47**: 135-141 [PMID: 17400325 DOI: 10.1016/j.jhep.2007.02.013]

7 **Athyros VG**, Tziomalos K, Gossios TD, Griva T, Anagnostis P, Kargiotis K, Pagourelias ED, Theocharidou E, Karagiannis A, Mikhailidis DP. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study: a post-hoc analysis. *Lancet* 2010; **376**: 1916-1922 [PMID: 21109302 DOI: 10.1016/s0140-6736(10)61272-x]

8 **Mahady SE**, Webster AC, Walker S, Sanyal A, George J. The role of thiazolidinediones in non-alcoholic steatohepatitis - a systematic review and meta analysis. *J Hepatol* 2011; **55**: 1383-1390 [PMID: 21703200 DOI: 10.1016/j.jhep.2011.03.016]

9 **Boettcher E**, Csako G, Pucino F, Wesley R, Loomba R. Meta-analysis: pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2012; **35**: 66-75 [PMID: 22050199 DOI: 10.1111/j.1365-2036.2011.04912.x]

10 **Shyangdan D**, Clar C, Ghouri N, Henderson R, Gurung T, Preiss D, Sattar N, Fraser A, Waugh N. Insulin sensitisers in the treatment of non-alcoholic fatty liver disease: a systematic review. *Health Technol Assess* 2011; **15**: 1-110 [PMID: 22059955 DOI: 10.3310/hta15380]

11 **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]

12 **Hardwick RN**, Fisher CD, Canet MJ, Lake AD, Cherrington NJ. Diversity in antioxidant response enzymes in progressive stages of human nonalcoholic fatty liver disease. *Drug Metab Dispos* 2010; **38**: 2293-2301 [PMID: 20805291 DOI: 10.1124/dmd.110.035006]

13 **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]

14 **Rockey DC**, Caldwell SH, Goodman ZD, Nelson RC, Smith AD.

Liver biopsy. *Hepatology* 2009; **49**: 1017-1044 [PMID: 19243014 DOI: 10.1002/hep.22742]
15 **Bota S**, Herkner H, Sporea I, Salzl P, Sirli R, Neghina AM, Peck-Radosavljevic M. Meta-analysis: ARFI elastography versus transient elastography for the evaluation of liver fibrosis. *Liver Int* 2013; **33**: 1138-1147 [PMID: 23859217 DOI: 10.1111/liv.12240]
16 **Nishikawa T**, Hashimoto S, Kawabe N, Harata M, Nitta Y, Murao M, Nakano T, Mizuno Y, Shimazaki H, Kan T, Nakaoka K, Takagawa Y, Ohki M, Ichino N, Osakabe K, Yoshioka K. Factors correlating with acoustic radiation force impulse elastography in chronic hepatitis C. *World J Gastroenterol* 2014; **20**: 1289-1297 [PMID: 24574802 DOI: 10.3748/wjg.v20.i5.1289]
17 **Yoneda M**, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, Saito S, Nakajima A. Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography. *Radiology* 2010; **256**: 640-647 [PMID: 20529989 DOI: 10.1148/radiol.10091662]
18 **Palmeri ML**, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, Diehl AM, Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. *J Hepatol* 2011; **55**: 666-672 [PMID: 21256907 DOI: 10.1016/j.jhep.2010.12.019]
19 **Fierbinteanu Braticevici C**, Sporea I, Panaitescu E, Tribus L. Value of acoustic radiation force impulse imaging elastography for non-invasive evaluation of patients with nonalcoholic fatty liver disease. *Ultrasound Med Biol* 2013; **39**: 1942-1950 [PMID: 23932277 DOI: 10.1016/j.ultrasmedbio.2013.04.019]
20 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
21 **Kawaguchi T**, Sumida Y, Umemura A, Matsuo K, Takahashi M, Takamura T, Yasui K, Saibara T, Hashimoto E, Kawanaka M, Watanabe S, Kawata S, Imai Y, Kokubo M, Shima T, Park H, Tanaka H, Tajima K, Yamada R, Matsuda F. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS One* 2012; **7**: e38322 [PMID: 22719876 DOI: 10.1371/journal.pone.0038322]
22 **Hotta K**, Yoneda M, Hyogo H, Ochi H, Mizusawa S, Ueno T, Chayama K, Nakajima A, Nakao K, Sekine A. Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease. *BMC Med Genet* 2010; **11**: 172 [PMID: 21176169 DOI: 10.1186/1471-2350-11-172]
23 **Zain SM**, Mohamed R, Mahadeva S, Cheah PL, Rampal S, Basu RC, Mohamed Z. A multi-ethnic study of a PNPLA3 gene variant and its association with disease severity in non-alcoholic fatty liver disease. *Hum Genet* 2012; **131**: 1145-1152 [PMID: 22258181 DOI: 10.1007/s00439-012-1141-y]
24 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]
25 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]
26 **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]
27 **Yoshioka K**, Hashimoto S, Kawabe N. Measurement of liver stiffness as a non-invasive method for diagnosis of non-alcoholic fatty liver disease. *Hepatol Res* 2015; **45**: 142-151 [PMID: 25040931 DOI: 10.1111/hepr.12388]
28 **Sookoian S**, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res* 2009; **50**: 2111-2116 [PMID: 19738004 DOI: 10.1194/jlr.P900013-JLR200]
29 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]
30 **Lonn E**, Bosch J, Yusuf S, Sheridan P, Pogue J, Arnold JM, Ross C, Arnold A, Sleight P, Probstfield J, Dagenais GR. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. *JAMA* 2005; **293**: 1338-1347 [PMID: 15769967 DOI: 10.1001/jama.293.11.1338]

P- Reviewer: Hassanain M, Lee MK, Montalto G, Streba LAM
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i27.2757

World J Hepatol 2015 November 28; 7(27): 2757-2764
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Alpha-fetoprotein and des-gamma-carboxy-prothrombin at twenty-four weeks after interferon-based therapy predict hepatocellular carcinoma development

Satoshi Shakado, Shotaro Sakisaka, Kazuaki Chayama, Takeshi Okanoue, Joji Toyoda, Namiki Izumi, Akihiro Matsumoto, Tetsuo Takehara, Akio Ido, Yoichi Hiasa, Kentaro Yoshioka, Hideyuki Nomura, Yoshiyuki Ueno, Masataka Seike, Hiromitsu Kumada

Satoshi Shakado, Shotaro Sakisaka, Department of Gastroenterology and Medicine, Faculty of Medicine, Fukuoka University, Fukuoka City, Fukuoka 814-0180, Japan

Kazuaki Chayama, Department of Gastroenterology and Metabolism, Applied Life Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima City, Hiroshima 734-8553, Japan

Takeshi Okanoue, Center of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Suita City, Osaka 564-0013, Japan

Joji Toyoda, Department of Gastroenterology and Hepatology, Sapporo Kousei Hospital, Sapporo City, Hokkaido 060-0033, Japan

Namiki Izumi, Department of Gastroenterology and Hepatology, Musashino Red-Cross Hospital, Musashino City, Tokyo 180-8610, Japan

Akihiro Matsumoto, Department of Medicine, Shinshu University School of Medicine, Matsumoto City, Nagano 390-8621, Japan

Tetsuo Takehara, Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Suita City, Osaka 565-0871, Japan

Akio Ido, Department of Gastroenterology and Hepatology, Kagoshima University, Kagoshima City, Kagoshima 890-8520, Japan

Yoichi Hiasa, Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Toon City, Ehime 791-0295, Japan

Kentaro Yoshioka, Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, Toyoake City, Aichi 470-1192, Japan

Hideyuki Nomura, the Center for Liver Disease, Shin-Kokura Hospital, Kitakyushu City, Fukuoka 803-8505, Japan

Yoshiyuki Ueno, Department of Gastroenterology, Yamagata University, Yamagata City, Yamagata 990-9585, Japan

Masataka Seike, Department of Gastroenterology, Faculty of Medicine, Oita University, Yufu City, Oita 879-5593, Japan

Hiromitsu Kumada, Department of Hepatology, Toranomon Hospital, Kawasaki City, Kanagawa 213-8587, Japan

Author contributions: Shakado S and Sakisaka S performed the study design, acquisition, analysis and interpretation of data, literature search, drafting of the manuscript; Chayama K, Okanoue T, Toyoda J, Izumi N, Matsumoto A, Takehara T, Ido A, Hiasa Y, Yoshioka K, Nomura H, Ueno Y, Seike M and Kumada H contributed to the acquisition of data.

Supported by A Grant-in-Aid from the Japanese Ministry of Health, Welfare and Labour.

Institutional review board statement: The study was not needed to approve by the institutional review board.

Informed consent statement: This study is a retrospective report. The additional informed consent from the patients does not apply.

Conflict-of-interest statement: Shotaro Sakisaka received Research Fund from MSD; Kazuaki Chayama received Research Fund from AbbVie, Dainippon Sumitomo, Janssen, Daiichi Sankyo, Roche, MSD, Toray, Otsuka and Tanabe; and Kazuaki Chayama received lecture fees form MSD and Bristol-Myers; Namiki Izumi received lecture fees from Chugai, MSD, Bristol-Myers, Dainippon Sumitomo, Janssen; Tetsuo Takehara received Research Fund from MSD; and Tetsuo Takehara received lecture fees form MSD; Akio Ido received Research Fund from Chugai, MSD, Dai-ichi Sankyo and Tanabe; Kentaro Yoshioka received Research Fund from MSD; and Kentaro Yoshioka had an advisory role to Sanwa Kagaku Kenkyusho; Yoshiyuki Ueno

received Research Fund from Tanabe, MSD, Chugai, BMS and Gilead Sciences; Hiromitsu Kumada received lecture fees from MSD K.K, Bristol-Myers Squibb, Janssn Pharmaceutical K.K, Glaxo Smith Kline K.K; and Hiromitsu Kumada had patentroyalties from SRL.

Data sharing statement: No additional data are available.

Correspondence to: Satoshi Shakado, MD, Associate Professor, Department of Gastroenterology and Medicine, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka City, Fukuoka 814-0180, Japan. shakado@cis.fukuoka-u.ac.jp
Telephone: +81-92-8011011
Fax: +81-92-8742663

Received: May 30, 2015
Peer-review started: June 1, 2015
First decision: July 6, 2015
Revised: August 27, 2015
Accepted: November 13, 2015
Article in press: November 17, 2015
Published online: November 28, 2015

Abstract

AIM: To investigate risk factors for development of hepatocellular carcinoma (HCC) in patients with hepatitis C virus-related liver cirrhosis (LC-C).

METHODS: To evaluate the relationship between clinical factors including virological response and the development of HCC in patients with LC-C treated with interferon (IFN) and ribavirin, we conducted a multicenter, retrospective study in 14 hospitals in Japan. All patients had compensated LC-C with clinical or histological data available. HCC was diagnosed by the presence of typical hypervascular characteristics on computed tomography and/or magnetic resonance imaging.

RESULTS: HCC was diagnosis in 50 (21.6%) of 231 LC-C patients during a median observation period of 3.8 years after IFN and ribavirin therapy. Patients who developed HCC were older (P = 0.018) and had higher serum levels of pretreatment alpha-fetoprotein (AFP) (P = 0.038). Multivariate analysis revealed the following independent risk factors for HCC development: history of treatment for HCC [P < 0.001, odds ratio (OR) = 15.27, 95%CI: 4.98-59.51], AFP levels of ⩾ 10 ng/mL (P = 0.009, OR = 3.89, 95%CI: 1.38-11.94), and des-γ-carboxy prothrombin (DCP) levels of ⩾ 40 mAU/mL at 24 wk after the completion of IFN and ribavirin therapy (P < 0.001, OR = 24.43, 95%CI: 4.11-238.67).

CONCLUSION: We suggested that the elevation of AFP and DCP levels at 24 wk after the completion of IFN and ribavirin therapy were strongly associated with the incidence of HCC irrespective of virological response among Japanese LC-C patients.

Key words: Des-γ-carboxy prothrombin; Hepatocellular carcinoma; Alpha-fetoprotein; Interferon; Hepatitis C virus; Liver cirrhosis; Ribavirin

Core tip: Interferon (IFN)-based therapy reduces the rate of hepatocellular carcinoma (HCC) development in patients with chronic hepatitis C virus (HCV) infection. However, HCC development has frequently been reported in HCV-related liver cirrhosis (LC-C) patients who achieved sustained virological response. We conducted a multicenter, retrospective study to evaluate the relationship between clinical factors and HCC development in Japanese LC-C patients treated with IFN and ribavirin therapy. We suggested that the elevation of Alpha-fetoprotein and des-γ-carboxy prothrombin levels at 24 wk after the completion of IFN and ribavirin therapy were strongly associated with the incidence of HCC irrespective of virological response among Japanese LC-C patients.

Shakado S, Sakisaka S, Chayama K, Okanoue T, Toyoda J, Izumi N, Matsumoto A, Takehara T, Ido A, Hiasa Y, Yoshioka K, Nomura H, Ueno Y, Seike M, Kumada H. Alpha-fetoprotein and des-gamma-carboxy-prothrombin at twenty-four weeks after interferon-based therapy predict hepatocellular carcinoma development. *World J Hepatol* 2015; 7(27): 2757-2764 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i27/2757.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i27.2757

INTRODUCTION

Chronically hepatitis C virus (HCV) infection is the commonest cause of liver cirrhosis in the world[1]. HCV-related liver cirrhosis (LC-C) patients are the high risk to the development of hepatocellular carcinoma (HCC)[2,3]. Many previous studies suggested that interferon (IFN)-based therapy reduces the rate of HCC development in patients with chronically HCV infection, especially those with sustained virological response (SVR)[4-8]. Non-SVR, male sex, older age, and advanced liver fibrosis have been shown to be risk factors for HCC development in patients treated with IFN[9-13]. Therapy with IFN and ribavirin has been used for LC-C patients, leading to significant effects including SVR. However, the development of HCC has frequently been reported

Table 1 Pretreatment clinical characteristics of 231 hepatitis C virus-related liver cirrhosis patients treated with interferon and ribavirin

	All patients (n = 231)	Non HCC (n = 181)	HCC (n = 50)	P-value[1]
Sex (M:F)	111:120	82:99	29:21	NS
Age (yr)	60.4 ± 9.2	59.6 ± 9.2	63.1 ± 9.1	0.018
BMI (kg/m^2)	23.7 ± 3.4	23.7 ± 3.5	23.9 ± 2.9	NS
Total bilirubin (mg/dL)	1.1 ± 1.2	1.2 ± 1.4	0.9 ± 0.3	NS
Albumin (g/dL)	3.8 ± 0.5	3.8 ± 0.5	3.8 ± 0.3	NS
Prothrombin (%)	86.1 ± 15.2	86.1 ± 15.8	86.1 ± 13.2	NS
ALT (IU/L)	84.6 ± 64.4	86.6 ± 65.8	77.1 ± 59.6	NS
GGT (IU/L)	89.0 ± 124.0	89.0 ± 125.2	89.3 ± 122.1	NS
Hemoglobin (g/dL)	13.2 ± 1.8	13.1 ± 1.9	13.5 ± 1.7	NS
Platelets (10^4/mm^3)	12.1 ± 6.8	12.2 ± 7.2	11.7 ± 5.1	NS
AFP (ng/mL)	94.1 ± 916.1	24.2 ± 38.0	355.1 ± 1994.9	0.038
DCP (mAU/mL)	261.5 ± 2687.8	328.6 ± 3057.3	26.5 ± 18.1	NS
IL28B (TT:non TT)	161:70	130:51	31:19	NS
Presence of EV	74/191 (38.7%)	60/146 (41.1%)	14/45 (31.1%)	NS
HCC treatment history	80 (34.6%)	44 (24.3%)	36 (72.0%)	NS
HCV genotype (1/2)	189:42	147:34	42:8	NS
IFN treatment (naive)	208 (90.0%)	162 (89.5%)	46 (92%)	NS

Data are expressed as number (%) or mean ± SD. All demographic and clinical data are those at the start of antiviral treatment. [1]Comparison between non HCC and HCC. HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; NS: Not significant; BMI: Body mass index; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transpeptidase; AFP: Alpha-fetoprotein; DCP: Des-gamma-carboxy prothrombin; IL28B: Interleukin 28B rs8099917; EV: Esophageal varices; IFN: Interferon.

in LC-C patients who achieved SVR[14,15]. The aim of this retrospective, multicenter study was to evaluate the relationship among pre- and post-treatment clinical factors, virological response, and HCC development in Japanese LC-C patients treated with IFN and ribavirin to elucidate the predictive markers for HCC development.

MATERIALS AND METHODS

Patient selection

We conducted a retrospective, multicenter study in 14 hospitals in Japan. All 290 patients with LC-C were treated with IFN plus ribavirin. A diagnosis of compensated LC-C was defined with the clinical or histological finding. We decided the presence of at least one of the following criteria: Liver biopsy demonstrating cirrhosis, multiple nodular appearance of liver surface on peritoneoscopy, liver stiffness greater than 12.5 kPa on transient elastography, presence of esophageal varices, or positive values of cirrhosis criteria[16-18].

Of the 290 patients, 59 developed HCC within 6 mo of completing IFN and ribavirin therapy and were excluded from the study. All analyses used data from the remaining 231 cases. Table 1 shows pretreatment clinical characteristics. Of 231 patients, 189 patients were infected with HCV genotype 1 and 80 patients (34.6%) had received treatment for HCC previously. Eighty patients were treated for HCC with hepatectomy, transcatheter chemoembolization, or radio frequency ablation therapy in each hospital. More detail of treatment history was not investigated in this study. The average follow-up period was 3.8 ± 2.2 years.

Combination therapy with IFN and ribavirin

All 231 patients were treated with INF and ribavirin. Pegylated-IFN alpha-2b, pegylated-IFN alpha-2a or IFN alpha-2b were administered to 297 (85.3%), 19 (8.2%), 15 (6.5%), respectively.

Surveillance for HCC

Hepatic ultrasonography, computed tomography (CT), and/or magnetic resonance imaging (MRI) were performed every 3 to 6 mo during follow-up period for HCC surveillance. HCC was diagnosed on the basis of the presence of typical hypervascular characteristics of CT and/or MRI findings.

Statistical analysis

We conducted statistical analyze with Fisher's exact test or Student's t-test. Univariate and multivariate analysis were used with JMP version 9.0 for Macintosh (SAS Institute, Cary, NC). The odds ratio and 95%CI were also calculated.

RESULTS

Pretreatment clinical factors associated with HCC development

HCC was diagnosed in 50 (21.6%) of 231 LC-C patients treated with IFN and ribavirin during a median follow-up period of 3.8 years (0.6-11.9 years). Patients who developed HCC were older (P = 0.018) and had higher serum levels of alpha-fetoprotein (AFP) (P = 0.038) than the non-HCC group (Table 1). In our study, no significant difference in HCC development was observed

Table 2 Risk factors for the development of hepatocellular carcinoma in hepatitis C virus-related liver cirrhosis patients treated with interferon and ribavirin

	Non HCC (n = 181)	HCC (n = 50)	P-value
IFN treatment duration (wk)	43.1 ± 21.5	44.1 ± 22.5	NS
Sustained virological response	63 (34.8%)	12 (24%)	NS
Albumin levels at the end of IFN treatment (g/dL)	3.7 ± 0.6	3.7 ± 0.6	NS
Prothrombin levels at the end of IFN treatment (%)	86.0 ± 21.5	83.5 ± 11.1	NS
AFP levels at the end of IFN treatment (ng/mL)	15.5 ± 34.9	42.8 ± 96.0	0.009
DCP levels at the end of IFN treatment (mAU/mL)	25.6 ± 47.2	255.6 ± 863.2	0.017
Albumin levels at 24 wk after IFN treatment (g/dL)	4.0 ± 0.5	3.7 ± 0.5	0.004
Prothrombin levels at 24 wk after IFN treatment (%)	87.8 ± 17.9	86.6 ± 14.2	NS
AFP levels at 24 wk after IFN treatment (ng/mL)	11.5 ± 15.8	63.2 ± 193.2	0.002
DCP levels at 24 wk after IFN treatment (mAU/mL)	18.4 ± 12.7	354.0 ± 1887.5	NS

Data are expressed as number (%) or mean ± SD. HCC: Hepatocellular carcinoma; IFN: Interferon; NS: Not significant; AFP: Alpha-fetoprotein; DCP: Des-gamma-carboxy prothrombin.

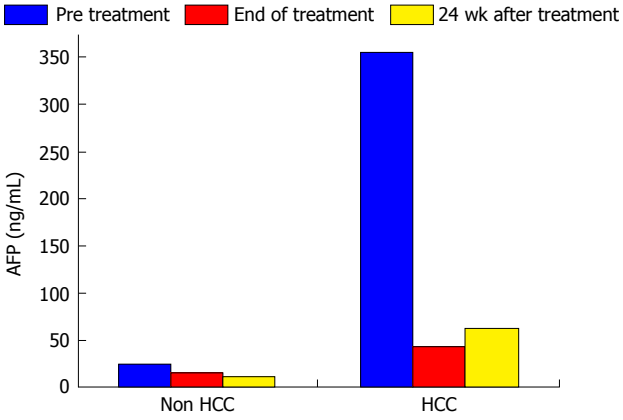

Figure 1 A change of the alpha-fetoprotein levels after interferon-based treatment. AFP: Alpha-fetoprotein; HCC: Hepatocellular carcinoma.

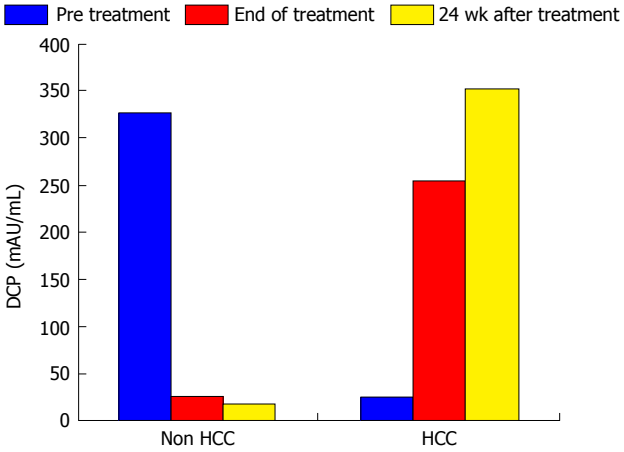

Figure 2 A change of the des-gamma-carboxy prothrombin levels after interferon-based treatment. DCP: Des-gamma-carboxy prothrombin; HCC: Hepatocellular carcinoma.

for male sex, platelet count, interleukin 28B genotype, presence of esophageal varices, HCV genotype, type of IFN or IFN treatment history (naive or non-naive).

Predictive factors associated with HCC development after IFN and ribavirin therapy

In this study, the duration of treatment with IFN and SVR were not associated with HCC development (Table 2). Serum levels of AFP and des-γ-carboxy prothrombin (DCP) were focus to be risk factors for HCC development. Normal range of AFP and DCP were under 10 ng/mL and under 40 mAU/mL, respectively. In patients who developed HCC, serum levels of AFP decreased from 355.1 ng/mL to 42.8 ng/mL during the course of IFN therapy and then increased from 42.8 ng/mL to 63.2 ng/mL at 24 wk after the completion of IFN and ribavirin therapy. In patients who did not develop HCC, serum AFP levels at the beginning, completion, and 24 wk after IFN and ribavirin therapy were 94.1, 15.5 and 11.5 ng/mL, respectively. In patients who did not develop HCC, serum DCP levels at the beginning, completion, and 24 wk after IFN and ribavirin therapy were 328.6, 25.6 and 18.4 mAU/mL, respectively. As with AFP, serum DCP levels were increased in patients who developed HCC. Serum levels of AFP and DCP were the greatest risk factors for HCC development at 24 wk after the completion of IFN and ribavirin therapy. In patients who did not develop HCC, albumin levels increased from 3.7 g/dL at the completion of IFN and ribavirin therapy to 4.0 g/dL at 24 wk after the completion of treatment. A change of those tumor markers after IFN and ribavirin therapy was shown in Figures 1 and 2.

In our study, SVR was not associated to HCC development. And the levels of AFP and DCP were not associated with HCC development in patients with a SVR (Table 3).

As shown in Table 4, the multivariate analysis revealed the following independent risk factors for HCC development after IFN and ribavirin therapy: History of treatment for HCC (P < 0.001, OR = 15.27, 95%CI: 4.98-59.51), AFP levels of ⩾ 10 ng/mL at 24 wk after the completion of IFN and ribavirin therapy (P = 0.009, OR = 3.89, 95%CI: 1.38-11.94), and DCP levels of ⩾ 40 mAU/mL at 24 wk after the completion of IFN and ribavirin therapy (P < 0.001, OR = 24.43, 95%CI: 4.11-238.67).

Table 3 The levels of alpha-fetoprotein and des-gamma-carboxy prothrombin with development of hepatocellular carcinoma in patients with sustained virological response

	Patients with SVR (n = 75)	Non HCC (n = 63)	HCC (n = 12)	P-value[1]
AFP levels at pretreatment (ng/mL)	16.1 ± 20.2	17.8 ± 22.2	12.6 ± 14.9	NS
AFP levels at the end of IFN treatment (ng/mL)	13.7 ± 47.8	17.9 ± 57.8	5.1 ± 1.8	NS
AFP levels at 24 wk after IFN treatment (ng/mL)	6.7 ± 10.0	5.8 ± 4.1	8.0 ± 15.1	NS
Pre DCP levels at pretreatment (mAU/mL)	93.7 ± 374.1	131.3 ± 481.4	40.3 ± 69.8	NS
Post DCP levels at the end of IFN treatment (mAU/mL)	140.0 ± 637.9	226.9 ± 864.2	42.1 ± 83.3	NS
24 wk DCP levels at 24 wk after IFN treatment (mAU/mL)	33.9 ± 52.6	28.9 ± 37.4	39.3 ± 66.5	NS

Data are expressed as number (%) or mean ± SD. [1]Comparison between non HCC and HCC. HCC: Hepatocellular carcinoma; AFP: Alpha-fetoprotein; DCP: Des-gamma-carboxy prothrombin; NS: Not significant; SVR: Sustained virological response.

Table 4 Factors associated with hepatocellular carcinoma development (n = 231)

Risk factor	Univariate analysis P-value	Multivariate analysis P-value	OR	95%CI
Age (over 60 yr)	0.012	Not significant		
HCC treatment history	< 0.001	< 0.001	15.27	4.98-59.51
AFP levels at 24 wk after IFN treatment ⩾ 10 ng/mL	0.003	0.009	3.89	1.38-11.94
DCP levels at 24 wk after IFN treatment ⩾ 40 mAU/mL	< 0.001	< 0.001	24.43	4.11-238.67

HCC: Hepatocellular carcinoma; AFP: Alpha-fetoprotein; IFN: Interferon; DCP: Des-gamma-carboxy prothrombin.

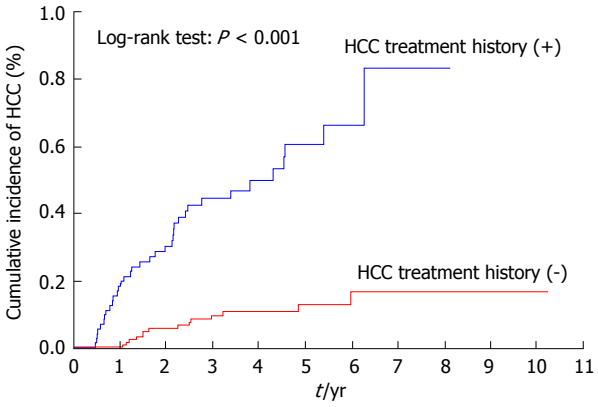

Figure 3 Cumulative incidence of hepatocellular carcinoma according to the history of treatment for hepatocellular carcinoma. HCC: Hepatocellular carcinoma.

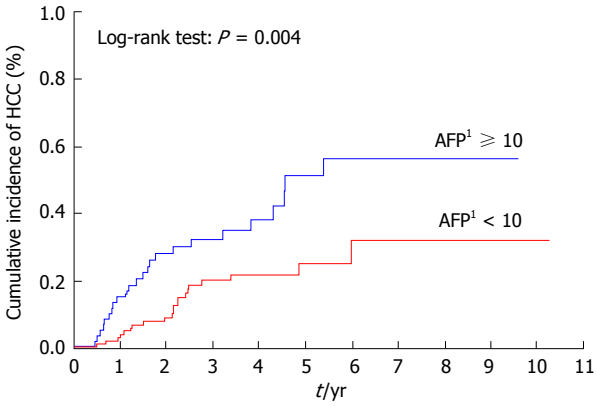

Figure 4 Cumulative incidence of hepatocellular carcinoma according to alpha-fetoprotein levels. [1]The value at 24 wk after the completion of interferon-based therapy (ng/mL). AFP: Alpha-fetoprotein; HCC: Hepatocellular carcinoma.

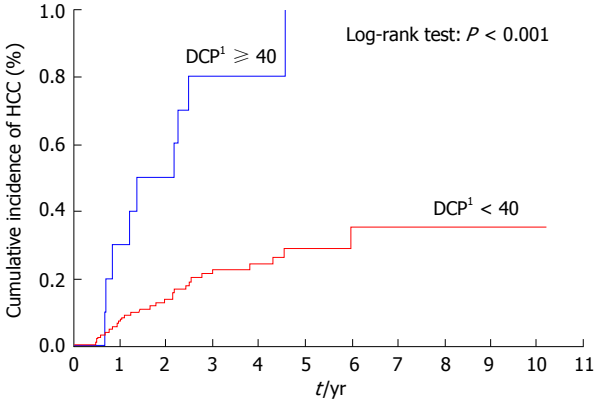

Figure 5 Cumulative incidence of hepatocellular carcinoma according to des- gamma-carboxy prothrombin levels at 24 wk after the completion of interferon-based therapy. [1]The value at 24 wk after the completion of interferon-based therapy (mAU/mL). DCP: Des-gamma-carboxy prothrombin; HCC: Hepatocellular carcinoma.

Cumulative incidence of HCC after IFN and ribavirin therapy

Patients with a previous history of treatment for HCC had a significantly higher cumulative incidence of HCC development after IFN and ribavirin therapy (P < 0.001) (Figure 3). The incidence of HCC was significantly lower in patients with AFP levels of < 10 ng/mL than in those with AFP levels of ⩾ 10 ng/mL at 24 wk after the completion of IFN and ribavirin therapy (P = 0.004) (Figure 4) as in patients with DCP levels of < 40 mAU/mL than in those with DCP levels of ⩾ 40 mAU/mL at 24 wk after the completion of IFN and ribavirin therapy (P < 0.001) (Figure 5).

DISCUSSION

In this retrospective, multicenter, cooperative study

conducted in Japan, we evaluated risk factors of HCC development in LC-C patients treated with IFN and ribavirin. A history of treatment for HCC was a strong risk factor for the development of HCC in our patients. Although HCC has a high recurrence rate, even after curative surgery, a suppressive effect of IFN on HCC recurrence after previous curative treatment has been reported in several studies[19-23]. Furthermore, particularly in Japan, IFN is used as an anti-cancer drug for the treatment of HCC[24-27]. Unfortunately, in LC-C patients treated with IFN and ribavirin, most notably in those with a history of treatment for HCC, IFN therapy did not reduce recurrence rates in our study. Many studies reported that IFN-based therapy not only improves hepatic fibrosis and inflammation but also reduces the incidence of HCC, particularly in patients who achieve SVR[4-13]. In our study, a history of treatment for HCC was a stronger risk factor for HCC development than achieving SVR. In those patients receiving IFN and ribavirin therapy, long-term surveillance for HCC should be conducted even after antiviral therapy with SVR.

In our study, serum levels of AFP decreased after IFN and ribavirin therapy compared to baseline levels in both HCC and non-HCC groups. Serum levels of AFP were further decreased at 24 wk after IFN and ribavirin therapy in the non-HCC group. However, serum levels of AFP were increased at 24 wk after the completion of IFN and ribavirin therapy in patients who developed HCC. Therefore, serum AFP levels at 24 wk after the completion of IFN and ribavirin therapy may be a strong predictor of HCC development in LC-C patients treated with IFN and ribavirin. Serum levels of DCP were also increased at 24 wk after the completion of IFN and ribavirin therapy in patients who developed HCC. Both AFP and DCP serum levels at 24 wk after the completion of IFN and ribavirin therapy were more strongly associated with HCC development than those of pre- and/or post-IFN treatment.

Previous studies reported that a low or decreased AFP level during IFN therapy is associated with a reduced incidence of HCC[28-30]. Serum levels of AFP after IFN-based therapy are also informative, and a higher post-treatment AFP ($\geqslant$ 6 ng/mL) was a risk factor for HCC development[11,31].

Recently, DCP was demonstrated as a tumor marker for the detection of HCC[32,33]. However, it was unclear whether DCP had value in detecting HCC in patients with LC-C who received IFN and ribavirin therapy. We demonstrated by multivariate analysis that elevated serum levels of AFP ($\geqslant$ 10 ng/mL) and DCP ($\geqslant$ 40 mAU/mL) at 24 wk after the completion of IFN and ribavirin therapy were independently associated with HCC development. In clinical practice, even in patients with SVR, careful surveillance for HCC is required in patients with LC-C with an AFP of $\geqslant$ 10 ng/mL or a DCP of $\geqslant$ 40 mAU/mL at 24 wk after the completion of IFN and ribavirin therapy. A randomized controlled study demonstrated that the LC-C patients who were treated with long-term pegylated-IFN had a low risk of HCC development[34]. Therefore, LC-C patients with an AFP of $\geqslant$ 10 ng/mL or a DCP of $\geqslant$ 40 mAU/mL at 24 wk after the completion of IFN and ribavirin therapy should be considered for long-term maintenance treatment with pegylated-IFN, irrespective of whether SVR is achieved.

Recently, therapies with direct-acting antivirals without IFN have demonstrated great efficacy against HCV[35-38]. However, it is currently unknown whether serum AFP levels and HCC incidence are decreased in patients treated with IFN-free regimens using direct-acting antivirals.

Although this present study had some limitations, all included patients were diagnosed with well-established cirrhosis without chronic hepatitis. Thus, our findings provide valuable information.

In conclusion, we suggested that elevated serum levels of both AFP and DCP at 24 wk after the completion of IFN and ribavirin therapy are strongly associated with HCC development, irrespective of the virological response, among Japanese LC-C patients. In these patients, additional surveillance for the development of HCC may be required.

COMMENTS

Background

Chronically hepatitis C virus (HCV) infection is the commonest cause of liver cirrhosis in the world. HCV-related liver cirrhosis (LC-C) patients are the high risk to the development of hepatocellular carcinoma (HCC).

Research frontiers

Interferon (IFN)-based therapy reduces the rate of HCC development in patients with chronically HCV infection, especially those with sustained virological response (SVR). However, HCC development has frequently been reported in LC-C patients who achieved SVR. In those patients receiving IFN-based therapy, long-term surveillance for HCC should be conducted even after antiviral therapy with SVR. Knowing risk factors for HCC development is required in aged patients with LC-C treated with anti-viral agents.

Innovations and breakthroughs

Previous studies included chronic hepatitis and cirrhosis. In this study, all included patients were diagnosed with well-established cirrhosis. SVR was not associated with HCC development in LC-C patients.

Applications

The authors suggested that elevated serum levels of both alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) at 24 wk after the completion of IFN and ribavirin therapy are strongly associated with HCC incidence, irrespective of the virological response, among LC-C patients. In those patients, additional surveillance for the development of HCC may be required.

Terminology

Serum levels of both AFP and DCP at 24 wk after the completion of anti-viral agent provide valuable information that can be used to clinical decisions.

Peer-review

The authors showed elevated serum levels of both AFP and DCP at 24 wk after the completion of IFN and ribavirin therapy are strongly associated with HCC incidence. This study has a certain clinical impact.

REFERENCES

1 **Niederau C**, Lange S, Heintges T, Erhardt A, Buschkamp M, Hürter D, Nawrocki M, Kruska L, Hensel F, Petry W, Häussinger D. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 1998; **28**: 1687-1695 [PMID: 9828236 DOI: 10.1002/hep.510280632]

2 **Hu KQ**, Tong MJ. The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. *Hepatology* 1999; **29**: 1311-1316 [PMID: 10094980 DOI: 10.1002/hep.510290424]

3 **Sangiovanni A**, Prati GM, Fasani P, Ronchi G, Romeo R, Manini M, Del Ninno E, Morabito A, Colombo M. The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. *Hepatology* 2006; **43**: 1303-1310 [PMID: 16729298 DOI: 10.1002/hep.21176]

4 **Nishiguchi S**, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; **346**: 1051-1055 [PMID: 7564784 DOI: 10.1016/S0140-6736(95)91739-X]

5 **Yoshida H**, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174-181 [PMID: 10428733 DOI: 10.7326/0003-4819-131-3-199908030-00003]

6 **Imai Y**, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, Maeda Y, Shirai Y, Fukuzaki T, Kaji I, Ishikawa H, Matsuda Y, Nishikawa M, Seki K, Matsuzawa Y. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998; **129**: 94-99 [PMID: 9669992 DOI: 10.7326/0003-4819-129-2-199807150-00005]

7 **Serfaty L**, Aumaître H, Chazouillères O, Bonnand AM, Rosmorduc O, Poupon RE, Poupon R. Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology* 1998; **27**: 1435-1440 [PMID: 9581703 DOI: 10.1002/hep.510270535]

8 **Shiratori Y**, Ito Y, Yokosuka O, Imazeki F, Nakata R, Tanaka N, Arakawa Y, Hashimoto E, Hirota K, Yoshida H, Ohashi Y, Omata M. Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; **142**: 105-114 [PMID: 15657158 DOI: 10.7326/0003-4819-142-2-200501180-00009]

9 **Bruno S**, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, Asti M, Rossi S, Larghi A, Cerino A, Podda M, Mondelli MU. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. *Hepatology* 1997; **25**: 754-758 [PMID: 9049231 DOI: 10.1002/hep.510250344]

10 **Cardoso AC**, Moucari R, Figueiredo-Mendes C, Ripault MP, Giuily N, Castelnau C, Boyer N, Asselah T, Martinot-Peignoux M, Maylin S, Carvalho-Filho RJ, Valla D, Bedossa P, Marcellin P. Impact of peginterferon and ribavirin therapy on hepatocellular carcinoma: incidence and survival in hepatitis C patients with advanced fibrosis. *J Hepatol* 2010; **52**: 652-657 [PMID: 20346533 DOI: 10.1016/j.jhep.2009.12.028]

11 **Oze T**, Hiramatsu N, Yakushijin T, Miyazaki M, Yamada A, Oshita M, Hagiwara H, Mita E, Ito T, Fukui H, Inui Y, Hijioka T, Inada M, Katayama K, Tamura S, Yoshihara H, Inoue A, Imai Y, Hayashi E, Kato M, Miyagi T, Yoshida Y, Tatsumi T, Kasahara A, Hamasaki T, Hayashi N, Takehara T. Post-treatment levels of α-fetoprotein predict incidence of hepatocellular carcinoma after interferon therapy. *Clin Gastroenterol Hepatol* 2014; **12**: 1186-1195 [PMID: 24321207 DOI: 10.1016/j.cgh.2013.11.033]

12 **Hung CH**, Lee CM, Wang JH, Hu TH, Chen CH, Lin CY, Lu SN. Impact of diabetes mellitus on incidence of hepatocellular carcinoma in chronic hepatitis C patients treated with interferon-based antiviral therapy. *Int J Cancer* 2011; **128**: 2344-2352 [PMID: 20669224 DOI: 10.1002/ijc.25585]

13 **Asahina Y**, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, Yasui Y, Hosokawa T, Ueda K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Enomoto N, Izumi N. Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. *Hepatology* 2010; **52**: 518-527 [PMID: 20683951]

14 **Ogawa E**, Furusyo N, Kajiwara E, Takahashi K, Nomura H, Maruyama T, Tanabe Y, Satoh T, Nakamuta M, Kotoh K, Azuma K, Dohmen K, Shimoda S, Hayashi J. Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: a prospective, multicenter study. *J Hepatol* 2013; **58**: 495-501 [PMID: 23099187 DOI: 10.1016/j.jhep.2012.10.017]

15 **Yamashita N**, Ohho A, Yamasaki A, Kurokawa M, Kotoh K, Kajiwara E. Hepatocarcinogenesis in chronic hepatitis C patients achieving a sustained virological response to interferon: significance of lifelong periodic cancer screening for improving outcomes. *J Gastroenterol* 2014; **49**: 1504-1513 [PMID: 24317936 DOI: 10.1007/s00535-013-0921-z]

16 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54 [PMID: 15690481 DOI: 10.1002/hep.20506]

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

18 **Ikeda K**, Saitoh S, Kobayashi M, Suzuki Y, Tsubota A, Suzuki F, Arase Y, Murashima N, Chayama K, Kumada H. Distinction between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection. Practical discriminant function using common laboratory data. *Hepatol Res* 2000; **18**: 252-266 [PMID: 11058829 DOI: 10.1016/S1386-6346(00)00074-7]

19 **Liver Cancer Study Group of Japan**. Primary liver cancer in Japan. Clinicopathologic features and results of surgical treatment. *Ann Surg* 1990; **211**: 277-287 [PMID: 2155591]

20 **Nagasue N**, Uchida M, Makino Y, Takemoto Y, Yamanoi A, Hayashi T, Chang YC, Kohno H, Nakamura T, Yukaya H. Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. *Gastroenterology* 1993; **105**: 488-494 [PMID: 8392955]

21 **Miyake Y**, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *J Viral Hepat* 2010; **17**: 287-292 [PMID: 19732321]

22 **Shen YC**, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. *J Hepatol* 2010; **52**: 889-894 [PMID: 20395009 DOI: 10.1016/j.jhep.2009.12.041]

23 **Hsu YC**, Ho HJ, Wu MS, Lin JT, Wu CY. Postoperative peg-interferon plus ribavirin is associated with reduced recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatology* 2013; **58**: 150-157 [PMID: 23389758 DOI: 10.1002/hep.26300]

24 **Sakon M**, Nagano H, Dono K, Nakamori S, Umeshita K, Yamada A, Kawata S, Imai Y, Iijima S, Monden M. Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer* 2002; **94**: 435-442 [PMID: 11900229 DOI: 10.1002/cncr.10246]

25 **Uka K**, Aikata H, Takaki S, Miki D, Kawaoka T, Jeong SC, Takahashi S, Toyota N, Ito K, Chayama K. Pretreatment predictor of response, time to progression, and survival to intraarterial 5-fluorouracil/interferon combination therapy in patients with advanced hepatocellular carcinoma. *J Gastroenterol* 2007; **42**: 845-853 [PMID: 17940838 DOI: 10.1007/s00535-007-2099-8]

26 **Kasai K**, Ushio A, Kasai Y, Sawara K, Miyamoto Y, Oikawa

K, Kuroda H, Takikawa Y, Suzuki K. Therapeutic efficacy of combination therapy with intra-arterial 5-fluorouracil and systemic pegylated interferon α-2b for advanced hepatocellular carcinoma with portal venous invasion. *Cancer* 2012; **118**: 3302-3310 [PMID: 22072099 DOI: 10.1002/cncr.26648]

27 **Shakado S**, Iwata K, Tsuchiya N, Kunimoto H, Yotsumoto K, Fukunaga A, Kuno S, Tanaka T, Sakurai K, Iwashita H, Hirano G, Yokoyama K, Morihara D, Nishizawa S, Takeyama Y, Irie M, Sohda T, Kora S, Yoshimitsu K, Sakisaka S. Pilot Study of Hepatic Arterial Infusion Chemotherapy with Interferon-beta and 5-fluorouracil: A New Chemotherapy for Patients with Advanced Hepatocellular Carcinoma. *Hepatogastroenterology* 2014; **61**: 557-562 [PMID: 26176035 DOI: 10.5754/hge13950]

28 **Nomura H**, Kashiwagi Y, Hirano R, Tanimoto H, Tsutsumi N, Higashi M, Ishibashi H. Efficacy of low dose long-term interferon monotherapy in aged patients with chronic hepatitis C genotype 1 and its relation to alpha-fetoprotein: A pilot study. *Hepatol Res* 2007; **37**: 490-497 [PMID: 17539990 DOI: 10.1111/j.1872-034X.2007.00073.x]

29 **Chen TM**, Huang PT, Tsai MH, Lin LF, Liu CC, Ho KS, Siauw CP, Chao PL, Tung JN. Predictors of alpha-fetoprotein elevation in patients with chronic hepatitis C, but not hepatocellular carcinoma, and its normalization after pegylated interferon alfa 2a-ribavirin combination therapy. *J Gastroenterol Hepatol* 2007; **22**: 669-675 [PMID: 17444854 DOI: 10.1111/j.1440-1746.2007.04898.x]

30 **Osaki Y**, Ueda Y, Marusawa H, Nakajima J, Kimura T, Kita R, Nishikawa H, Saito S, Henmi S, Sakamoto A, Eso Y, Chiba T. Decrease in alpha-fetoprotein levels predicts reduced incidence of hepatocellular carcinoma in patients with hepatitis C virus infection receiving interferon therapy: a single center study. *J Gastroenterol* 2012; **47**: 444-451 [PMID: 22105231 DOI: 10.1007/s00535-011-0505-8]

31 **Asahina Y**, Tsuchiya K, Nishimura T, Muraoka M, Suzuki Y, Tamaki N, Yasui Y, Hosokawa T, Ueda K, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Enomoto N, Nakagawa M, Kakinuma S, Watanabe M, Izumi N. α-fetoprotein levels after interferon therapy and risk of hepatocarcinogenesis in chronic hepatitis C. *Hepatology* 2013; **58**: 1253-1262 [PMID: 23564522 DOI: 10.1002/hep.26442]

32 **Ertle JM**, Heider D, Wichert M, Keller B, Kueper R, Hilgard P, Gerken G, Schlaak JF. A combination of α-fetoprotein and des-γ-carboxy prothrombin is superior in detection of hepatocellular carcinoma. *Digestion* 2013; **87**: 121-131 [PMID: 23406785 DOI: 10.1159/000346080]

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

34 **Lok AS**, Everhart JE, Wright EC, Di Bisceglie AM, Kim HY, Sterling RK, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Morgan TR. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. *Gastroenterology* 2011; **140**: 840-849; quiz e12 [PMID: 21129375 DOI: 10.1053/j.gastro.2010.11.050]

35 **Poordad F**, Lawitz E, Kowdley KV, Cohen DE, Podsadecki T, Siggelkow S, Heckaman M, Larsen L, Menon R, Koev G, Tripathi R, Pilot-Matias T, Bernstein B. Exploratory study of oral combination antiviral therapy for hepatitis C. *N Engl J Med* 2013; **368**: 45-53 [PMID: 23281975 DOI: 10.1056/NEJMoa1208809]

36 **Kumada H**, Suzuki Y, Ikeda K, Toyota J, Karino Y, Chayama K, Kawakami Y, Ido A, Yamamoto K, Takaguchi K, Izumi N, Koike K, Takehara T, Kawada N, Sata M, Miyagoshi H, Eley T, McPhee F, Damokosh A, Ishikawa H, Hughes E. Daclatasvir plus asunaprevir for chronic HCV genotype 1b infection. *Hepatology* 2014; **59**: 2083-2091 [PMID: 24604476 DOI: 10.1002/hep.27113]

37 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]

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

P- Reviewer: Enomoto H, Kanda T **S- Editor**: Gong XM
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.i27.2765

World J Hepatol 2015 November 28; 7(27): 2765-2773
ISSN 1948-5182 (online)

SYSTEMATIC REVIEWS

Comparative study and systematic review of laparoscopic liver resection for hepatocellular carcinoma

Wei Qi Leong, Iyer Shridhar Ganpathi, Alfred Wei Chieh Kow, Krishnakumar Madhavan, Stephen Kin Yong Chang

Wei Qi Leong, Iyer Shridhar Ganpathi, Alfred Wei Chieh Kow, Krishnakumar Madhavan, Stephen Kin Yong Chang, Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, National University Health System, Singapore 119228, Singapore

Author contributions: Chang SKY conceptualized and designed the review together with Leong WQ; Leong WQ carried out the analysis; Leong WQ drafted the initial manuscript; Ganpathi IS, Kow AWC, Madhavan K and Chang SKY provided the data for analysis; all authors reviewed and approved the final manuscript as submitted.

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

Data sharing statement: Technical appendix, statistical code, and the dataset are available from the corresponding author at cfscky@nus.edu.sg. As this study comprises a review in literature and a retrospective study on patient's data in our own hospital, informed consent from patients was not taken. The presented data are anonymized and risk of identification is low. All data generated during the project will be made available *via* the National University Hospital (Singapore)'s research data repository. There is no security, licensing or ethical issues related to the data, and all data used in the project was generated directly as a result of the project, without any pre-existing data being used.

Correspondence to: Stephen Kin Yong Chang, Associate Professor, Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, National University Health System, 1E Kent Ridge Road, NUHS Tower Block, Level 8, Singapore 119228, Singapore. cfscky@nus.edu.sg
Telephone: +65-67725300
Fax: +65-67778427

Received: March 23, 2015
Peer-review started: March 26, 2015
First decision: May 13, 2015
Revised: September 22, 2015
Accepted: October 20, 2015
Article in press: October 27, 2015
Published online: November 28, 2015

Abstract

AIM: To compare the surgical outcomes between laparoscopic liver resection (LLR) and open liver resection (OLR) as a curative treatment in patients with hepatocellular carcinoma (HCC).

METHODS: A PubMed database search was performed systematically to identify comparative studies of LLR *vs* OLR for HCC from 2000 to 2014. An extensive text word search was conducted, using combinations of search headings such as "laparoscopy", "hepatectomy", and "hepatocellular carcinoma". A comparative study was also performed in our institution where we analysed surgical outcomes of 152 patients who underwent liver resection between January 2005 to December 2012, of which 42 underwent laparoscopic or hand-assisted laparoscopic resection and 110 underwent open resection.

RESULTS: Analysis of our own series and a review of 17 high-quality studies showed that LLR was superior to OLR in terms of short-term outcomes, as patients in the laparoscopic arm were found to have less intraoperative blood loss, less blood transfusions, and a shorter length

of hospital stay. In our own series, both LLR and OLR groups were found to have similar overall survival (OS) rates, but disease-free survival (DFS) rates were higher in the laparoscopic arm.

CONCLUSION: LLR is associated with better short-term outcomes compared to OLR as a curative treatment for HCC. Long-term oncologic outcomes with regards to OS and DFS rates were found to be comparable in both groups. LLR is hence a safe and viable option for curative resection of HCC.

Key words: Hepatocellular carcinoma; Laparoscopy; Open liver resection; Hepatectomy; Surgery

Core tip: Surgical resection is the standard treatment for hepatocellular carcinoma (HCC), and provides the best outcomes for patients eligible for resection. Laparoscopic liver resection (LLR) is a relatively new advancement in treatment of HCC and has raised concerns on its feasibility and safety. We reviewed 17 studies and performed our own comparative study on surgical outcomes of LLR *vs* open liver resection for the curative treatment of HCC. We showed that LLR resulted in more desirable short-term outcomes, whereas long-term oncologic outcomes were comparable. Hence, LLR is a safe and feasible option in the surgical treatment of HCC.

Leong WQ, Ganpathi IS, Kow AWC, Madhavan K, Chang SKY. Comparative study and systematic review of laparoscopic liver resection for hepatocellular carcinoma. *World J Hepatol* 2015; 7(27): 2765-2773 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i27/2765.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i27.2765

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third most frequent cause of cancer-related death, with about 750000 new cases diagnosed and approximately 700000 deaths worldwide each year[1,2]. Potentially curative treatment options for HCC include surgical resection[3], liver transplantation[4], and local ablation[5]. Surgical resection remains the standard treatment, and provides the best outcomes, for candidates who are eligible for resection[6]. In 1991, Reich *et al*[7] performed the first laparoscopic hepatic resection (LLR) for a benign liver tumour; subsequently, Hashizume *et al*[8] reported the first LLR performed for HCC. However, many barriers have hindered the popularity of LLR, including concerns of uncontrollable bleeding, resection margins, tumour seeding, and port-site metastases. LLR may also be perceived as a challenge especially in cirrhotic patients, who are at increased risk of complications related to underlying synthetic and metabolic dysfunction[9]. Nevertheless, over the past 2 decades, it has become a widely accepted mode of curative resection for HCC by being established as both safe and feasible. It has also evolved to encompass more difficult anatomic resections.

A number of comparative studies have been published on the surgical outcomes of LLR *vs* open liver resection (OLR) as a curative treatment for HCC, and most suggest that while LLR and OLR both have similar overall survival (OS) and disease-free survival (DFS) rates, LLR confers the additional advantages of shorter duration of hospitalization and lower complication rates. To our knowledge, there has been so far no prospective, randomized controlled study done on this subject.

In this review article, we systematically reviewed 17 comparative studies from 2001 to 2014 to look at the surgical outcomes of LLR *vs* OLR for curative resection of HCC. We also conducted our own comparative study by analysing data from 152 patients who underwent surgical resection of HCC from 2005 to 2012 at the National University Hospital (Singapore) and compared our results to those of the 17 comparative studies.

MATERIALS AND METHODS

A PubMed database search was performed systematically to identify comparative studies of LLR *vs* OLR for HCC from 2000 to 2014. An extensive text word search was conducted, using combinations of search headings such as "laparoscopy", "laparoscopic", "minimally invasive surgery", "hepatectomy", "hepatic resection", "hepatic lobectomy", "liver resection", "hepatocellular carcinoma", "HCC", and "primary liver cancer". The search was restricted to comparative studies and human studies only. All studies identified for screening were manually reviewed. References from these articles were also searched for relevant studies. The most recent search was conducted on 6 June 2014 (Figure 1).

Studies were included in the analysis if they: (1) were comparative studies on humans and in the English language; (2) focused on outcomes of LLR *vs* OLR for HCC; (3) had more than 10 patients in each group included in the study; and (4) if multiple studies were reported by the same institution or authors, the most recent publication was included. Studies were excluded from the analysis if they: (1) were reviews lacking original data, abstracts, editorials, or expert opinions; (2) did not show clear comparisons between outcomes on LLR *vs* OLR; and (3) included resections of benign tumours or metastatic lesions other than HCC. Our own series was analysed together with the selected studies.

National University Hospital series

Case records of 152 patients who underwent curative resection for HCC at the National University Hospital (NUH) in Singapore from January 2005 to December 2012 were prospectively retrieved and manually culled for clinical data. Of the patients included in our study, 42

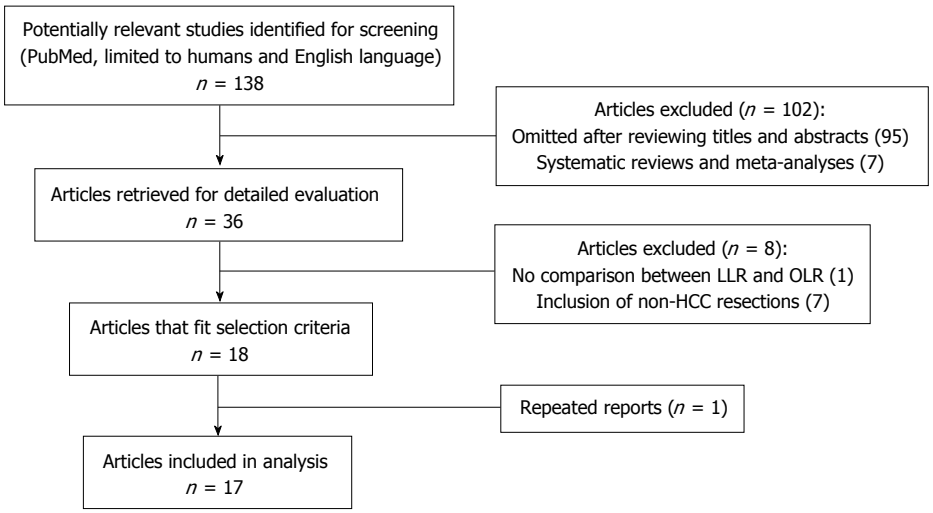

Figure 1 Flow chart illustrating the screening and selection process. LLR: Laparoscopic liver resection; OLR: Open liver resection; HCC: Hepatocellular carcinoma.

underwent laparoscopic or hand-assisted laparoscopic resection and 110 underwent open resection. All patients were followed up for recurrence at least 3-mo for the 1st year, 4-mo for the 2nd year, then every 6 mo subsequently. Patients were stratified according to the type of operation they underwent (OLR *vs* LLR). Vital status and the death date for subjects were obtained from the National Death Registry Database, and death data was supplemented with data from hospital records. For long-term oncologic outcomes, the study endpoints analyzed were OS and DFS. OS was calculated from the date of operation to the date of death. DFS was calculated from the date of operation to the date of 1st recurrence or HCC-related death.

Statistical analysis

The clinical characteristics of patients and postoperative results were expressed as means with standard deviations. The χ^2 or Fisher's exact test was used to compare categorical variables and the Mann-Whitney *U* test was used to compare continuous variables. Survival analysis was performed using the time of disease-free survival *vs* recurrence of a tumor or death. Survival curves were computed using the Kaplan-Meier method and compared between open and laparoscopic groups by the log-rank test. A *P* value of < 0.05 was considered as being statistically significant. All statistical calculations were performed using SPSS version 21.0.

RESULTS

NUH series

One hundred and fifty-two patients undergoing liver resection for HCC were retrospectively reviewed at the National University Hospital in Singapore, from January 2005 to December 2012. Of the patients included in our study, 42 underwent laparoscopic or hand-assisted laparoscopic resection and 110 underwent open resection. All patients were followed up for recurrence at least 3-mo for the 1st year, 4-mo for the 2nd year, then every 6 mo subsequently.

Demographics

The demographic data and clinical characteristics of both groups are shown in Table 1. Both groups did not differ in terms of age, gender, Child-Pugh score, pre-operative laboratory investigations, and tumour locations; however, there was a significant difference in the ASA score ($P = 0.045$), number of co-morbidities (mean 3 *vs* 2.32, $P = 0.028$), and tumour size (mean 3.85 cm *vs* 7.15 cm, $P < 0.001$).

Intraoperative results

Table 2 shows the intraoperative results of the two groups. In the LLR group, conversion from LLR to OLR occurred in 5 patients (11.9%). The duration of operation in the LLR group was significantly shorter compared to the OLR group (mean 250.43 min *vs* 349.90 min, $P < 0.001$). The intraoperative blood loss was significantly lower in the LLR group (495.83 mL *vs* 1085.00 mL, $P < 0.001$), as was the requirement for blood transfusion (9.5% *vs* 39.1%, $P < 0.001$). However, there was no difference in the amount of blood transfused in patients who required transfusion in both groups.

Pathologic results

As for the pathologic results shown in Table 3, there was no difference in the condition of the surrounding liver parenchyma in both groups, except for a larger proportion of patients with cirrhosis in the LLR group (59.5% *vs* 35.5%, $P = 0.007$). Microscopic vascular invasion occurred more often in the OLR group (14.3% *vs* 30.9%, $P = 0.037$). There was no difference between both groups in the histological grade of the tumours as well as the number of patients with local tumour invasion and positive resection margins.

Table 1 Preoperative characteristics *n* (%)

Variable	LLR (*n* = 42)	OLR (*n* = 110)	*P* value
Age	61.07 (11.91)	59.45 (11.15)	0.400
Gender			0.359
Male	32 (76.2)	91 (82.7)	
Female	10 (23.8)	19 (17.3)	
Child-Pugh score			0.094
A	42 (100.0)	103 (93.6)	
B	0 (0.0)	7 (6.4)	
No. of comorbidities	3 ± 1.86	2.32 ± 1.64	0.028
HBsAg	6 (42.9)	25 (55.6)	0.406
Anti-HCV	1 (7.1)	1 (2.7)	0.466
Alpha-fetoprotein	734.33 ± 2978.62	2126.96 ± 8456.88	0.654
ALT	40.64 ± 28.86	46.57 ± 35.59	0.408
AST	46.83 ± 36.03	56.93 ± 50.26	0.280
ALP	90.26 ± 35.51	102.29 ± 44.24	0.099
Total bilirubin	13.12 ± 7.18	12.91 ± 14.26	0.367
PT	13.71 ± 0.94	13.61 ± 1.64	0.176
ASA class			0.045
Ⅰ	3 (7.1)	9 (8.2)	
Ⅱ	24 (57.1)	62 (56.4)	
Ⅲ	15 (35.7)	39 (35.5)	
No. of tumours			0.469
Solitary	37 (88.1)	91 (82.7)	
Multiple	5 (11.9)	19 (17.3)	
Size of largest tumour (cm)	3.85 ± 2.60	7.15 ± 4.88	< 0.001
Tumour location			0.256
Left lobe	15 (35.7)	27 (24.5)	
Right lobe	21 (50.0)	71 (64.5)	
Bilobar	6 (14.3)	12 (10.9)	

Data are mean ± SD or *n* (%) unless otherwise indicated. HBsAg: Hepatitis B virus surface antigen; Anti-HCV: Anti-hepatitis C virus antibody; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; PT: Prothrombin time; ASA: American Society of Anaesthesiologists; LLR: Laparoscopic liver resection; OLR: Open liver resection.

Table 3 Pathologic results *n* (%)

Variable	LLR (*n* = 42)	OLR (*n* = 110)	*P* value
Condition of non-tumourous liver			
Normal	8 (19.0)	35 (31.8)	0.118
Chronic hepatitis	12 (28.6)	31 (28.2)	0.962
Cirrhosis	25 (59.5)	39 (35.5)	0.007
Steatosis	16 (38.1)	29 (26.4)	0.157
Others	2 (4.8)	5 (4.5)	0.955
Microscopic vascular invasion	6 (14.3)	34 (30.9)	0.037
Invasion into adjacent organs	0 (0.00)	2 (1.8)	0.379
Histological grade			0.077
Well differentiated	9 (21.4)	20 (18.2)	
Moderately differentiated	30 (71.4)	61 (55.5)	
Poorly differentiated	3 (7.1)	28 (25.5)	
Undifferentiated	0 (0.0)	1 (0.9)	
Positive resection margin	1 (2.4)	8 (7.3)	0.253

LLR: Laparoscopic liver resection; OLR: Open liver resection.

Post-operative outcomes

With regards to post-operative outcomes (Table 4), there was no difference in the overall complications rate as well as the specific complications (cardiac, pulmonary, gastrointestinal, wound infections, bleeding, prolonged

Table 2 Perioperative data *n* (%)

Variable	LLR (*n* = 42)	OLR (*n* = 110)	*P* value
Type of resection			< 0.001
Right hepatectomy	4 (9.5)	33 (30.0)	
Left hepatectomy	4 (9.5)	11 (10.0)	
Extended right hepatectomy	0 (0.0)	9 (8.2)	
Extended left hepatectomy	0 (0.0)	6 (5.5)	
Right anterior sectionectomy	0 (0.0)	2 (1.8)	
Right posterior sectionectomy	6 (14.3)	2 (1.8)	
Left lateral sectionectomy	8 (19.0)	3 (2.7)	
Wedge resection	6 (14.3)	10 (9.1)	
Segmentectomy	14 (33.3)	31 (28.2)	
Others	0 (0.0)	3 (2.7)	
Conversion from LLR to OLR	5 (11.9)	-	-
Duration of operation (min), means ± SD	250.43 ± 98.85	349.90 ± 132.29	< 0.001
Intra-operative blood loss (mL), mean ± SD	495.83 ± 501.94	1085.00 ± 943.55	< 0.001
Blood transfusion	4 (9.5)	43 (39.1)	< 0.001
Amount transfused (mL), mean ± SD	709.25 ± 726.18	1349.30 ± 1532.32	0.269

LLR: Laparoscopic liver resection; OLR: Open liver resection.

Table 4 Postoperative outcomes *n* (%)

Variable	LLR (*n* = 42)	OLR (*n* = 110)	*P* value
Patients with complications	16 (38.1)	50 (45.5)	0.413
Bleeding	1 (2.4)	1 (0.9)	0.476
Prolonged ascites	1 (2.4)	4 (3.6)	0.698
Intra-abdominal sepsis	0 (0.0)	3 (2.7)	0.280
Liver failure	2 (4.8)	1 (0.9)	0.127
Cardiac	3 (7.1)	10 (9.1)	0.701
Pulmonary	8 (19.0)	15 (13.6)	0.405
Gastrointestinal	1 (2.4)	9 (8.2)	0.197
Wound infections	0 (0.0)	5 (3.3)	0.160
Postoperative mortality	1 (2.4)	3 (2.7)	0.905
Length of hospital stay (d), means ± SD	7.55 ± 11.74	11.42 ± 9.35	< 0.001

LLR: Laparoscopic liver resection; OLR: Open liver resection.

ascites, intra-abdominal sepsis, liver failure) among the LLR and OLR groups. There was no difference in postoperative mortality as well. The total length of hospital stay was significantly shorter in the LLR group (7.55 d *vs* 11.42 d, $P < 0.001$).

Long-term oncologic outcomes

Table 5 shows the long-term oncologic outcomes of the two groups. In the LLR group, the 5-year overall survival was 80.5%. In the OLR group, the 5-year overall survival was 83.8% ($P = 0.949$) (Figure 2). For disease-free survival rates, the LLR group had a survival rate of 52.5% whereas their counterparts in the OLR group had a survival rate of 38.2% ($P = 0.035$) (Figure 3). Hence, there was a significant difference in the disease-free survival rates between both groups but not in overall

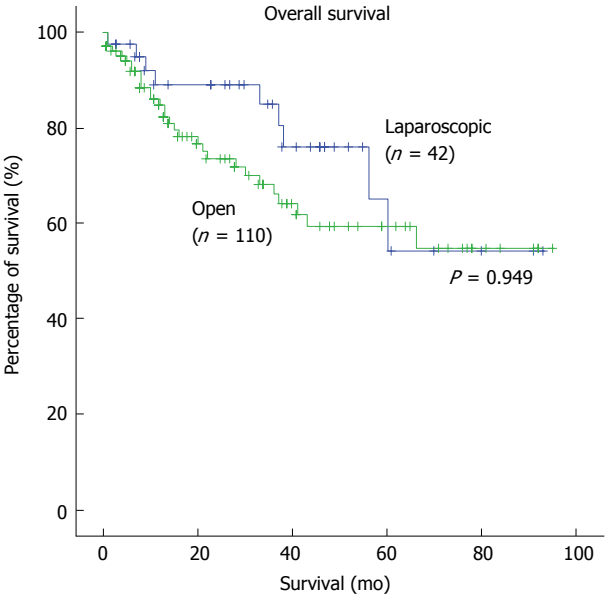

Figure 2 Kaplan-Meier survival curves of overall survival in laparoscopic and open liver resection.

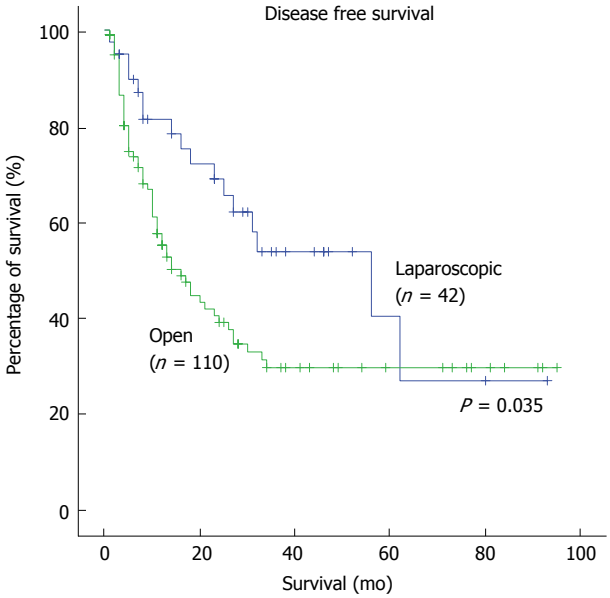

Figure 3 Kaplan-Meier survival curves of disease-free survival in laparoscopic and open liver resection.

Table 5 Oncological outcomes

Variable	LLR (n = 42)	OLR (n = 110)	P value
Overall survival time (mo), mean ± SD	71.25 ± 6.59	76.42 ± 4.468	0.949
Disease-free survival time (mo), mean ± SD	46.81 ± 7.132	34.390 ± 4.254	0.035

LLR: Laparoscopic liver resection; OLR: Open liver resection.

survival rates.

Systematic review

After an extensive literature search and screening, a total of 138 references were identified. The flow of reference selection is depicted in Figure 1. A total of 17 studies published between 2001 and 2014 were identified as eligible for analysis[10-26].

Our review of the above selected articles, as well as the results of our own comparative study, showed that post-operative outcomes in the OLR cohort were significantly and consistently poorer compared to the LLR cohort. The characteristics of the selected articles are summarised in Table 6, and some of the post-operative outcomes analysed using Forest plots (Figures 4-6).

Fourteen high-quality studies (including the NUH series) reported on length of hospital stay (Figure 4); pooled outcome measure favored LLR [patients 1340; WMD: -5.08; 95%CI: -6.82-(-3.33); P < 0.00001]. The results of 18 studies on post-operative complications (Figure 5) showed that patients who underwent LLR experienced significantly fewer complications than their counterparts who underwent OLR (patients: 1653; WMD: 0.40; 95%CI: 0.30-0.54; P < 0.0001). No significant differences were observed between LLR and OLR with regards to post-operative mortality in the 11 studies analysed, as shown in Figure 6 (patients: 1173; WMD: 0.41; 95%CI: 0.14-1.08; P = 0.07).

DISCUSSION

Intraoperative bleeding is a significant problem faced during liver resection, and is frequently the most common reason for conversion from laparoscopic to open hepatectomy[27,28]. The number of transfusions required intraoperatively has also been shown to be an independent risk factor for a worse post-operative prognosis[29,30]. The worldwide acceptance of LLR was delayed due to concerns of the technical difficulties of controlling hemorrhage and obtaining hemostasis. However, our study showed that intraoperative blood loss and the number of patients requiring transfusion were significantly lower in the laparoscopic arm. Reasons for this include image magnification during LLR, usage of intra-operative ultrasonography to visualize the tumour and surrounding intrahepatic vessels and equipment such as ultrasonic laparoscopic coagulation shears and argon beam coagulators to provide rapid hemostasis in the event of hepatic hemorrhage. The pneumoperitoneum in LLR results in increased intra-abdominal pressure, which also reduces visceral blood flow, in turn decreasing blood loss[31,32].

Another major concern regarding LLR for malignant lesions is difficulty assessing resection margins, due to the lack of tactile sensation and distance perception in laparoscopic resection. However, our study showed that there was no difference in resection margins in both series. We are able to make up for the lack of palpation in LLR and hence achieve the intended margins laparoscopically, with pre-operative surgical planning using a variety of imaging techniques and the use of intra-operative ultrasonography to demarcate surgical

Table 6 Characteristics of included studies

Ref.	No. of patients		Child-Pugh score A			Solitary tumour			Mean tumour size (cm)		
	LLR	OLR	LLR *n* (%)	OLR *n* (%)	*P* value	LLR *n* (%)	OLR *n* (%)	*P* value	LLR	OLR	*P* value
Shimada *et al*[10]	17	38	-	-	-	-	-	-	2.6 ± 0.9	2.5 ± 1.0	0.89
Laurent *et al*[11]	13	14	13 (100.0)	14 (100.0)	0.49	-	-	-	3.35 ± 0.89	3.43 ± 1.05	0.48
Kaneko *et al*[12]	30	28	22 (73.3)	22 (57.9)	NS	-	-	-	3.0 ± 0.8	3.1 ± 0.9	NS
Sarpel *et al*[13]	20	56	-	-	-	-	-	-	4.3 ± 2.1	4.3 ± 2.2	0.876
Lai *et al*[14]	25	33	23 (92.0)	31 (93.9)	0.90						
Belli *et al*[15]	54	125	49 (90.7)	117 (93.6)	0.499	44 (81.5)	96 (76.8)	0.486	3.8 ± 1.3	6.0 ± 2.3	0.006
Endo *et al*[16]	10	11	10 (100.0)	7 (63.6)	NS	9 (90.0)	10 (90.9)	NS	3.0 ± 1.5	4.1 ± 0.8	NS
Aldrighetti *et al*[17]	16	16	9 (56.2)	9 (56.2)	NS	-	-	-	4 ± 2.2	4.6 ± 2.5	NS
Tranchart *et al*[18]	42	42	30 (71.4)	33 (78.6)	-	-	-	-	3.58 ± 1.75	3.68 ± 2.09	0.95
Ker *et al*[19]	116	209	98 (84.5)	197 (94.3)	0.08	-	-	-	2.5 ± 1.2	5.4 ± 3.5	0.001
Kim *et al*[20]	26	29	-	-	-	-	-	-	-	-	-
Truant *et al*[21]	36	53	32 (88.9)	47 (88.7)	1	34 (94.4)	44 (83.0)	0.2	2.9 ± 1.2	3.1 ± 1.2	0.5
Hu *et al*[22]	30	30	29 (96.7)	24 (80.0)	NS	-	-	-	6.7 ± 3.1	8.7 ± 2.3	NS
Lee *et al*[23]	33	50	33 (100.0)	50 (100.0)	NS	31 (93.9)	41 (82.0)	0.186	-	-	-
Cheung *et al*[24]	32	64	32 (100.0)	60 (93.8)	0.367	-	-	-	-	-	-
Kim *et al*[25]	70	76	-	-	-	-	-	-	2.58 ± 1.44	2.45 ± 1.27	0.550
Kim *et al*[26]	29	29	28 (96.6)	29 (100.0)	0.317	24 (82.8)	28 (96.6)	0.103	3.59 ± 2.17	4.28 ± 2.55	0.278
Our reports	42	110	42 (100.0)	103 (93.6)	0.094	37 (88.1)	91 (82.7)	0.469	3.85 ± 2.60	7.15 ± 4.88	< 0.001

LLR: Laparoscopic liver resection; OLR: Open liver resection.

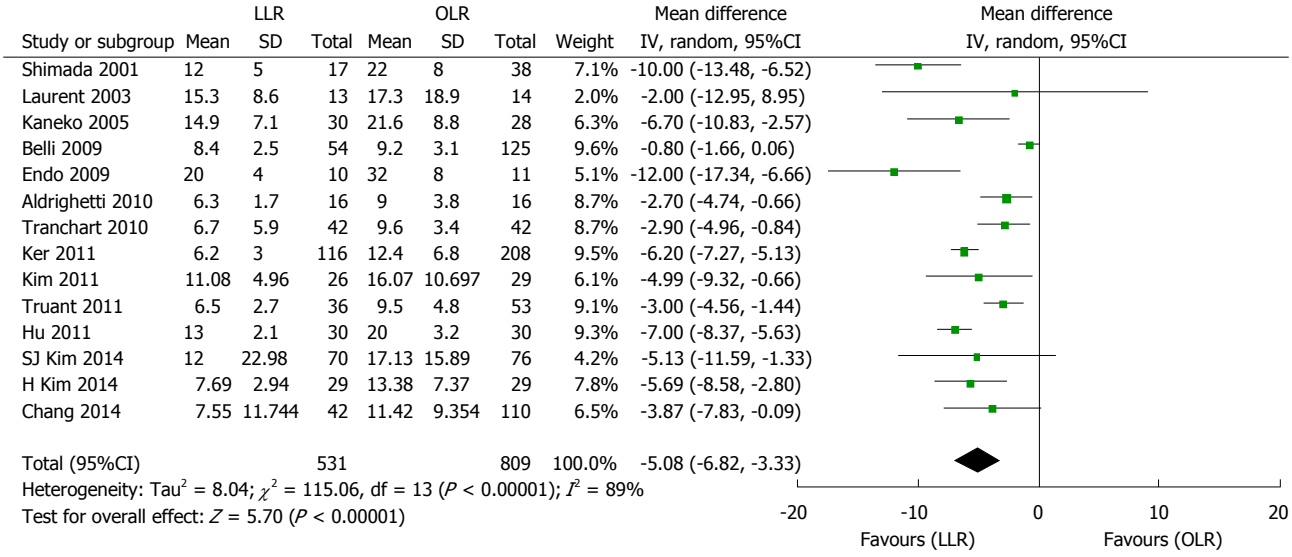

Figure 4 Forest plots depicting length of hospital stay in the included studies. LLR: Laparoscopic liver resection; OLR: Open liver resection.

margins.

Our analysis of the 17 studies showed that the rates of postoperative complications were significantly lower in patients who underwent LLR. Possible reasons for this include less mobilization and manipulation of the liver and other intra-abdominal organs, avoidance of long incisions and division of the abdominal muscles hence minimizing disruption to the abdominal wall collateral circulation, less severe pain, earlier ambulation and oral food intake, and more post-operative cough and expectoration. However, the findings in our comparative study were not significant. Nevertheless, it is worthy to note that even though there was a significantly higher number of co-morbidities in patients in the LLR group, and a significantly greater number of patients found to have cirrhosis in the LLR group, the LLR cohort experienced fewer postoperative complications, though this result was not statistically significant.

Liver resection in HCC patients with chronic liver disease (CLD) or cirrhosis has been a major issue due to the high rates of postoperative morbidity from decompensation due to their underlying liver disease. In these patients, portal hypertension is a major risk factor for development of postoperative decompensation[33,34]. The studies we analysed which were specific to HCC patients with underlying CLD or cirrhosis showed that LLR resulted in fewer postoperative complications compared to OLR. Belli *et al*[15] showed that a significantly decreased postoperative morbidity rate in the laparoscopic group. The studies by Laurent *et al*[11] and Truant *et al*[21] showed lower rates of post-operative ascites and liver failure in the LLR group as well. Overall,

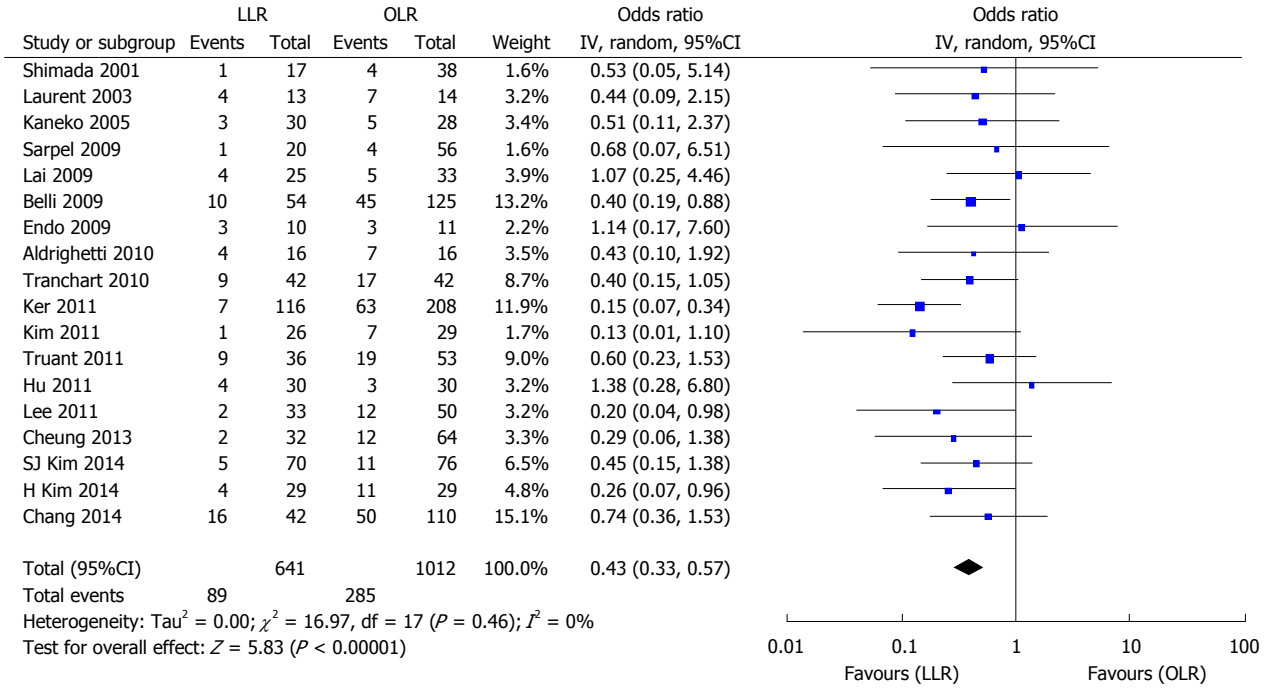

Figure 5 Forest plots depicting postoperative complications in the included studies. LLR: Laparoscopic liver resection; OLR: Open liver resection.

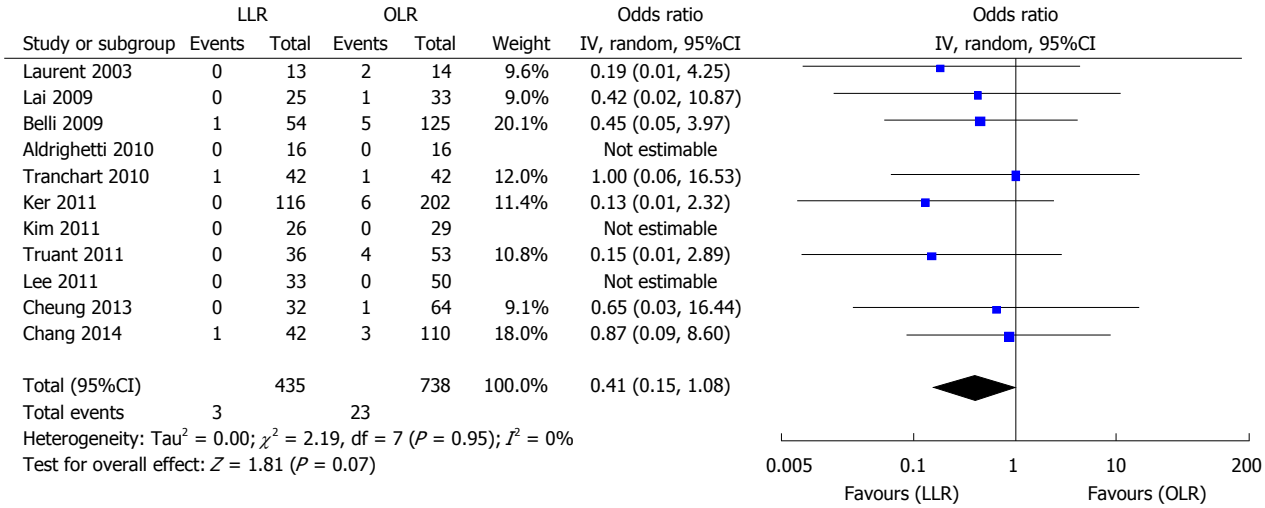

Figure 6 Forest plots depicting postoperative mortality in the included studies. LLR: Laparoscopic liver resection; OLR: Open liver resection.

fewer complications in the LLR group result in a shorter length of hospital stay. Furthermore, from our own comparative study, the rates of prolonged ascites and liver failure in both groups were not significantly different despite a significantly larger number of patients with cirrhosis in the LLR group.

Laparoscopic hepatectomy has not been shown to increase the risk of tumor recurrence and affect the oncologic outcomes (in terms of overall survival and disease-free survival). However, in our study, there was a significant increase in disease-free survival rates in the LLR group; this could be attributed to the higher incidence of microscopic vascular invasion found on histology in the OLR group, which is a significant underlying risk factor for tumour recurrence.

Although LLR has been shown to be superior to OLR in terms of surgical outcomes, the clinical significance of these results should be interpreted keeping in mind that they were based on selected patients who fulfill certain criteria. The size and location of the tumour are important considerations that influence a surgeon's decision to perform an open or a laparoscopic resection. As a general rule, small (< 5 cm) tumours, in superficial or peripheral locations, far away from major vessels, are considered for LLR. Large tumours and cases requiring vascular or biliary reconstruction are usually indications for open resection. Nevertheless, with improvement of the laparoscopic technique and new advances in technology over the past 2 decades, LLR is being performed more frequently and for more complex cases

with tumours in difficult anatomical locations[22,35,36].

Strengths and limitations

Our systematic review has some limitations which warrant discussion and should be considered when interpreting the results. Firstly, all comparative studies including our own are non-randomized controlled studies that are retrospective or retrospective matched. To our knowledge, no randomized control trial has been published on this subject. Also, as mentioned above, selection of patients in both the LLR and OLR groups followed certain criteria based on the pre-operative clinicopathologic characteristics of each case, as well as according to the experience and expertise of the surgeons. This tends to increase the risk of selection bias. However, many of the studies we analysed performed case-matched analysis and matched patients in both groups based on similar characteristics, such as tumour size, tumour location, and presence of CLD or cirrhosis[12-14,17,18,20-22,24,26]. This minimized the degree of selection bias to some extent.

The strengths of our review are, firstly, a substantial number of studies analysed from various centres around the world, in addition to our own. Also, strict inclusion and exclusion criteria were implemented to select the highest quality and most recent studies after an extensive literature search.

In conclusion, our systematic review and comparative study show that as a curative treatment for HCC, LLR provides better short-term outcomes than OLR in terms of intraoperative blood loss, blood transfusions, and length of hospital stay, while both LLR and OLR provide similar long-term oncologic outcomes. Further research should be undertaken in the form of prospective randomized control trials to substantiate our findings even further.

COMMENTS

Background

For hepatocellular carcinoma (HCC), surgical resection is the standard treatment and provides the best outcomes for candidates who are eligible for resection. With advances in technology, laparoscopic liver resection (LLR) is becoming more widely accepted as a safe and effective approach to the management of HCC. Studies comparing various outcomes of the open *vs* laparoscopic approach to surgical resection of HCC have reported that LLR results in better short-term outcomes, both methods of resection give rise to similar long-term oncologic results.

Research frontiers

Since LLR for HCC was first reported in 1995, it has been constantly evolving to encompass more difficult anatomic resections, including larger tumours, and tumours located in the posterosuperior segments of the liver, which were previously traditionally done *via* the open method.

Innovations and breakthroughs

In this study, the authors analysed a substantial number of studies from various established and reputable centres all around the world, including the authors' own. Strict inclusion and exclusion criteria were implemented to select the highest quality and most recent studies after an extensive literature search.

Applications

The study results suggest that LLR is associated with better short-term outcomes compared to open liver resection as a curative treatment for HCC, with comparable long-term oncologic outcomes between both groups. LLR is hence a safe and viable option for curative resection of HCC.

Peer-review

This is an excellent paper dealing with comparison between laparoscopic and open liver resection in the treatment of HCC. The manuscript is well written and provides important clinical information that is potentially useful to readers.

REFERENCES

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

2 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

3 **Ryder SD**. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. *Gut* 2003; **52** Suppl 3: iii1-iii8 [PMID: 12692148 DOI: 10.1136/gut.52.suppl_3.iii1]

4 **Lewis JH**, Mortensen ME, Zweig S, Fusco MJ, Medoff JR, Belder R. Efficacy and safety of high-dose pravastatin in hypercholesterolemic patients with well-compensated chronic liver disease: Results of a prospective, randomized, double-blind, placebo-controlled, multicenter trial. *Hepatology* 2007; **46**: 1453-1463 [PMID: 17668878 DOI: 10.1002/hep.21848]

5 **Lau WY**, Leung TW, Yu SC, Ho SK. Percutaneous local ablative therapy for hepatocellular carcinoma: a review and look into the future. *Ann Surg* 2003; **237**: 171-179 [PMID: 12560774 DOI: 10.1097/01.SLA.0000048443.71734.BF]

6 **Bruix J**, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: 24531850 DOI: 10.1136/gutjnl-2013-306627]

7 **Reich H**, McGlynn F, DeCaprio J, Budin R. Laparoscopic excision of benign liver lesions. *Obstet Gynecol* 1991; **78**: 956-958 [PMID: 1833688]

8 **Hashizume M**, Takenaka K, Yanaga K, Ohta M, Kajiyama K, Shirabe K, Itasaka H, Nishizaki T, Sugimachi K. Laparoscopic hepatic resection for hepatocellular carcinoma. *Surg Endosc* 1995; **9**: 1289-1291 [PMID: 8629211 DOI: 10.1007/bf00190161]

9 **Nagasue N**, Kohno H, Tachibana M, Yamanoi A, Ohmori H, El-Assal ON. Prognostic factors after hepatic resection for hepatocellular carcinoma associated with Child-Turcotte class B and C cirrhosis. *Ann Surg* 1999; **229**: 84-90 [PMID: 9923804 DOI: 10.1097/00000658-199901000-00011]

10 **Shimada M**, Hashizume M, Maehara S, Tsujita E, Rikimaru T, Yamashita Y, Tanaka S, Adachi E, Sugimachi K. Laparoscopic hepatectomy for hepatocellular carcinoma. *Surg Endosc* 2001; **15**: 541-544 [PMID: 11591936 DOI: 10.1007/s004640080099]

11 **Laurent A**, Cherqui D, Lesurtel M, Brunetti F, Tayar C, Fagniez PL. Laparoscopic liver resection for subcapsular hepatocellular carcinoma complicating chronic liver disease. *Arch Surg* 2003; **138**: 763-769; discussion 769 [PMID: 12860758 DOI: 10.1001/archsurg.138.7.763]

12 **Kaneko H**, Takagi S, Otsuka Y, Tsuchiya M, Tamura A, Katagiri T, Maeda T, Shiba T. Laparoscopic liver resection of hepatocellular carcinoma. *Am J Surg* 2005; **189**: 190-194 [PMID: 15720988 DOI: 10.1016/j.amjsurg.2004.09.010]

13 **Sarpel U**, Hefti MM, Wisnievsky JP, Roayaie S, Schwartz ME, Labow DM. Outcome for patients treated with laparoscopic versus open resection of hepatocellular carcinoma: case-matched analysis. *Ann Surg Oncol* 2009; **16**: 1572-1577 [PMID: 19259738 DOI: 10.1245/s10434-009-0414-8]

14 **Lai EC**, Tang CN, Ha JP, Li MK. Laparoscopic liver resection for hepatocellular carcinoma: ten-year experience in a single center.

Arch Surg 2009; **144**: 143-147; discussion 148 [PMID: 19221325 DOI: 10.1001/archsurg.2008.536]

15 **Belli G**, Limongelli P, Fantini C, D'Agostino A, Cioffi L, Belli A, Russo G. Laparoscopic and open treatment of hepatocellular carcinoma in patients with cirrhosis. *Br J Surg* 2009; **96**: 1041-1048 [PMID: 19672933 DOI: 10.1002/bjs.6680]

16 **Endo Y**, Ohta M, Sasaki A, Kai S, Eguchi H, Iwaki K, Shibata K, Kitano S. A comparative study of the long-term outcomes after laparoscopy-assisted and open left lateral hepatectomy for hepatocellular carcinoma. *Surg Laparosc Endosc Percutan Tech* 2009; **19**: e171-e174 [PMID: 19851245 DOI: 10.1097/SLE.0b013e3181bc4091]

17 **Aldrighetti L**, Guzzetti E, Pulitanò C, Cipriani F, Catena M, Paganelli M, Ferla G. Case-matched analysis of totally laparoscopic versus open liver resection for HCC: short and middle term results. *J Surg Oncol* 2010; **102**: 82-86 [PMID: 20578084 DOI: 10.1002/jso.21541]

18 **Tranchart H**, Di Giuro G, Lainas P, Roudie J, Agostini H, Franco D, Dagher I. Laparoscopic resection for hepatocellular carcinoma: a matched-pair comparative study. *Surg Endosc* 2010; **24**: 1170-1176 [PMID: 19915908 DOI: 10.1007/s00464-009-0745-3]

19 **Ker CG**, Chen JS, Kuo KK, Chuang SC, Wang SJ, Chang WC, Lee KT, Chen HY, Juan CC. Liver Surgery for Hepatocellular Carcinoma: Laparoscopic versus Open Approach. *Int J Hepatol* 2011; **2011**: 596792 [PMID: 21994865 DOI: 10.4061/2011/596792]

20 **Kim HH**, Park EK, Seoung JS, Hur YH, Koh YS, Kim JC, Cho CK, Kim HJ. Liver resection for hepatocellular carcinoma: case-matched analysis of laparoscopic versus open resection. *J Korean Surg Soc* 2011; **80**: 412-419 [PMID: 22066068 DOI: 10.4174/jkss.2011.80.6.412]

21 **Truant S**, Bouras AF, Hebbar M, Boleslawski E, Fromont G, Dharancy S, Leteurtre E, Zerbib P, Pruvot FR. Laparoscopic resection vs. open liver resection for peripheral hepatocellular carcinoma in patients with chronic liver disease: a case-matched study. *Surg Endosc* 2011; **25**: 3668-3677 [PMID: 21688080 DOI: 10.1007/s00464-011-1775-1]

22 **Hu BS**, Chen K, Tan HM, Ding XM, Tan JW. Comparison of laparoscopic vs open liver lobectomy (segmentectomy) for hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 4725-4728 [PMID: 22180716 DOI: 10.3748/wjg.v17.i42.4725]

23 **Lee KF**, Chong CN, Wong J, Cheung YS, Wong J, Lai P. Long-term results of laparoscopic hepatectomy versus open hepatectomy for hepatocellular carcinoma: a case-matched analysis. *World J Surg* 2011; **35**: 2268-2274 [PMID: 21842300 DOI: 10.1007/s00268-011-1212-6]

24 **Cheung TT**, Poon RT, Yuen WK, Chok KS, Jenkins CR, Chan SC, Fan ST, Lo CM. Long-term survival analysis of pure laparoscopic versus open hepatectomy for hepatocellular carcinoma in patients with cirrhosis: a single-center experience. *Ann Surg* 2013; **257**: 506-511 [PMID: 23299521 DOI: 10.1097/SLA.0b013e31827b947a]

25 **Kim SJ**, Jung HK, Lee DS, Yun SS, Kim HJ. The comparison of oncologic and clinical outcomes of laparoscopic liver resection for hepatocellular carcinoma. *Ann Surg Treat Res* 2014; **86**: 61-67 [PMID: 24761410 DOI: 10.4174/astr.2014.86.2.61]

26 **Kim H**, Suh KS, Lee KW, Yi NJ, Hong G, Suh SW, Yoo T, Park MS, Choi Y, Lee HW. Long-term outcome of laparoscopic versus open liver resection for hepatocellular carcinoma: a case-controlled study with propensity score matching. *Surg Endosc* 2014; **28**: 950-960 [PMID: 24149856 DOI: 10.1007/s00464-013-3254-3]

27 **Yoshioka K**, Hashimoto S, Kawabe N. Measurement of liver stiffness as a non-invasive method for diagnosis of non-alcoholic fatty liver disease. *Hepatol Res* 2015; **45**: 142-151 [PMID: 25040931 DOI: 10.1111/hepr.12388]

28 **Ai JH**, Li JW, Chen J, Bie P, Wang SG, Zheng SG. Feasibility and safety of laparoscopic liver resection for hepatocellular carcinoma with a tumor size of 5-10 cm. *PLoS One* 2013; **8**: e72328 [PMID: 23991092]

29 **Silva JM**, Cezario TA, Toledo DO, Magalhães DD, Pinto MA, Victoria LG. Complications and prognosis of intraoperative blood transfusion. *Rev Bras Anestesiol* 2008; **58**: 454-461, 447-454 [PMID: 19382404]

30 **Kooby DA**, Stockman J, Ben-Porat L, Gonen M, Jarnagin WR, Dematteo RP, Tuorto S, Wuest D, Blumgart LH, Fong Y. Influence of transfusions on perioperative and long-term outcome in patients following hepatic resection for colorectal metastases. *Ann Surg* 2003; **237**: 860-869; discussion 869-870 [PMID: 12796583 DOI: 10.1097/00000658-200306000-00015]

31 **Srivastava A**, Niranjan A. Secrets of safe laparoscopic surgery: Anaesthetic and surgical considerations. *J Minim Access Surg* 2010; **6**: 91-94 [PMID: 21120064 DOI: 10.4103/0972-9941.72593]

32 **Papp A**, Vereczkei J, Horváth OP. The effect of different levels of peritoneal CO2 pressure on bleeding time of spleen capsule injury. *Surg Endosc* 2003; **17**: 1125-1128 [PMID: 12728370 DOI: 10.1007/s00464-002-9204-0]

33 **Bruix J**, Castells A, Bosch J, Feu F, Fuster J, Garcia-Pagan JC, Visa J, Bru C, Rodés J. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 1996; **111**: 1018-1022 [PMID: 8831597 DOI: 10.1016/S0016-5085(96)70070-7]

34 **Powell-Jackson P**, Greenway B, Williams R. Adverse effects of exploratory laparotomy in patients with unsuspected liver disease. *Br J Surg* 1982; **69**: 449-451 [PMID: 7104630 DOI: 10.1002/bjs.1800690805]

35 **Gigot JF**, Glineur D, Santiago Azagra J, Goergen M, Ceuterick M, Morino M, Etienne J, Marescaux J, Mutter D, van Krunckelsven L, Descottes B, Valleix D, Lachachi F, Bertrand C, Mansvelt B, Hubens G, Saey JP, Schockmel R. Laparoscopic liver resection for malignant liver tumors: preliminary results of a multicenter European study. *Ann Surg* 2002; **236**: 90-97 [PMID: 12131090 DOI: 10.1097/00000658-200207000-00014]

36 **Santambrogio R**, Aldrighetti L, Barabino M, Pulitanò C, Costa M, Montorsi M, Ferla G, Opocher E. Laparoscopic liver resections for hepatocellular carcinoma. Is it a feasible option for patients with liver cirrhosis? *Langenbecks Arch Surg* 2009; **394**: 255-264 [PMID: 18553101 DOI: 10.1007/s00423-008-0349-8]

P- Reviewer: Ding MX, He ST, Ikuta S **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i27.2774

World J Hepatol 2015 November 28; 7(27): 2774-2780
ISSN 1948-5182 (online)

META-ANALYSIS

Portal vein thrombosis, mortality and hepatic decompensation in patients with cirrhosis: A meta-analysis

Jonathan G Stine, Puja M Shah, Scott L Cornella, Sean R Rudnick, Marwan S Ghabril, George J Stukenborg, Patrick G Northup

Jonathan G Stine, Sean R Rudnick, Patrick G Northup, Division of Gastroenterology and Hepatology, University of Virginia, Charlottesville, VA 22908, United States

Puja M Shah, Department of Surgery, University of Virginia, Charlottesville, VA 22908, United States

Scott L Cornella, Department of Medicine, University of Virginia, Charlottesville, VA 22908, United States

Marwan S Ghabril, Division of Gastroenterology and Hepatology, Indiana University, Indianapolis, IN 46202, United States

George J Stukenborg, Department of Public Health Science, University of Virginia, Charlottesville, VA 22908, United States

Author contributions: Stine JG and Shah PM contributed equally to this work; Stine JG, Shah PM, Ghabril MS, Stukenborg GJ and Northup PG designed research; Stine JG, Shah PM, Cornella SL and Rudnick SR performed research; Stine JG and Shah PM analyzed data; Stine JG, Shah PM, Cornella SL, Rudnick SR, Ghabril MS, Stukenborg GJ and Northup PG wrote the paper.

Supported by (In part) grant funding from the National Institutes of Health (Grant 5T32DK007769-15); and NIH-Surgical Oncology grant (T32 CA163177).

Conflict-of-interest statement: We have no conflicts of interest to report.

Data sharing statement: No additional data are available.

Correspondence to: Jonathan G Stine, MD, MSc, Division of Gastroenterology and Hepatology, University of Virginia, JPA and Lee Street, MSB 2145, PO Box 800708, Charlottesville, VA 22908, United States. jgs9f@virginia.edu
Telephone: +1-434-9242959
Fax: +1-434-2447529

Received: September 18, 2015
Peer-review started: September 19, 2015
First decision: October 21, 2015
Revised: November 2, 2015
Accepted: November 10, 2015
Article in press: November 11, 2015
Published online: November 28, 2015

Abstract

AIM: To determine the clinical impact of portal vein thrombosis in terms of both mortality and hepatic decompensations (variceal hemorrhage, ascites, portosystemic encephalopathy) in adult patients with cirrhosis.

METHODS: We identified original articles reported through February 2015 in MEDLINE, Scopus, Science Citation Index, AMED, the Cochrane Library, and relevant examples available in the grey literature. Two independent reviewers screened all citations for inclusion criteria and extracted summary data. Random effects odds ratios were calculated to obtain aggregate estimates of effect size across included studies, with 95%CI.

RESULTS: A total of 226 citations were identified and reviewed, and 3 studies with 2436 participants were included in the meta-analysis of summary effect. Patients with portal vein thrombosis had an increased risk of mortality (OR = 1.62, 95%CI: 1.11-2.36, P = 0.01). Portal vein thrombosis was associated with an increased risk of ascites (OR = 2.52, 95%CI: 1.63-3.89, P < 0.001). There was insufficient data available to determine the pooled effect on other markers of

decompensation including gastroesophageal variceal bleeding or hepatic encephalopathy.

CONCLUSION: Portal vein thrombosis appears to increase mortality and ascites, however, the relatively small number of included studies limits more generalizable conclusions. More trials with a direct comparison group are needed.

Key words: Hepatology; Coagulopathy; Liver; Ascites; Hepatic encephalopathy; Portal hypertension

Core tip: Portal vein thrombosis (PVT) is a common complication of cirrhosis with resultant downstream hepatic decompensation and mortality. Treatment options carry risk and are not without complications. To date, there is a lack of systematic evidence on the clinical importance of PVT. We performed a systematic review and meta-analysis to determine the aggregate estimates of effect of PVT on hepatic decompensation and mortality. PVT appears to significantly increase mortality (OR = 1.62, 95%CI: 1.11-2.36) and ascites (OR = 2.52, 95%CI: 1.63-3.89), however, the small number of included studies limits more generalizable conclusions. More trials with a direct comparison group are needed.

Stine JG, Shah PM, Cornella SL, Rudnick SR, Ghabril MS, Stukenborg GJ, Northup PG. Portal vein thrombosis, mortality and hepatic decompensation in patients with cirrhosis: A meta-analysis. *World J Hepatol* 2015; 7(27): 2774-2780 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i27/2774.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i27.2774

INTRODUCTION

Portal vein thrombosis (PVT) is defined as an obstruction of the portal vein or its branches, which include the splenic, superior mesenteric, and inferior mesenteric veins[1]. It is associated with numerous conditions including malignancy, myeloproliferative disorders, inflammatory conditions (such as pancreatitis), intra-abdominal infections (such as secondary peritonitis), and cirrhosis[2,3]. PVT is common in patients with cirrhosis; over 30% of liver transplant recipients have PVT on direct explant examination at the time of transplant (LT)[2,4,5]. Incidence rates of PVT while variable, are reported to be as high as 16%[6]. The mechanism of PVT development in cirrhosis is multifactorial and is due to a combination of changes in liver architecture leading to impaired blood flow and endothelial cell activation, hypercoagulability, and the potential development of hepatocellular carcinoma (HCC)[7]. The presence of PVT appears to be associated with the severity of underlying liver disease and hepatic decompensation from a mechanistic standpoint. However, the field of coagulation disorders and chronic liver disease is ever evolving and continues to generate controversy, in particular, when consideration is given to the impact of PVT on the development of hepatic decompensation. Multiple studies have been published indicating adverse clinical outcomes in the setting of PVT in both transplant and non-transplant populations, including hepatic decompensation, increased post-transplant mortality, and decreased quality of life[7-10]. Others have argued that PVT does not affect clinically relevant outcomes[11]. Due to this uncertainty, we sought to determine the clinical impact of PVT on transplant free survival and hepatic decompensation in adult patients with cirrhosis.

MATERIALS AND METHODS

Literature search strategy and study selection

The investigators systematically searched the published medical literature for observational studies and clinical trials that compared mortality or hepatic decompensation outcomes in cirrhosis patients with and without PVT. Published studies were identified by searching the following electronic databases: MEDLINE, Scopus, Science Citation Index, AMED, and the Cochrane Library. The search criteria included all publications through February 2015 with English language restriction. Electronic search criteria included the following terms or keywords: "portal vein thrombosis", "mesenteric thrombosis", "splanchnic thrombosis", "cirrhosis", "mortality", "decompensation", and "humans". We reviewed the reference lists of included articles in order to identify articles missed in the database searches. Recent conference abstract lists and other relevant grey literature sources were also searched for examples of relevant studies using the same terms and keywords. Studies were excluded if PVT was associated with only malignancy, developed post-procedure (surgery or interventional), were found in non-cirrhotic patients with portal hypertension, were in LT-only recipients, if no control/comparison group without PVT was included, or if survival was not analyzed. This study did not require institutional review board approval.

Data extraction

Two study personnel (Stine JG and Shah PM) independently screened the abstracts and titles of all studies identified using the electronic and manual search criteria to identify studies meeting the inclusion criteria. Each study meeting requirements of the first-round inclusion criteria then underwent a full-text independent review by both reviewers. Disagreements about inclusion between reviewers were resolved by follow-up consultation, and if necessary by a third clinical reviewer (Cornella CL). Two reviewers independently extracted the following data from each study that met inclusion criteria: patient characteristics (age, gender, MELD, and etiology of liver disease), study-level characteristics (author, publication year, study design, enrollment period, target population, total number of enrolled

Table 1 Study level characteristics *n* (%)

Ref.	Date published	Dates enrolled	Enrollment	PVT	Death - no PVT	Death - PVT
John *et al*[16]	2013	2004-2009	290	70 (24.1)	62 (28.2)	24 (34.3)
Maruyama *et al*[17]	2013	1998-2009	150	42 (28.0)	21 (19.4)	9 (21.4)
Englesbe *et al*[7]	2010	1995-2007	3295	148 (4.5)	1171 (37.2)	81 (54.7)

PVT: Portal vein thrombosis.

patents, and percentage of patients with PVT) and outcomes (mortality and hepatic decompensation).

Primary and secondary outcomes

Mortality was the primary outcome assessed. Secondary outcomes included the presence of or development of new gastroesophageal variceal bleeding, ascites, hepatic encephalopathy, and an aggregate measure of the occurrence of any of these three hepatic decompensation outcomes.

Study quality and risk of bias assessment

The quality of observational studies was assessed using the methods described by Stroup *et al*[12]. Only studies deemed high-quality by the investigators were included in the analysis. The Newcastle-Ottowa Quality Assessment for Cohort Studies scale[13] was used to further characterize the quality of studies based on selection of study groups, comparability of groups, and ascertainment of outcome. The studies are rated on an 8-point scale separated by the three broad sections delineated above.

Statistical analysis

Descriptive analysis of the studies identified, excluded, and included, and meta-analysis of the reported study effect measures, was conducted utilizing review manager software (Rev-Man version 5.3; Copenhagen; The Nordic Cochrane Centre; The Cochrane Collaboration; 2014). We estimated pooled ORs and calculated corresponding 95%CIs using DerSimonian and Laird random-effects models, which account for both between and within study variability given that the included studies were not functionally identical[14,15]. Between study variability was separately assessed using the Cochran's Q statistic (with $P < 0.05$ considered significant). The proportion of heterogeneity accounted for by between-study variability was estimated using the I^2 index and adjudicated to be significant if I^2 was > 75%[14,15]. A post-hoc funnel plot was created to assess for the presence or absence of publication bias.

RESULTS

Included studies

The electronic search criteria identified 226 studies. After ensuring no duplicates were present, we screened titles and abstracts. The full text of eleven studies was assessed for eligibility. Following the qualitative systematic review process, three observational studies met the inclusion criteria for the current meta-analysis[7,16,17]. Of these, two were retrospective[7,17]. The third study followed a prospective cohort of patients[16]. No additional studies were appropriate for inclusion based on our a-priori determined inclusion and exclusion criteria. Nery *et al*[11] recently published a multicenter prospective series of 1243 adult patients with cirrhosis without baseline PVT in France and Belgium. About 118 patients developed *de novo* PVT during a median follow-up period of 47 mo. This study, while initially considered in full-text review, was excluded specifically because absolute numbers for mortality or individual types of hepatic decompensation were not provided; rather, univariate and multivariable analysis *P*-values were provided only and only a composite of hepatic decompensation was given in absolute number.

The 3 eligible reports evaluated cirrhotic patients that did not initially have PVT, but developed it sometime over the study period. They each excluded patients with HCC and prior transplant. All 3 studies evaluated long-term outcomes in cirrhotic patients with PVT compared to cirrhotic patients without PVT. Study level characteristics are found in Table 1. A summary of the search results is presented in Figure 1, reflecting the reporting standards of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[18].

The Englesbe *et al*[7] study assessed a total of 3295 (148 with PVT and 3147 without PVT) cirrhotic patients between 1995-2007. The study assessed patients that were being evaluated as candidates for liver transplantation that had thrombus in the main portal vein only. Patients with partial thrombus or thrombi in portal vein branches, without extension into the main portal vein were excluded. The Maruyama *et al*[17] article evaluated a total of 150 patients with viral hepatitis, 42 had PVT (and 108 did not have PVT). The study by John *et al*[16] found 290 patients with cirrhosis, 70 of these had PVT and 220 did not. Notably, both the Maruyama *et al*[17] and John *et al*[16] articles specified patients with complete and partial thrombus, unlike the Englesbe study[7].

In total, the three studies included 3735 cirrhotic patients, 260 of which had PVT. Lengths of follow-up ranged from less than one month to 136 mo, with mean follow-ups ranging from 25 mo to 50 mo. Baseline demographic characteristics were similar between PVT and non-PVT groups in all 3 studies. There were no differences in regards to race, age, gender, causes of cirrhosis, or model for end stage liver disease (MELD) scores. Demographic and etiologic characteristics of the

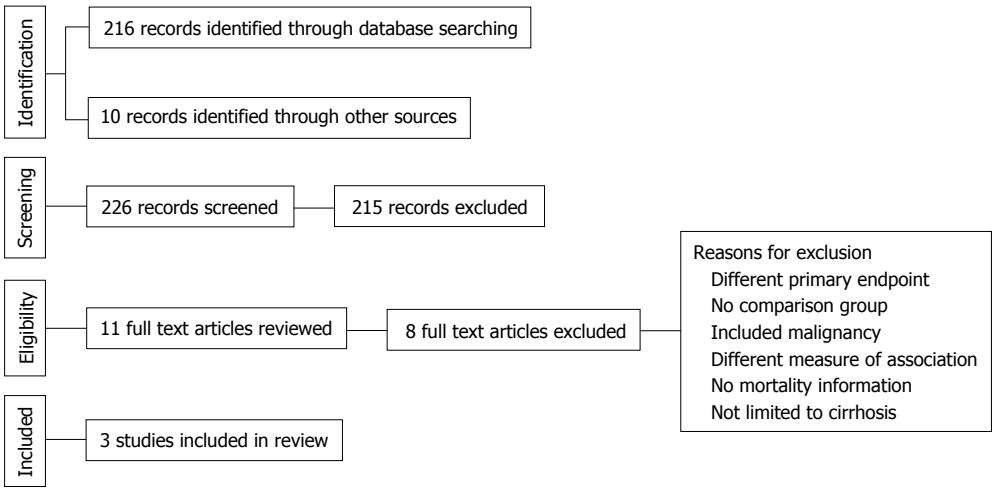

Figure 1 The preferred reporting items for systematic reviews and meta-analyses diagram. About 226 records were screened in aggregate; 11 full text articles were reviewed; 3 studies met inclusion criteria.

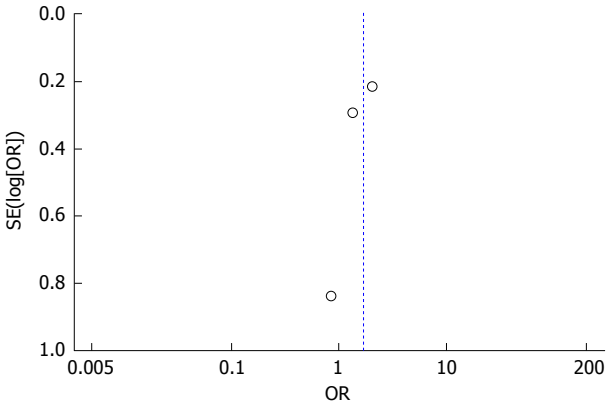

Figure 2 Funnel plot assessing publication bias. No significant publication bias was observed in this study.

patients included in each studies are summarized in Table 2.

Bias assessment

All 3 studies include cohorts drawn from their abdominal transplant clinic population. The patients were truly representative of the average transplant population. Exposed and unexposed patients (patients with cirrhosis with spontaneous PVT and those without PVT) were all drawn from their transplant clinic population and all information was obtained through medical records in the three studies. The Englesbe *et al*[7] study controlled for PVT along with MELD, age, and presence of hepatitis C virus in a multivariable logistic regression with survival as the outcome. The Maruyama *et al*[17] study does not include a model controlling for covariates. The John *et al*[16] study does control for ascites and renal function, however this study created a multivariable model to identify predictors for PVT development. All three studies had widely variable amounts of follow-up and there is no information available on patients lost to follow-up for any of the studies. Based on these characteristics, the Englesbe *et al*[7] study received a score of 7/8, while Maruyama *et al*[17] and John *et al*[16] both received 6/8. The potential for publication bias was assessed using a funnel plot of the relationship between reported effect variance SE(log[OR]) and the reported study OR. The plot illustrates the lack of evidence for potential publication bias in the three studies-the study with the largest effect size is the largest study included while the smaller studies have lower effect sizes (Figure 2).

Portal vein thrombosis and mortality

The Englesbe *et al*[7] article demonstrated an incidence rate of 4.5% for PVT. The patients with PVT were at significantly higher risk of mortality with an OR of 2.04 (95%CI: 1.46-2.84) (Figure 3) Conversely, the Maruyama *et al*[17] and John *et al*[16] studies found higher rates of mortality between PVT and non-PVT subjects. The OR for mortality for PVT in both studies were 1.13 and 1.27, respectively but these differences were non-significant. Pooled analysis of the results reported across all 3 studies demonstrates a significantly increased risk of mortality in PVT patients (OR = 1.62, 95%CI: 1.11-2.36, P = 0.01). The Cochran's Q statistic was non-significant at P = 0.23 and I^2 = 32%, demonstrating non-significant heterogeneity of effects reported across studies.

Portal vein thrombosis and hepatic decompensation

Secondary outcomes included episodes of hepatic decompensation, including individual cases of ascites, variceal bleeding, or portosystemic encephalopathy. Both John *et al*[16] and Maruyama *et al*[17] demonstrated similar effects of PVT on ascites development. John *et al*[16] showed an OR of 1.51 (95%CI: 0.87-2.60) compared to an OR of 7.46 (95%CI: 3.38-16.45) in the Maruyama *et al*[17] study (Figure 4). The Englesbe *et al*[7] study was excluded from this portion of analysis since they did not report on rates of hepatic decompensation. When the pooled OR was evaluated using random-effects modeling, PVT continued to have statistically

Table 2 Patient level characteristics *n* (%)

	Englesbe 2010[1]		John 2013[2]		Maruyama 2013[3]	
	No PVT (*n* = 3147)	PVT (*n* = 148)	No PVT (*n* = 220)	PVT (*n* = 70)	No PVT (*n* = 108)	PVT (*n* = 42)
Sex (M/F)	1905/1242	91/57	144/76	42/28	63/45	22/20
Race						
Black	218 (6.9)	7 (4.7)	-	-	-	-
White	-	-	184 (83.6)	60 (85.7)	-	-
Other	2545 (80.9)	131 (88.5)			-	-
	"Nonblack"	"Nonblack"	-	-		
Etiology						
AIH	95 (3)	8 (5.4)	-	-	-	-
Biliary/cholestatic	173 (5.4)	7 (5.4)	24 (11)	5 (7.1)	-	-
Alcohol	672 (21.4)	31 (20.9)	37 (16.9)	5 (7.1)	-	-
Viral	1253 (39.8)	50 (34.1)	62 (28.3)	16 (27.1)	108	42
Cryptogenic/NASH	72 (2.3)	0	35 (16)	12 (17.1)	-	-
Other	449 (14.3)	22 (14.9)	37 (16.9)	20 (28.6)	-	-
Age (yr)	51.5 ± 11.2	50.9 ± 10.8	55.8 ± 9.1	58.4 ± 8.8	63.3 ± 8.68	62.4 ± 11
MELD	12.1 ± 7.2	13.3 ± 8.3	13.8 ± 4.5	14.9 ± 5.9	10.2	10.6

[1]Presence of PVT at time of initial transplant evaluation or during the pre-transplant period; [2]PVT category includes patients with PVT at baseline (n = 47) and those that developed new PVT during the study period (n = 23); [3]Study only assessed patients with viral hepatitis. No data reported for race. PVT: Portal vein thrombosis; MELD: Model for end stage liver disease; NASH: Non-alcoholic steatohepatitis; M/F: Male/female; AIH: Autoimmune hepatitis.

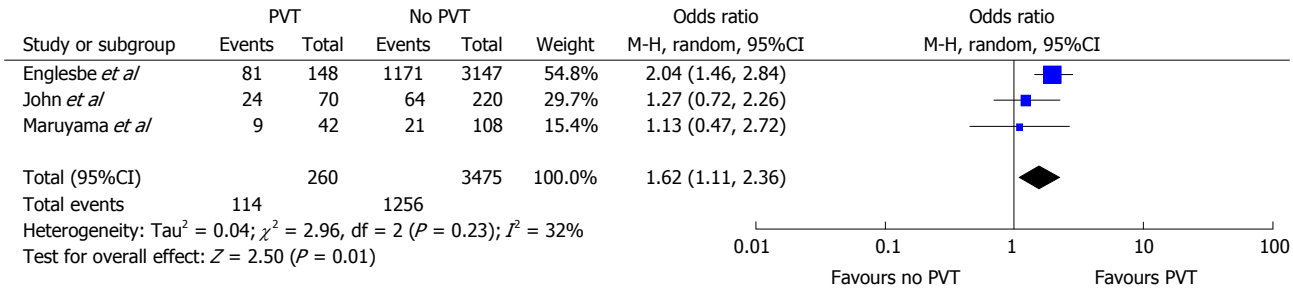

Figure 3 Portal vein thrombosis and mortality. PVT is associated with an increased pooled risk of death in the absence of significant heterogeneity. PVT: Portal vein thrombosis.

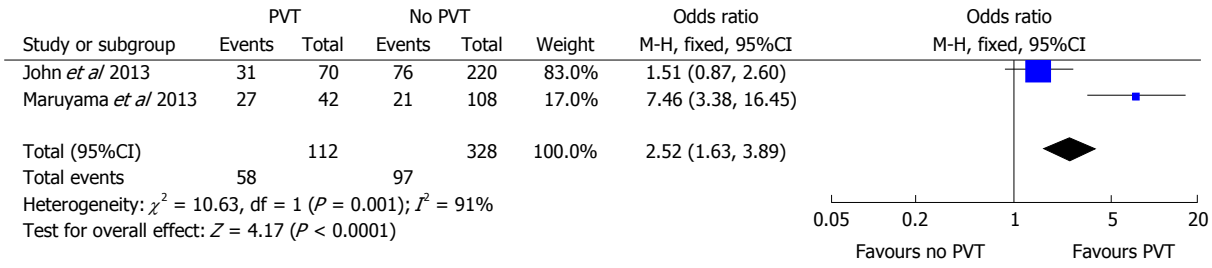

Figure 4 Portal vein thrombosis and ascites. PVT is associated with an increased pooled risk of hepatic decompensation manifested as ascites. This conclusion may be limited by heterogeneity in the included studies. PVT: Portal vein thrombosis.

significant higher odds of hepatic decompensation (OR = 2.52, 95%CI: 1.63-3.89, P < 0.001). The Cochran's Q statistic and I^2 were both significant for heterogeneity in this analysis. There was insufficient data available to determine the pooled effect on other markers of decompensation including gastroesophageal variceal bleeding or hepatic encephalopathy.

DISCUSSION

Our systematic review and meta-analysis is the first study to offer a pooled estimate and quantitative assessment of the clinical impact of PVT in terms of both mortality and hepatic decompensation. We have demonstrated a significantly increased rate of mortality for patients with cirrhosis and PVT when compared to those patients without PVT. This finding is important because PVT is a common finding in patients with cirrhosis[4,6,19] and one that significantly impairs quality of life and post-LT outcomes[7-9]. Several risk factors have been suggested to increase the risk of PVT in patients awaiting liver transplantation, including non-alcoholic steatohepatitis[20].

The risk of hepatic decompensation with ascites for patients with cirrhosis was also significantly greater in the presence of PVT. Evaluating a pooled estimate

of risk for gastroesophageal variceal hemorrhage or ascites resulting from PVT could not be performed due to the lack of data reporting by the included studies. While this has been shown in other studies, these were not included due to a lack of a comparison group.

PVT appears to increase mortality and hepatic decompensation (composite as well as variceal hemorrhage and ascites), however, the relatively small number of included studies limits generalizable conclusions. Our study has several other limitations. Multiple studies with a large number of patients were excluded due to a lack of a comparison group, and our literature search revealed a general lack of randomized controlled trials within the context of PVT. The large multicenter prospective study by Nery *et al*[11] was specifically excluded due to a lack of absolute numbers and component hepatic decompensation assessment. The inclusion of only three studies also limits the systematic assessment for publication bias and may also have resulted in the large degree of heterogeneity seen in calculation of the pooled measure of effect for PVT and the development of ascites.

Regardless, this current review represents the best available summary of the evidence to date and highlights a significant need for future research around the implications of PVT, especially prospective studies with a direct comparator group. Safety and efficacy data on both prevention and treatment of PVT is in general lacking. Villa *et al*[21] recently published their unblinded randomized, single center experience having found that daily prophylactic dosing of low molecular weight heparin (the equivalent of 40 mg/d) for twelve months prevented the development of PVT in patients with compensated cirrhosis. While the study was terminated at 48 wk, the effect persisted through follow-up at 5 years when compared to standard of care[21]. Additionally, the authors demonstrated less hepatic decompensation in the low molecular weight heparin arm ($P < 0.0001$) and a more importantly, a significant survival benefit[21]. Building on this work, Cui *et al*[22] published their single center randomized trial of 65 patients investigating therapeutic doses of low-molecular weight heparin (1 mg/kg every twelve hours or 1.5 mg/kg daily), where 78.5% ($n = 51$) responded to treatment with either complete or partial recanalization at 6 mo after starting therapy. These responders had regression of their liver disease when compared directly to the 14 non-responders. Similar to Villa *et al*[21], Cui *et al*[22] found no episodes of variceal hemorrhage, however, they did find much higher rates of non-variceal bleeding (6.4%-23.5%). While this study has several limitations including its generalizability as it only enrolled hepatitis B patients in China, it is nonetheless promising. With the development of new oral anticoagulants, the promise of treatment and possibly prevention is becoming a reality[23]. More rigorous study is needed in the field of coagulation disorders in a randomized, placebo controlled interventional or preventative trial with a direct comparator group using either heparin based or new direct acting oral anticoagulant therapy.

In conclusion, PVT appears to increase mortality and hepatic decompensation from ascites, however, the relatively small number of included studies limits generalizable conclusions and contributes significant heterogeneity in the pooled measures of effect. More prospective, randomized placebo controlled trials with a direct comparator group are needed.

COMMENTS

Background

Non-neoplastic portal vein thrombosis is a common complication of cirrhosis. Treatment options carry risk and are not without complications. To make appropriate clinical decisions, clinicians need to be cognizant of the available evidence.

Research frontiers

The field of coagulation disorders in chronic liver disease is ever expanding. Much of the research focuses on portal vein thrombosis and clinically relevant outcomes with the goal of preventing hepatic decompensation through either prevention or treatment of portal vein thrombosis.

Innovations and breakthroughs

In the present study, the authors investigated the impact of portal vein thrombosis on mortality and hepatic decompensation in patients with cirrhosis. This is the first report of a meta-analysis in patients with cirrhosis specifically excluding those who go on to receive liver transplantation.

Applications

The present report furthers understanding regarding the clinical importance of portal vein thrombosis in patients with underlying cirrhosis.

Peer-review

This systematic review and meta-analysis adds useful information for both clinical practice and further academic research with the goal of impacting patient centered outcomes.

REFERENCES

1 **Kinjo N**, Kawanaka H, Akahoshi T, Matsumoto Y, Kamori M, Nagao Y, Hashimoto N, Uehara H, Tomikawa M, Shirabe K, Maehara Y. Portal vein thrombosis in liver cirrhosis. *World J Hepatol* 2014; **6**: 64-71 [PMID: 24575165 DOI: 10.4254/wjh.v6.i2.64]

2 **DeLeve LD**, Valla DC, Garcia-Tsao G. Vascular disorders of the liver. *Hepatology* 2009; **49**: 1729-1764 [PMID: 19399912 DOI: 10.1002/hep.22772]

3 **Ogren M**, Bergqvist D, Björck M, Acosta S, Eriksson H, Sternby NH. Portal vein thrombosis: prevalence, patient characteristics and lifetime risk: a population study based on 23,796 consecutive autopsies. *World J Gastroenterol* 2006; **12**: 2115-2119 [PMID: 16610067]

4 **Wanless IR**, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G. Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension. *Hepatology* 1995; **21**: 1238-1247 [PMID: 7737629]

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

6 **Zocco MA**, Di Stasio E, De Cristofaro R, Novi M, Ainora ME, Ponziani F, Riccardi L, Lancellotti S, Santoliquido A, Flore R, Pompili M, Rapaccini GL, Tondi P, Gasbarrini GB, Landolfi R, Gasbarrini A. Thrombotic risk factors in patients with liver cirrhosis: correlation with MELD scoring system and portal vein

thrombosis development. *J Hepatol* 2009; **51**: 682-689 [PMID: 19464747 DOI: 10.1016/j.jhep.2009.03.013]
7 **Englesbe MJ**, Kubus J, Muhammad W, Sonnenday CJ, Welling T, Punch JD, Lynch RJ, Marrero JA, Pelletier SJ. Portal vein thrombosis and survival in patients with cirrhosis. *Liver Transpl* 2010; **16**: 83-90 [PMID: 20035521 DOI: 10.1002/lt.21941]
8 **Rodríguez-Castro KI**, Porte RJ, Nadal E, Germani G, Burra P, Senzolo M. Management of nonneoplastic portal vein thrombosis in the setting of liver transplantation: a systematic review. *Transplantation* 2012; **94**: 1145-1153 [PMID: 23128996 DOI: 10.1097/TP.0b013e31826e8e53]
9 **Hibi T**, Nishida S, Levi DM, Selvaggi G, Tekin A, Fan J, Ruiz P, Tzakis AG. When and why portal vein thrombosis matters in liver transplantation: a critical audit of 174 cases. *Ann Surg* 2014; **259**: 760-766 [PMID: 24299686 DOI: 10.1097/SLA.0000000000000252]
10 **Ponziani FR**, Zocco MA, Senzolo M, Pompili M, Gasbarrini A, Avolio AW. Portal vein thrombosis and liver transplantation: implications for waiting list period, surgical approach, early and late follow-up. *Transplant Rev* (Orlando) 2014; **28**: 92-101 [PMID: 24582320 DOI: 10.1016/j.trre.2014.01.003]
11 **Nery F**, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou PE, Plessier A, Roulot D, Chaffaut C, Bourcier V, Trinchet JC, Valla DC. Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study. *Hepatology* 2015; **61**: 660-667 [PMID: 25284616 DOI: 10.1002/hep.27546]
12 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670]
13 **Stang A**. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; **25**: 603-605 [PMID: 20652370]
14 **Haidich AB**. Meta-analysis in medical research. *Hippokratia* 2010; **14**: 29-37 [PMID: 21487488]
15 **Murad MH**, Montori VM, Ioannidis JP, Jaeschke R, Devereaux PJ, Prasad K, Neumann I, Carrasco-Labra A, Agoritsas T, Hatala R, Meade MO, Wyer P, Cook DJ, Guyatt G. How to read a systematic review and meta-analysis and apply the results to patient care: users' guides to the medical literature. *JAMA* 2014; **312**: 171-179 [PMID: 25005654]
16 **John BV**, Konjeti R, Aggarwal A, Lopez R, Atreja A, Miller C, Zein NN, Carey WD. Impact of untreated portal vein thrombosis on pre and post liver transplant outcomes in cirrhosis. *Ann Hepatol* 2013; **12**: 952-958 [PMID: 24114826]
17 **Maruyama H**, Okugawa H, Takahashi M, Yokosuka O. De novo portal vein thrombosis in virus-related cirrhosis: predictive factors and long-term outcomes. *Am J Gastroenterol* 2013; **108**: 568-574 [PMID: 23381015 DOI: 10.1038/ajg.2012.452]
18 **Hutton B**, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, Ioannidis JP, Straus S, Thorlund K, Jansen JP, Mulrow C, Catalá-López F, Gøtzsche PC, Dickersin K, Boutron I, Altman DG, Moher D. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann Intern Med* 2015; **162**: 777-784 [PMID: 26030634]
19 **Gayowski TJ**, Marino IR, Doyle HR, Echeverri L, Mieles L, Todo S, Wagener M, Singh N, Yu VL, Fung JJ, Starzl TE. A high incidence of native portal vein thrombosis in veterans undergoing liver transplantation. *J Surg Res* 1996; **60**: 333-338 [PMID: 8598664 DOI: 10.1006/jsre.1996.0053]
20 **Stine JG**, Shah NL, Argo CK, Pelletier SJ, Caldwell SH, Northup PG. Increased risk of portal vein thrombosis in patients with cirrhosis due to nonalcoholic steatohepatitis. *Liver Transpl* 2015; **21**: 1016-1021 [PMID: 25845711 DOI: 10.1002/lt.24134]
21 **Villa E**, Cammà C, Marietta M, Luongo M, Critelli R, Colopi S, Tata C, Zecchini R, Gitto S, Petta S, Lei B, Bernabucci V, Vukotic R, De Maria N, Schepis F, Karampatou A, Caporali C, Simoni L, Del Buono M, Zambotto B, Turola E, Fornaciari G, Schianchi S, Ferrari A, Valla D. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* 2012; **143**: 1253-1260.e1-4 [PMID: 22819864 DOI: 10.1053/j.gastro.2012.07.018]
22 **Cui SB**, Shu RH, Yan SP, Wu H, Chen Y, Wang L, Zhu Q. Efficacy and safety of anticoagulation therapy with different doses of enoxaparin for portal vein thrombosis in cirrhotic patients with hepatitis B. *Eur J Gastroenterol Hepatol* 2015; **27**: 914-919 [PMID: 25856686 DOI: 10.1097/meg.0000000000000351]
23 **Intagliata NM**, Maitland H, Northup PG, Caldwell SH. Treating thrombosis in cirrhosis patients with new oral agents: ready or not? *Hepatology* 2015; **61**: 738-739 [PMID: 24829112 DOI: 10.1002/hep.27225]

P- Reviewer: Liang Y, Lim EC, Wang SK **S- Editor**: Wang JL
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