ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2017 June 8; 9(16): 715-756

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 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Repoublic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), 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 (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

Chile

Luis A Videla, *Santiago*

China

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

Pratika Yuhyi Hernanda, *Surabaya*
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*
Matteo Garcovich, *Rome*
Edoardo G Giannini, *Genova*
Rossano Girometti, *Udine*
Alessandro Granito, *Bologna*
Alberto Grassi, *Rimini*
Alessandro Grasso, *Savona*
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*
Jeong Heo, *Busan*
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*
José M González-Navajas, *Alicante*
Juan C Laguna, *Barcelona*
Elba Llop, *Madrid*
Laura Ochoa-Callejero, *La Rioja*
Albert Pares, *Barcelona*
Sonia Ramos, *Madrid*
Francisco Rodriguez-Frias, *Córdoba*
Manuel L Rodriguez-Peralvarez, *Córdoba*
Marta R Romero, *Salamanca*
Carlos J Romero, *Madrid*
Maria Trapero-Marugan, *Madrid*

Sri Lanka

Niranga M Devanarayana, *Ragama*

Sudan

Hatim MY Mudawi, *Khartoum*

Sweden

Evangelos Kalaitzakis, *Lund*

Switzerland

Christoph A Maurer, *Liestal*

Thailand

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

Turkey

Osman Abbasoglu, *Ankara*
Mesut Akarsu, *Izmir*
Umit Akyuz, *Istanbul*
Hakan Alagozlu, *Sivas*
Yasemin H Balaban, *Istanbul*
Bulent Baran, *Van*
Mehmet Celikbilek, *Yozgat*
Levent Doganay, *Istanbul*
Fatih Eren, *Istanbul*
Abdurrahman Kadayifci, *Gaziantep*
Ahmet Karaman, *Kayseri*
Muhsin Kaya, *Diyarbakir*
Ozgur Kemik, *Van*
Serdar Moralioglu, *Uskudar*
A Melih Ozel, *Gebze - Kocaeli*
Seren Ozenirler, *Ankara*
Ali Sazci, *Kocaeli*
Goktug Sirin, *Kocaeli*
Mustafa Sunbul, *Samsun*
Nazan Tuna, *Sakarya*
Ozlem Yonem, *Sivas*

Ukraine

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

United Kingdom

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

United States

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

World Journal of Hepatology

Contents

Three issues per month Volume 9 Number 16 June 8, 2017

Contents

World Journal of Hepatology
Volume 9 Number 16 June 8, 2017

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Mehmet Celikbilek, MD, Assistant Professor, Department of Gastroenterology, Bozok University, 66200 Yozgat, Turkey

AIM AND SCOPE

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

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

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

INDEXING/ABSTRACTING

World Journal of Hepatology is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, and Scopus.

FLYLEAF

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

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

EDITORIAL OFFICE
Xiu-Xia Song, Director
World Journal of Hepatology
Baishideng Publishing Group Inc
7901 Stoneridge Drive,
Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive,
Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
June 8, 2017

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2017 June 8; 9(16): 715-732

DOI: 10.4254/wjh.v9.i16.715

ISSN 1948-5182 (online)

REVIEW

Non-alcoholic fatty liver disease: An expanded review

Mark Benedict, Xuchen Zhang

Mark Benedict, Xuchen Zhang, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510, United States

Author contributions: Benedict M wrote the paper; Zhang X edited, revised and contributed with conceptual development.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

Correspondence to: Xuchen Zhang, MD, PhD, Department of Pathology, Yale University School of Medicine, 310 Cedar Street, LH 108, PO Box 208023, New Haven, CT 06520, United States. xuchen.zhang@yale.edu
Telephone: +1-203-7856010
Fax: +1-203-7872922

Received: December 16, 2016
Peer-review started: December 19, 2016
First decision: January 28, 2017
Revised: February 8, 2017
Accepted: April 18, 2017
Article in press: April 20, 2017
Published online: June 8, 2017

Abstract

Non-alcoholic fatty liver disease (NAFLD) encompasses the simple steatosis to more progressive steatosis with associated hepatitis, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma. NAFLD is a growing epidemic, not only in the United States, but worldwide in part due to obesity and insulin resistance leading to liver accumulation of triglycerides and free fatty acids. Numerous risk factors for the development of NAFLD have been espoused with most having some form of metabolic derangement or insulin resistance at the core of its pathophysiology. NAFLD patients are at increased risk of liver-related as well as cardiovascular mortality, and NAFLD is rapidly becoming the leading indication for liver transplantation. Liver biopsy remains the gold standard for definitive diagnosis, but the development of noninvasive advanced imaging, biochemical and genetic tests will no doubt provide future clinicians with a great deal of information and opportunity for enhanced understanding of the pathogenesis and targeted treatment. As it currently stands several medications/supplements are being used in the treatment of NAFLD; however, none seem to be the "magic bullet" in curtailing this growing problem yet. In this review we summarized the current knowledge of NAFLD epidemiology, risk factors, diagnosis, pathogenesis, pathologic changes, natural history, and treatment in order to aid in further understanding this disease and better managing NAFLD patients.

Key words: Non-alcoholic fatty liver disease; Metabolic syndrome; Steatohepatitis; Hepatocellular carcinoma; Steatosis

Core tip: Non-alcoholic fatty liver disease (NAFLD) is a growing epidemic, not only in the United States, but worldwide in part due to obesity and insulin resistance leading to liver accumulation of triglycerides and free fatty acids. NAFLD patients are at increased risk of liver-related as well as cardiovascular mortality, and NAFLD is rapidly becoming the leading indication for liver transplantation. Numerous risk factors for the development of NAFLD have been espoused with most having some form of metabolic derangement or insulin resistance at the core of its pathophysiology. However, the exact pathogenic mechanism of NAFLD still remains unclear, and there

is no effective treatment yet so far. In this review we summarized the current knowledge of NAFLD epidemiology, risk factors, diagnosis, pathogenesis, pathologic changes, natural history, and treatment.

Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol* 2017; 9(16): 715-732 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i16/715.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i16.715

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an umbrella term and encompasses the simple deposition of adipose tissue in the liver to more progressive steatosis with associated hepatitis, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma (HCC)[1]. For the sake of terminology, NAFLD is comprised of non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH)[1]. NAFL is characterized by steatosis of the liver, involving greater than 5% of parenchyma, with no evidence of hepatocyte injury[2]. Whereas, NASH is defined by histologic terms, that is a necroinflammatory process whereby the liver cells become injured in a background of steatosis[2]. Although the natural history of NAFLD remains incompletely characterized, what is clear from the published data is a risk of progression to cirrhosis and HCC[3-7]. However, whether there is a clear progression of NAFL to NASH is under active investigation, but early evidence suggests this could be the case[1]. In terms of epidemiology, several studies have tried to quantify the true worldwide incidence of NAFL/NASH; however, due to extreme variations in study parameters and available testing, a clear and reliable occurrence rate is not currently available[1]. With that being said, estimates have been posited suggesting the incidence of NAFLD to be 20%-30% in Western countries and 5%-18% in Asia[1]. It is no surprise that the prevalence of NAFLD is increasing worldwide with each passing year, given the current trends in dietary irresponsibility and preponderance of a sedentary lifestyle[1]. Additionally, there has been a linear rise of NAFLD with that of diabetes and metabolic syndrome[3]. In one study from the United States, it was shown that the incidence of NAFLD was 10% higher in overweight individuals compared to lean persons[8]. In fact, NAFLD has been projected, within the next 20 years, to become the major cause of liver related morbidity and mortality as well as a leading indication for liver transplantation[3]. As it currently stands, NAFLD represents the second most common reason to be listed for a liver transplant[9]. Additionally, not only does NAFLD place a strain on the medical system and its resources, it also is associated with a 34%-69% chance of dying over the next 15 years when compared with the general population[9]. The pathogenetic processes that underscore NAFLD typically lead to death by cardiovascular disease with liver related mortality only accounting for 5% in these individuals[9,10]. In the forthcoming sections we will provide context for how and why NAFLD develops, current genetic proposals, histologic criteria, differential diagnoses, and prognosis of this very important disease affecting not only the United States but much of the world.

RISK FACTORS AND ETIOLOGY

Metabolic syndrome and type 2 diabetes mellitus

Metabolic syndrome is a conglomerate of cardiovascular risk factors which predispose a person to developing type Ⅱ diabetes and cardiovascular disease[2]. The current diagnostic criteria require having 3 of 5 of the following factors: Triglycerides 150 mg/dL or greater, high-density lipoprotein-cholesterol of less than 40 mg/dL in men and less than 50 mg/dL in women, hyperglycemia (fasting glucose of 100 g/dL or greater), an increased waist circumference (defined by population specific data), and hypertension (systolic blood pressure of 130 mmHg or greater or diastolic blood pressure of 85 mmHg or greater)[2]. As previously mentioned the incidence of NAFLD has been increasing in concert with the rising rates of metabolic syndrome. In fact it has been stated that the incidence of NAFLD increases with increasing number of metabolic syndrome criteria met[2]. When compared to non-diabetic patients (matched for age, sex, and body weight), type 2 diabetes mellitus (T2DM) patients have liver fat contents that are 80% higher[11]. Interestingly, it has been shown that T2DM patients with NAFLD can have normal liver function tests, which may lead one to believe that the prevalence of NAFLD in T2DM patients is much higher than reported in this patient population[11]. Additionally, T2DM patients display a very high risk of developing NASH as well as a two-to-four-fold increased risk of fatty liver associated complications[11,12].

Ethnic differences

The rate at which NAFLD develops has been shown to be greatest in Hispanic patients[13]. Also, NAFLD in the Asian population has been increasing, and interestingly, can be seen in those who have a normal body mass index[13]. In a United States based study, the investigators found a lower degree of steatosis in African Americans when compared to whites and also showed a higher degree of NAFLD findings in Asians and Hispanics[14]. The Hispanic population also has been shown to have a higher occurrence of steatohepatitis and cirrhosis, while those who are African American enjoy a decreased chance of developing liver failure[15]. With further genetic investigation by genome wide association, it was noted that Hispanics had a twofold higher liver fat content if they possessed the homozygous PNPLA3 allele (patatin-like phospholipase domain-containing protein 3 rs738409)[15]. The PNPLA3 gene family has been shown to affect lipid metabolism and patients who harbor this polymorphism were found to have increased hepatic fat content, triglyceride stores,

and inflammation[13]. In fact, the mutation of *PNPLA3 rs738409* gene (encoding I148M) has revealed more severe histologic features of NAFLD in those carrying the mutation[13]. More information on the genetic basis for NAFLD can be found under the "genetics" heading.

Gender and age

Unfortunately, the role of gender in the development of NAFLD has been met with differing conclusions in the literature. Several studies provide data to suggest a higher prevalence in males while others proposed the opposite[1]. However, according to Lonardo *et al*[11] epidemiological review, NAFLD is more common in men and has been shown to increase in those who are younger to middle aged with a decline noted after the age of 50-60 years. In contrast, NAFLD has been shown to spare those women who are pre-menopausal and then a rise in incidence occurs after the age of 50 with a peak at 60-69 years, and the preponderance of evidence does seem to suggest that NASH is histologically more severe in women when compared to men[11]. It has been reported that the prevalence of NAFLD increases with age (20% in people younger than age 20) to greater than 40% in those who are older than 60 years of age[16]. Not only does the prevalence of NAFLD increase with increasing age, but the incidence of NASH and cirrhosis also increases in those patients who are 50 years of age or greater compared with younger age groups[1]. Notably, it has been suggested that NAFLD begins in utero based on several studies, using magnetic resonance spectroscopy, showing steatosis in infants born to mothers with gestational diabetes (GD)[17]. In a study using hepatic fat fraction (HFF), performed at 1-3 wk of age in neonates born to normal mothers compared to those with gestational diabetes, neonates born to obese mothers with GD had a mean HFF that was 68% higher than those born to normal weight mothers[18]. In another study by Patel *et al*[19], 33 stillborn babies of diabetic mothers were compared with 48 stillborn babies of mothers without diabetes and there was a markedly increased rate of hepatic steatosis in neonates born to mothers with diabetes (79%) *vs* controls (17%). A study with 191 Italian children with biopsy confirmed NAFLD, showed hepatic steatosis, inflammation, hepatocyte ballooning, and fibrosis were worse in those children who were not breast-fed compared to those who were[20]. Similar to what has been observed in adults, obesity is a considerable risk factor for the development of NAFLD in children[21]. According to the Study of Child and Adolescent Liver Epidemiology, approximately one-third of obese children have NAFLD[22]. With that being said, a fatty liver is the most common liver abnormality found in children aged 2-19 years[22]. Again like that seen in adulthood, there is also an association of pediatric NAFLD and cardiovascular disease with higher levels of total cholesterol, LDL, triglycerides, and systolic blood pressure reported[21]. As it currently stands the incidence of HCC in the pediatric population with NALFD is not known but thought to be rare[17]. Only one case report of HCC with concurrent NAFLD in a 7-year-old boy has been reported[23]. Longitudinal outcomes are sparse for pediatric patients with NAFLD; however, what is known is that children can present with cirrhosis at diagnosis and may progress from NASH to cirrhosis[24].

Diet, smoking and life style

Diet has been thought of as an independent risk factor for the development of NAFLD, specifically, a diet high in fats[15]. It has been shown, through energy restriction and manipulation of dietary macronutrients, namely, restriction of carbohydrates, fat, or enrichment with monounsaturated fatty acids, that dietary modifications can reduce metabolic syndrome[25,26]. Diets that model after a Westernized pattern, such as those high in red meat consumption, refined grains, pastries, and sugar laden beverages are associated with a greater likelihood for the development of metabolic syndrome and subsequent NAFLD[15]. In a retrospective study with 2029 participants, cigarette smoking was found to be an independent risk factor for the onset of NAFLD[27]. The use of tobacco predisposes a person for the development of insulin resistance[28-30]. Additionally, in a study looking at adolescents in the United States, passive and active smoke exposure are strong independent predictors of metabolic syndrome[31]. As to life style, associations have been shown between a person's fitness and sedentary behavior with the risk of developing NAFLD and NASH; the severity of NAFLD also intensifies with lower physical activity[15]. In fact, as part of the EASL-EASD-EASO Clinical practice guidelines for the management of NAFLD, a recommendation for the assessment of physical activity habits should be included as part of a comprehensive NAFLD screening exam[32]. Additionally, part of the treatment regimen for NAFLD incorporates diet and physical activity to address obesity and insulin resistance. Several studies have evaluated the effect of a balanced diet with gradual weight reduction and their effects of NAFLD biologic parameters. Overwhelmingly, gradual weight reduction through diet, with or without exercise, have shown improvements in serum liver enzymes, reduced hepatic fatty infiltration, decreased hepatic inflammation and reduced levels of fibrosis[33]. Also there is a clear benefit of exercise on hepatic fatty infiltration; this benefit is even evident with minimal or no weight loss and exercise levels that fall below those which are recommended for obesity management[34]. According to a systematic review, NAFLD is also improved with resistance exercise (as opposed to the therapeutic benefits of aerobic activities such as running), which may be more tolerable for the NAFLD patients who suffer from poor cardiorespiratory fitness and cannot tolerate intense aerobic exercise[35].

Polycystic ovarian syndrome

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder in reproductive aged women and is typically

characterized by obesity and insulin resistance[36]. Hence, women with PCOS are at a heightened risk of developing T2DM[36]. In a study that evaluated 600 women with PCOS and 125 body mass index (BMI)-matched healthy control women, the prevalence of NAFLD was found to be higher in those with PCOS[36]. Insulin resistance and obesity, as have been previously examined in this paper, are known to contribute to the development of NAFLD. Women with PCOS are typically hyperandrogenemic and insulin resistance worsens the hyperandrogenemia by increasing ovarian androgen synthesis and decreasing liver SHBG production, which results in elevated circulating levels of free androgens[36]. The subsequent hyperandrogenemia is associated with a more prominent insulin resistance in patients with PCOS, which endangers these patients for developing NAFLD[36]. Numerous other investigations into the association of PCOS and NAFLD have been performed and similar results were obtained[37-40].

Obstructive sleep apnea

Obstructive sleep apnea (OSA) is characterized by complete or partial airway obstruction caused by pharyngeal collapse during sleep[41]. A budding association of OSA with diabetes mellitus, metabolic syndrome, and cardiovascular disease has started to appear in the last few years[41]. In the general population, obstructive sleep apnea has a prevalence of around 4% with that number jumping to 35%-45% in obese individuals[15]. In a study performed by Tanné *et al*[42], patients with severe OSA were found to be more insulin resistant and had a higher percentage of steatosis as well as increased necrosis and fibrosis scores (on liver biopsy) when compared to those patients without OSA and a similar BMI. The pathogenic mechanisms that underpin this association is believed to be due to the alteration of gas exchange (repetitive hypoxemic and hypercapnic events), termed chronic intermittent hypoxia, which can lead to an increase in proinflammatory cytokines, endothelial dysfunction, oxidative stress, metabolic dysregulation, and finally insulin resistance[41]. Interestingly, OSA may be one of the elements promoting the evolution of NAFLD from steatosis to NASH[41]. Additionally, using animal models, OSA was shown to promote the digression of NAFLD to NASH[15]. Investigational evidence has suggested that chronic intermittent hypoxia may trigger liver injury, inflammation, and fibrogenesis with several studies showing an intriguing relationship between OSA and NASH[41,43-48].

GENETICS

Data from numerous studies have given evidence for a heritable component to NAFLD and includes: Familial aggregation, twin studies, and interethnic differences in susceptibility[49-57]. Whole exome sequencing studies performed on obese Caucasian participants with NAFLD have revealed deleterious mutations in Bardet-Biedl syndrome 1 gene as well as the Melanocortin 3 receptor gene[58]. In 2008, the first genome wide association study was published; it examined hepatic triacylglycerol (HTAG) accumulation and identified association with increased HTAG and the *PNPLA3* gene[59]. This single nucleotide polymorphism is a nonsynonymous cytosine to guanine nucleotide transversion mutation that results in an isoleucine to methionine amino acid change. Subsequent work has confirmed this variant (PNPLA3 rs738409) in Japanese, Indian, and Chinese NAFLD patients[60-65]. In a meta-analysis of 24 studies with 9915 participants, Singal *et al*[66] found that PNPLA3 was associated with fibrosis severity. Additionally, among nine studies, totaling 2937 participants, the PNPLA3 was again linked with increased risk for the development of HCC in those with cirrhosis[66]. A separate meta-analysis, 16 studies included, revealed the rs738409 GG genotype compared to the CC genotype was linked to a 73% greater liver fat content as well as a 3.24-fold increased risk of more pronounced necroinflammatory scores and a 3.2-fold increased risk of developing fibrosis[67]. Xu *et al*[68], by way of meta-analysis totaling 23 case-control studies (totaling 6071 NAFLD participants and 10366 controls) found the PNPLA3 rs738409 polymorphism to have a significant association with a high cross-ethnicity risk for NAFLD as well as NASH. Genome-wide association study performed on 236 non-Hispanic white women with NAFLD (324623 single nucleotide polymorphisms in total from 22 autosomal chromosomes) found the NAFLD activity score to be associated with the SNP rs2645424, the degree of fibrosis associated with SNP rs343062, lobular inflammation with SNPs rs1227756, rs6591182, and rs887304, increased levels of ALT was associated with SNPs rs2499604, rs6487679, rs1421201, and finally rs2710833[69]. Using exome-wide association, Kozlitina *et al*[70] found three variants to be associated with higher liver fat levels: Two in the aforementioned PNPLA3 and one in the *TM6SF2* gene, which likely is required for normal VLDL secretion. The variant frequency in *TM6SF2* gene was found to be highest in those of European, African-American, and Hispanic ancestry[58]. In a later study by Mahdessian *et al*[71]; the *TM6SF2* gene was found to be a regulator of liver fat metabolism, which influenced triglyceride secretion and hepatic lipid droplet content. As it stands currently, approximately 7 categories of genes have been associated with NAFLD and are broken down as follows: (1) hepatic lipid export/oxidation in steatosis (PNPLA3, TM6SF2, NR1I2, PPAR-alpha, PEMT, MTTP, APOC3 and APOE); (2) glucose metabolism and insulin resistance (ENPP1/IRS1, GCKR, SLC2A1, GOAT, TCF7L2 and PPARG); (3) steatosis-hepatic lipid import or synthesis (SLC27A5, FADS1, and LPIN1); (4) steatohepatitis-oxidative stress (HFE, GCLC/GCLM, ABCC2 and SOD2); (5) steatohepatitis-endotoxin response (TLR4 and CD14); (6) cytokines (TNF and IL6); and (7) fibrosis (AGTR1 and KLF6)[49,72].

PATHOGENESIS

Non-alcoholic fatty liver disease, not surprisingly, as its name implies revolves around the deposition of fat within the liver. Specifically, free fatty acids and triglyceride accumulation is

the hallmark feature and has been attributed, at least in part, to insulin resistance and obesity[73]. With that being said, the pathogenic components of NAFLD are complex and multifactorial with different theories presented in the literature[74]. A two-hit model of NAFLD development has been proposed with the first hit consisting of: Hepatic lipid accumulation, sedentary lifestyle, high fat diet, obesity, and insulin resistance[74]. The second hit activates an inflammatory event with associated fibrogenesis[75]. This two-hit model has lost some favor as it was believed to be too simplistic to fully describe the intricacy of human NAFLD where a multitude of factors are acting in concert with one another in a genetically predisposed individual[74]. As was described in the risk factors, a multitude of factors contribute and have some association with the development of NAFLD[76]. However, it is insulin resistance that plays a key role in the development of steatosis/NASH, which results in hepatic *de novo* lipogenesis and subsequent reduction of adipose tissue lipolysis, with a consequent increase of fatty acids in the liver[77]. Alterations in the production and secretion of adipokines and inflammatory cytokines are a consequence of adipose tissue dysfunction, which is brought about by insulin resistance[78]. The production of reactive oxygen species and endoplasmic reticulum stress coupled with mitochondrial dysfunction occurs as a result of fat accumulations in the liver, specifically in the form of triglycerides[79]. An excess of nutrients essentially overwhelms the endoplasmic reticulum, which then turns on the unfolded protein response and as a consequence, triggers the development of insulin resistance through a number of mechanisms, including c-jun N-terminal kinase activation and inflammation[79]. The gut microbiota has been recognized as one of the key players in the pathogenesis of NAFLD. Gut microbiota not only influences absorption and disposal of nutrients to the liver, but also conditions hepatic inflammation by supplying toll-like receptor ligands, which can stimulate liver cells to produce proinflammatory cytokines. Accordingly, the modification of intestinal bacterial flora by specific probiotics has been proposed as a therapeutic approach for the treatment of NASH[80]. Interestingly, dysfunctional adipose tissue, as seen in obesity, T2DM and NAFLD, impairs glucose and lipid metabolism by two mechanisms: One, by acting as an endocrine organ, which is releasing a number of fat-derived cytokines; and two, by free fatty acid-induced ectopic fat deposition and lipotoxicity[79].

Liver transplantation is performed for a variety of reasons: Liver failure, end-stage liver disease, tumors; however, after surgery these patients often develop an increase in body weight, subsequent insulin resistance, and metabolic perturbations[81]. Additionally, patients who undergo a liver transplant may also fall prey to diabetes mellitus, hyperlipidemia, and arterial hypertension[81]. In part, some the metabolic derangements that occur after liver transplantation are due to medication effects (*i.e.,* corticosteroids, calcineurin inhibitors, and sirolimus promote hyperglycemia, hypertension, and hyperlipidemia)[81]. Many of the effects aforementioned can be found in the diagnostic criteria of metabolic syndrome, and as previously discussed, NAFLD is essentially the liver's manifestation of this syndrome. Hence, it is not surprising to see recurrent or *de novo* NAFLD/NASH after a liver transplant[82]. It is important to note that 15.5% and 26.3% of liver transplant patients, at one and three years, respectively, become clinically obese[83]. Likewise, post-transplant development of DM is reported to range from 10%-64%, although the underlying mechanisms for this is yet to be entirely worked out[84]. However, it does appear that the main risk factors for the development of post liver transplant DM would include: Male gender, obesity, family history, hepatitis C virus (HCV), older age range, and high dose immunosuppresives[84]. Additionally, the rate of metabolic syndrome development post liver transplant is approximately 50%-60%[85]. In a cohort comprising 170 transplant patients followed for two years, the researchers showed the presence of metabolic syndrome in approximately one-third[86]. Not surprisingly, the incidence of NAFLD after having received a liver transplantation ranges from 18%-40% and the incidence of NASH ranges from 9%-13%[87]. Intriguingly, post-transplant NAFLD risk has also been tied to polymorphisms in PNPLA3, which has been shown to mediate triglyceride hydrolysis and is also associated with pretransplant obesity and NAFLD[87]. Overall, the natural history of post-liver transplant NAFLD is incompletely understood, however, it may contribute to increased cardiovascular disease mortality in these patients[87].

HISTOPATHOLOGY

Non-alcoholic fatty liver disease shows a wide range of histologic manifestations, which can range from a very mild steatosis (5% or more of hepatocytes involved), to more aggressive forms showing lobular and/or portal inflammation, ballooning hepatocytes, fibrosis, and ultimately cirrhosis[88]. The presence of less than 5% of steatosis is not regarded as clinically significant. In adult patients, steatosis typically affects the centrilobular hepatocytes first; whereas in children the periportal or panacinar patterns are more likely seen[89]. Steatosis comes in a few morphologic appearances, the macrovesicular terminology is used when large lipid droplets inhabit the cytoplasm and displace the nucleus[90]. However, macrovesicular steatosis also encompasses small lipid droplets, which varying in size and keep their nuclear central location[90]. Finally, the terminology of microvesicular steatosis denotes the accumulation of innumerable lipid droplets with the hepatocyte nucleus remaining essentially in its original location[90,91]. It is important to note that microvesicular steatosis is rare in isolation but has been reported to occur in a patchy distribution (approximately 10% of NAFLD cases)[90,91]. With that being said the presence of pure microvesicular steatosis has been reported somewhat more commonly in the diagnosis of alcoholic fatty liver disease (so-named alcoholic foamy degeneration)[92]. As was alluded to earlier in this paper,

lipid is a dynamic and metabolically active substance and the same holds true for fatty lipid droplets in the liver. Lipid droplets are comprised of a core of triacylglycerols with or without cholesterol esters and a peripheral monolayer of phospholipids[93]. Inactive PNPLA3 has been shown to accumulate on the surface of lipid droplets and is linked to an increase in macrovesicular steatosis[94]. Recent studies have espoused that the loss of reticulin seen in those patients with extensive steatosis may not be related to the presence of inflammation or fibrosis; the effects of such a loss in connective framework has yet to be determined, however, this finding should be remembered when HCC enters the differential diagnosis[95].

Assessment of the extent of steatosis

With the starting point of at least 5% steatotic involvement being pathologic, the affected parenchyma is then divided into thirds: 5%-33%, 34%-66% and > 66%[96]. The rule of thirds has allowed a three-tiered classification system with 5%-33% designated as mild, 34%-66% designated as moderate, and > 66% corresponding to severe steatosis[96]. Steatosis, when not in abundance, is typically centered in a zone 3 distribution but when prominent can be found in a panacinar location[90]. In a patient who has resolving hepatic steatosis, the fat droplets can be found in an irregular distribution throughout the acinus[90]. In a more rare occurrence, the steatosis may be found in a zone 1 location with disease progression to cirrhosis leading to a more irregular distribution or complete loss of steatotic droplets[90]. There has been a documented tendency to overestimate the degree by which the liver parenchyma is involved by steatosis among pathologists, hence more accurate and objective methods have employed the use of digital imagining analysis[97]. It is important to point out that conventional imaging (ultrasound, computed tomography, or magnetic resonance imaging), are not sensitive enough to detect hepatic steatosis when the percent involvement is less than 30%[91]. More advanced imaging techniques such as controlled attenuation parameter, magnetic resonance imaging-estimated proton density fat fraction, and ^{1}H-magnetic resonance spectroscopy have been shown to correlate well with histologic steatosis assessment in both the adult and pediatric NAFLD populations[98,99].

Steatosis with inflammation and/or fibrosis

In the realm of NAFLD, steatosis rarely is identified as the only finding and is oftentimes accompanied by a chronic inflammatory infiltrate (typically mononuclear) with varied severity, few plasma cells and monocytes may also be encountered[91]. Neutrophils make a rare appearance with occasional eosinophils in the presence of a lipogranuloma (a structure composed of a central steatotic hepatocyte or fat droplet and a peripheral accumulation of mononuclear cells and macrophages)[91]. Kupffer cell density in NAFLD has correlated with the degree of necroinflammatory activity, injury, and degree of fibrosis[100]. In fact, it is the Kupffer cell that is believed to play a commanding role in the pathogenesis of NAFLD with its regulation of hepatic triglyceride storage, mediation of inflammatory activity, and hepatocyte injury to include parenchymal fibrosis[100]. In the strictest and most traditional of viewpoints of NAFLD, the presence of hepatocyte injury and fibrosis were thought to be a product of disease progression to steatohepatitis[89]. However, some mild NAFLD cases encountered in adults have shown a very mild degree of fibrosis, mainly centered on the portal area or occasionally zone 3[91]. A note of clarification is in order due to some confusion which may occur with NASH. In NASH, most experts would agree that the most basic criteria of hepatocyte ballooning in addition to steatosis and inflammation must be met in order to render a diagnosis of NASH[88,101]. It is, as of yet, still unclear whether these patients with NAFLD (*i.e.,* not NASH) and a mild component of inflammation/fibrosis have as benign of a course when compared with those who have steatosis alone[90]. Conflicting reports on progression are found in the literature with some suggesting that these cases may evolve to more severe disease, typically at a slower rate, while others have shown these lesions may stabilize or regress[102,103].

Steatohepatitis

Ballooned hepatocytes with accompanied steatosis and inflammation are typically found in zone 3 of the hepatic microanatomy[91]. Some recent work using immunohistochemistry, specifically CK8/18, have shown that ballooned hepatocytes display significantly decreased expression compared to normal hepatocytes[90]. As it currently stands, the use of immunohistochemical stains for differentiating ballooned hepatocytes is not currently a common practice[90]. Although the exact mechanisms by which a hepatocyte takes on a ballooned appearance are not entirely elucidated, some proposed mechanisms include: Oxidative stress alteration of microtubules, loss of intermediate filament cytoskeleton, retention of fluid, modifications to small droplet fat and endoplasmic reticulum dilatation[104-108]. Mallory-Denk bodies, glycogenated nuclei, acinar lipogranulomas, megamitochondria, pericellular fibrosis, and acidophilic bodies are frequently seen in NASH, but are not required for the diagnosis[101]. Ductular reaction can be seen in NASH as well and is usually associated with fibrosis[90]. It is important to keep in mind that no single feature is entirely specific for the diagnosis of NASH[91].

Fibrosis

The impact of fibrosis cannot be overstated when discussing NAFL/NASH. In fact, literature has shown a substantial impact regarding the stage of fibrosis and overall morality[90]. Fibrosis, when seen in NAFLD, has a characteristic appearance with early lesions showing a perisinusoidal deposition in zone 3[90]. Collagen fibers may be seen to encircle hepatocytes with more progressed lesions[90]. Additionally, pericellular fibrosis has been shown to progress without any appreciable periportal

fibrosis[90]. Periportal fibrosis develops after the perisinusoidal fibrosis and is demonstrated as trapping of hepatocytes around the portal area and extension of short strands of collagen into the parenchyma. Bridging fibrosis may eventually form single bands between the portal area and central vein without hepatocyte trapping or island formation. Evidence suggests that portal fibrosis in association with pericentral fibrosis is a necessary component for bridging fibrosis to develop[90]. Masson trichrome stain can highlight the fibrosis and are useful in identifying early fibrosis of steatohepatitis. Of note, NASH may retain all of the active steatohepatitis changes but the steatosis may decrease below the 5% level. On the other hand, the active steatohepatitis changes may disappear in cirrhosis as well, resulting in a diagnosis of "cryptogenic cirrhosis"[109].

HCC: Steatohepatitic variant

In the United States HCC has increased by 80% in the last twenty years with HCC being the fifth most common malignancy worldwide and the third most common cause of cancer-related death[110,111]. Hepatitis B and C, alcoholic liver disease, hemochromatosis, and several others represent the mainstay of risk factors for the development of HCC; recent studies have reported NAFLD to be an underlying cause of HCC in a number of cases even in the absence of cirrhosis[112-116]. A new variant of HCC has been described, that is the steatohepatitic variant of HCC, which is reminiscent of steatohepatitis (inflammation, hepatocyte ballooning, Mallory-Denk bodies, and pericellular fibrosis), and was first seen in a population of patients with HCV-related HCCs[117]. In one study, examining 118 cases of HCC over a 3.5 year period, 13.5% represented the steatohepatitic HCC with all but one case occurring in patients with underlying steatohepatitis[116]. When examining patient characteristics, the steatohepatitic HCC variant patients showed higher numbers of metabolic syndrome risk factors as well as at least 3 components of metabolic syndrome[116]. In a separate study, Jain *et al*[118] found the steatohepatitic variant of HCC (SH-HCC) in approximately 19% of their cases over a period of 7 years, with 50% of those cases being seen in NAFLD patients and the other 50% were largely of HCV etiology. It is important to note, in a study performed by Yeh *et al*[119], that SH-HCC can occur outside the morphology of that seen in fatty liver disease or metabolic syndrome and was posited to be more likely attributable to genetic changes of shared genes or metabolic pathways. Yeh *et al*[119] also found a loss of 9q12-q31.1 in a subset of cases, in this regard more investigation needs to be done to further ascertain the molecular driver for such a morphologic variant.

Pediatric NAFLD histology

The main histological differences seen in some pediatric NAFLD when compared to adults has been the distribution of hepatocyte lipid droplets, inflammation and fibrosis location[120]. In some pediatric patients with NAFLD, the lipid vacuoles are largest in the periportal hepatocytes and tend to decrease in diameter in pericentral area (zone 3). Similarly, inflammation and fibrosis is also seen around the portal tract (that is zone 1 predominance opposed to zone 3). When bridging fibrosis develops, the bridges connect portal to portal areas, leaving the central veins alone[120]. However, these features are not specific for pediatric NAFLD and many cases have similar picture as that of adult NAFLD.

Grading and staging in NAFLD/NASH

In order to provide a consistent and reproducible assessment of NAFLD, the evaluation of morphological features must be semiquantified *via* an agreed upon scoring system to guide clinical decision making and for use in clinical trials[96,121-124]. Three histological scoring systems are currently in place: NASH clinical research network's NAFLD activity score (NASH CRN-NAS), steatosis, activity, and fibrosis (SAF), and the Brunt staging system[96,121,124]. The NAS uses numerical scores (Table 1) to develop an activity grade, which includes steatosis (0-3 points), hepatocellular ballooning (0-2 points), and acinar inflammation (0-3 points), as well as a separate fibrosis stage (0-4)[121]. Using a threshold of < 3 (activity score), the NAS showed a good correlation with the absence of a histological diagnosis of NASH[121]. Likewise, using a threshold of greater than or equal to 5, the NAS showed good correlation with having a diagnosis of NASH[121]. In validation by Hjelkrem *et al*[125], a total of 386 liver biopsies were evaluated, the sensitivity and specificity were 57% and 95%, respectively, when using a NAS $\geqslant$ 5 (indicating NASH) and NAS < 5 (indicating no NASH). When using an activity score of $\geqslant$ 4, the sensitivity increased to 85% with a slight decrease in specificity to 81%[125]. The $\geqslant$ 4 threshold has been recommended for any admission to an interventional trial for NASH[125]. In contrast, the SAF scoring algorithm (Table 2) was originally intended for the grading and staging of NAFLD in those patients who were morbidly obese about to undergo bariatric surgery[124]. Since then it has been used in patients with metabolic syndrome and concomitant NAFLD[91]. When using the SAF scoring system, the activity score (consisting of ballooning and lobular inflammation), enabled the discrimination of NASH (NASH patients had A > 2, whereas no patients with an A < 2 had NASH)[124]. Finally, the Brunt system uses a three tiered grading system (mild, moderate, and severe) with three parameters under histological investigation: Steatosis, ballooning, and inflammation (Table 3)[96]. The Brunt system also uses a four tiered staging system based on the location and degree of fibrosis (Table 3)[96]. It should be noted that regardless of every effort to devise a scoring system that is standardized and highly reproducible, the classification of NAFLD will always be plagued by observer bias and a lack of complexity which would be necessary to describe an intricate disease process[91].

DIFFERENTIAL DIAGNOSIS

As would be intuitive by the name of the disease, non-

Table 1 Non-alcoholic fatty liver disease activity scoring system[121]

Steatosis, grade (0-3)	
< 5%	0
5%-33%	1
34%-66%	2
> 66%	3
Lobular inflammation	
No foci	0
< 2 foci per 200 × field	1
2-4 foci per 200 × field	2
> 4 foci per 200 × field	3
Hepatocyte ballooning	
None	0
Few balloon cells	1
Many cells/prominent ballooning	2
Fibrosis stage	
None	0
Perisinusoidal or periportal	1
Mild, zone 3, perisinusoidal	1A
Moderate, zone 3, perisinusoidal	1B
Portal/periportal	1C
Perisinusoidal and portal/periportal	2
Bridging fibrosis	3
Cirrhosis	4

Table 2 Steatosis, activity, and fibrosis scoring system[91,124]

Steatosis score (S): Assessed the quantities of large or medium-sized lipid droplets (0-3)
S0: < 5%
S1: 5%-33%
S2: 34%-66%
S3: > 67%
Activity grade (0-4): Sum of scores for ballooning and lobular inflammation
A1: Mild activity
A2: Moderate activity
A3 and A4: Severe activity
Hepatocyte ballooning (0-2)
0: None
1: Foci of hepatocytes with rounded shape, pale or reticulated cytoplasm
2: Foci of hepatocytes with rounded shape, pale or reticulated cytoplasm and enlargement (> 2 × normal size)
Lobular inflammation (0-2)
0: None
1: < 2 foci per 20 × field
2: > 2 foci per 20 × field
Fibrosis stage (F)
F0: No relevant fibrosis
F1: 1a - mild zone 3 perisinusoidal fibrosis
1b - moderate zone 3 perisinusoidal fibrosis
1c - portal fibrosis
F2: Zone 3 perisinusoidal fibrosis with periportal fibrosis
F3: Bridging fibrosis
F4: Cirrhosis

alcoholic fatty liver disease/non-alcoholic steatohepatitis, the presence of alcohol driving these changes must be ruled out. However, many other disease settings are associated with liver injury which may resemble histological changes that are typically observed in NAFLD/NASH[91]. One category that may mimic NAFLD/NASH is termed chemotherapy (CASH)- or drug-associated steatohepatitis[91,126-128].

Alcoholic steatosis, alcoholic steatohepatitis, alcoholic cirrhosis and HCC are the entities that a patient may develop with chronic alcohol use and abuse[129]. The distinction of alcoholic liver disease (ALD) and NASH can simply be made by delving into the history for the affirmation of alcohol use; however, there are histologic features that may help differentiate one form over the other in the absence of being able to obtain a detailed history (Table 4)[129]. The diagnostic criteria for rendering an ALD diagnosis rests on evidence of liver injury and a reported history of alcohol intake[101]. The amount of alcohol ingested is the strongest predictor of ALD development; just 60 g/d of alcohol consumed leads to the develop fatty liver in more than 90% of individuals[130]. In fact, the risk of developing alcohol related cirrhosis increases greatly with consumption of > 60-80 g/d for more 10 years in men, and > 20 g/d in women[130].

There has been a rapid increase in the number of novel cytotoxic chemotherapeutic agents over the last few years and with the liver's role of drug metabolism it is not surprising that these drugs wreak havoc and produce hepatic injury[131]. Hepatotoxicity is neither predictable nor dose-dependent with most drug reactions occurring in an idiosyncratic manner[132]. Drug induced hepatic steatosis is a fairly rare event with several drugs/classes implicated: Methotrexate, amiodarone, tetracycline, glucocorticoids, tamoxifen, chemotherapeutics, and nucleoside analogues to name a few[133]. Drug-induced hepatic steatosis is thought to result from the exuberant accumulation of intracellular phospholipids due in part by a drug therapy that has lasted several weeks to months[133]. Mechanistically, drug-related hepatic injury is due in part to mitochondrial toxicity resulting in inhibition of beta oxidation, oxidative phosphorylation, and mitochondrial respiration[134]. Since beta oxidation is one of the main ways lipids are metabolized, drug induced inhibition results in the accumulation lipids within the hepatocytes[134]. The steatosis that occurs in the setting of drug/chemotherapeutic treatment often resembles that seen in NAFLD with several notable exceptions[91].

As previously outlined, the prevalence of NAFLD is growing and expanding, which allows the likely overlap of this disease with a concurrent disease, specifically: Chronic hepatitis B, chronic hepatitis C, human immunodeficiency virus, autoimmune hepatitis, biliary diseases, or other inherited metabolic disturbances[135-141]. In fact it has been reported that half of patients with human immunodeficiency virus (HIV) who undergo testing for liver test aberrations have concurrent NAFLD, which can result from HIV itself or the HAART therapy used in treatment[138]. In terms of autoimmune hepatitis, routine autoantibodies are present in NAFLD patients 23% of the time, necessitating the need for a liver biopsy for differentiation[139,140]. When looking at virally infected livers, specifically by HCV, hepatic steatosis has been reported in approximately 40%-85% of infected patients[142]. HCV is interesting in terms of its two pathway approach to liver steatosis: Viral and non-viral[142]. HCV, especially genotype 3a, has

Table 3 Brunt grading and staging of nonalcoholic steatohepatitis[96]

Grading	Staging
Mild (Grade 1)	Stage 1
Steatosis (mostly macrovesicular)	Zone 3 perisinusoidal/pericellular fibrosis (focal or extensive)
Involves up to 66% of biopsy	
Occasional ballooned zone 3 hepatocytes	Stage 2
Scattered rare intra-acinar neutrophils with/without associated lymphocytes	Zone 3 perisinusoidal/pericellular fibrosis with associated focal
No/mild portal chronic inflammation	or extensive periportal fibrosis
Moderate (Grade 2)	
Steatosis-any degree	
Ballooning hepatocytes-zone 3	
Intra-acinar neutrophils-may be associated with zone 3 pericellular fibrosis	Stage 3
Portal and intra-acinar chronic inflammation	Zone 3 perisinusoidal/pericellular fibrosis and portal fibrosis
Severe (Grade 3)	with associated focal or extensive bridging fibrosis
Panacinar steatosis	
Ballooning-zone 3	
Intra-acinar inflammation with scattered neutrophils	Stage 4
Neutrophils associated with ballooned hepatocytes with/without chronic inflammation	Cirrhosis
Chronic portal inflammation-mild or moderate	

Table 4 Histologic comparison of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis and alcoholic liver disease[129]

Characteristic	NAFLD and NASH	Alcoholic liver disease
Disease severity	Mild	Varying
Mallory-Denk body	Poorly formed	Well formed
Glycogenated nuclei	Common	Less common
Ductular proliferation	Less prominent	More prominent
Fibrosis/cirrhosis	Less common	More common
Sclerosing hyaline necrosis	None/rare	Present
Phlebosclerosis	None/rare	Present
Canalicular cholestasis	None/rare	Present
Foamy degeneration	None/rare	Present

NASH: Non-alcoholic steatohepatitis; NAFLD: Non-alcoholic fatty liver disease.

been reported to up-regulate the expression of fatty acid synthase in infected hepatocytes leading to increased fatty acids, impaired beta oxidation and reduced export of triglycerides[143]. As a part of its pathogenesis, HCV causes the inhibition of the microsomal triglyceride transfer protein, which is involved in the release of triglycerides from hepatocytes and as a consequence leads to triglyceride accumulation[142]. The non-viral approach to liver steatosis is typified by interference of insulin signaling resulting in insulin resistance[142]. The mode by which hepatitis B virus (HBV) causes hepatic steatosis is not entirely agreed upon[142]. It is postulated that HBV X protein may lead to lipid accumulation in hepatocytes with inhibition of apolipoprotein B secretion while at the same time PPARgamma and SREBP-1c activation with resultant nuclear factor-kappa B activation and TNF production[144].

PROGNOSIS, PROGRESSION AND CLINICAL COURSE

Numerous studies have tracked the progression of steatosis, steatohepatitis, and fibrosis in NAFLD patients through paired liver biopsies[103,145-151]. Wong *et al*[145], *via* a prospective longitudinal hospital based cohort study, found that of patients with simple steatosis, 39% developed a borderline NASH picture and 23% developed full blown NASH. In another study, totaling 108 patients (81 with NASH and 27 with NAFL), 42% had fibrosis progression, 40% had no change in fibrosis, and 18% had fibrosis regression[103]. Interestingly, 22% of patients with NAFL at baseline developed stage 3 fibrosis at follow-up biopsy (median biopsy interval 6.6 years, range of 1.3-22.6)[103]. Overall, when evaluating the bulk of progression data it appears as though 33% of patients with NAFL and NASH will progress to fibrosis and up to 20% may have some regression of their disease[3]. Progressive fibrosis in NASH has been shown to be as high as 2 times that of NAFL and some patients with NASH and NAFL may progress rapidly from no fibrosis to severe fibrosis over the course of several years[102]. Clinically, cirrhosis and liver decompensation in NAFLD patients has been shown to be on the order of 3.1% over a mean 7.6 years[152]. The development of complications, specifically portal hypertension, with the development of cirrhosis is 17% (at one year), 23% (at three years), and 52% (at 10 years)[153]. A median survival of two years is seen in those patients with NASH who have experienced decompensation[154].

Several investigations have found that men, postmenopausal women, those who underwent early menopause, and duration of menopause have an increased chance of fibrosis[155,156]. Although Hispanic patients have an increase prevalence of NAFLD, this feature does not seem to confer an increased risk of progression of their disease[57,157]. In contrast, Asian patients have been shown in some studies to have a more severe histologic picture[14]. Single nucleotide polymorphisms, namely, PNPLA3 rs738409 and rs58542926 are associated with severe histology to include NASH and cirrhosis[158,159]. Although increasing age is shown to be prone for the development of more severe fibrosis in NASH, it is

unclear whether this finding just underscores the fact that these patients have cumulative metabolic insults and a longer duration of disease exposure[160]. Additionally, higher rates of fibrosis progression have been seen in diabetics, those who are obese, hypertension (although several studies looking at NASH patients found no increased risk of progression due to hypertension), and degree of inflammation found on biopsy[102,103,145,147,161].

In studies where biopsies were taken at the time of bariatric surgery and after subsequent weight loss, changes in hepatic histology were reported to improve[162,163]. However, some degree of worsening of either the fibrosis or steatosis has also been documented[164]. In an extreme case, one patient was reported to progress from mild fatty change before surgery to severe NASH and death due to liver failure[165]. The obvious mechanisms by which bariatric surgery improved the features of NAFLD would be related to weight loss, improvements in T2DM, reduced insulin resistance, reduced hyperlipidemia, and improved components of metabolic syndrome[162]. Other proposed mechanisms would include the altered route of food delivery, which results in changes to the release of gut and pancreatic hormones, changes in fat distribution, hepatic insulin and free fatty acid metabolism, and changes in adipocytokines and other cytokines[166]. These alterations in hormone secretion affect carbohydrate and lipid metabolism and interfere with hepatic glucose release[166]. Changes in gene expression may also play a pivotal role. In a study of 28 severely obese participants, PNPLA3 expression was measured by rtPCR before and after gastric banding-induced weight loss with the results showing a restoration of PNPLA3 expression in adipose tissue, but not in liver specimens[167].

A study, evaluating NASH and steatosis improvement by weight loss, found that NASH resolution was obtained in 25% and NAS score improvement was seen in 47% of participants[168]. Likewise, 48% had improvement of their steatosis, 39% reduced the ballooning hepatocyte score and 50% showed improved lobular inflammation[168]. In terms of fibrosis, 65% had no change, 19% showed improvement, and 16% progressed[168]. Not altogether surprising, those participants who had the greatest weight loss also showed the most improvement of their histologic endpoint[168]. In another study with 180 participants, those who showed weight reduction had a 18.37-fold increase in the odds of NAFLD resolution[169]. One recommendation is a weight loss of at least 5% to decrease the burden of steatosis and 10% weight reduction to have an effect on liver necroinflammation[170].

Investigations have proposed a link between metabolic syndrome, T2DM, obesity and the development of HCC[171,172]. NAFLD, even in the absence of fibrosis, provides a nurturing environment for the development of HCC with insulin resistance and steatosis providing the inflammation, adipokines, oxidative stress, and lipotoxicity needed for hepatocellular carcinogenesis[172,173]. In a study examining 1500 American veterans, NASH was found to be the third most common risk factor for the development of HCC[174]. With that being said, the appearance of HCC is relatively rare in NAFLD, on the order of 0.2% (after eight year follow-up); however, the development of HCC in NASH cirrhosis ranges from 2.4% and 12.8% over a 3.2 and 7.2-year period, respectively[175,176]. In fact, once HCC develops in these cirrhotic patients their survival appears to be shorter than that seen in patients with HCV induced HCC[114].

DIAGNOSIS, TREATMENT AND SCREENING

Non-alcoholic fatty liver disease, in most instances, represents an incidental diagnosis due to alterations noted on a chemistry profile or when imaging for other purposes finds a steatosis pattern in the liver[9]. In the absence of incidental discovery, often patients are asymptomatic until liver decompensation occurs; however, if the evaluation of the patient reveals such factors as insulin resistance, obesity, or factors associated with metabolic syndrome, the diagnosis can be achieved much earlier than decompensation[9]. In the physical evaluation of the patient, BMI and visceral adiposity are helpful clues to the possible presence of NAFLD; however, in lean patients the diagnosis becomes much more challenging[9]. Screening of patients who are at risk for the development of NAFLD seems to be a worthy undertaking, but liver function tests can be in the normal range in patients with NAFLD/NASH and ultrasound is too expensive and burdensome for use in screening large portions of a population (although it is a good starting point when suspicion is high)[177]. The diagnosis of NAFLD is a four-pronged approach (Table 5): (1) hepatic steatosis (*via* imaging or histology); (2) alcohol consumption is ruled out; (3) there are no rival etiologies; and (4) no other causes for chronic liver disease are identified[177]. The entities discussed in the differential diagnosis section of this paper should be ruled out, namely, alcohol use, chronic hepatitis B and C, medication use, parenteral nutrition, Wilson's disease, biliary disease, autoimmune hepatitis, and malnutrition to name a few of the major considerations. Although mild elevations in serum ferritin can be seen in NAFLD, marked increases should be worked-up for hemochromatosis and *HFE* gene mutations (*i.e.,* C282Y)[177]. As mentioned previously, NAFLD patients may have elevations in serum autoantibodies; however, increased serum autoantibodies in the presence of features to suggest an autoimmune liver disease should result in a more complete work-up for autoimmune disease/autoimmune liver disease[177]. Biomarker development in NAFLD has been a topic of great interest and research. Numerous potential biomarkers have been investigated, for example, cytokeratin 18 fragments were evaluated in potential NAFLD patients at the time of liver biopsy and then correlated with histologic findings[178]. In this study, CK18 fragments found in the plasma showed a significant ($P < 0.001$) and marked increase in patients with NASH when compared with those having steatosis

Table 5 Factors to be assessed in the evaluation of a patient with suspected non-alcoholic fatty liver disease[32]

Factor
Personal and family history of diabetes, hypertension and CVD
Alcohol use: < 20 g/d (women), < 30 g/d (man)
Waist circumference, BMI, change in body weight
Hepatitis B/C infection
Liver enzymes
History of steatosis-associated drug use
Fast blood glucose, hemoglobin A1c
Serum total and HDL-cholesterol, triacylglycerol, uric acid
Undertaken due to clinical suspicion
Ultrasound
Hemochromatosis testing: Ferritin and transferrin saturation
Celiac disease: IgA and tissue transglutaminase
Thyroid disease: TSH level (T3/T4)
Polycystic ovarian syndrome
Wilson's disease: Ceruloplasmin
Autoimmune disease: ANA, AMA, SMA
Alpha-1 antitrypsin deficiency: Alpha-1-antitrypsin level

ANA: Anti-nuclear antibody; AMA: Anti-mitochondrial antibody; SMA: Anti-smooth muscle antibody; CVD: Cardiovascular disease; BMI: Body mass index; HDL: High density lipoprotein; TSH: Thyroid stimulating hormone.

or normal findings (median 765.7 U/L *vs* 202.4 U/L *vs* 215.5 U/L, respectively)[178]. These findings were further investigated by several subsequent studies and a meta-analysis revealed CK18 fragment levels to have a sensitivity and specificity of 78% and 87%, respectively, for steatohepatitis in those with NAFLD[179]. Other studies have offered insight into miRNAs as a biomarker for NAFLD and HCC spectrum; however, more investigation is needed to determine its true place in the diagnostic algorithm of NAFLD[180]. Extracellular vesicles shed from the liver have also caught the attention of many investigators and they are being actively researched for a possible role in NAFLD detection[181].

Perhaps the most important treatment option, lifestyle modification (to include diet and exercise), as well as surgical interventions for the treatment of NAFLD have already been discussed. Medications and supplements are also part of the treatment consideration when dealing with NAFLD. Hence, there are four main pathways currently available in the treatment of NAFLD. First, targeting hepatic fat accumulation (pioglitazone, elafibranor, saroglitazar), bile acid-farnesoid X receptor axis (obeticholic acid), *de novo* lipogenesis inhibitors (aramchol, NDI-010976), incretins (liraglutide) and fibroblast growth factor FGF-21 or FGF-19 analogues[182]. Second, oxidative stress alle*via*tion through the use of antioxidants and medications that target the tumor necrosis factor alpha pathway (emricasan, pentoxyifylline) as well as immune modulators (amlexanox, cenicriviroc)[182]. Third, antiobesity medications such as orlistat and finally antifibrotics (simtuzumab and GR-MD-02) will be important players in therapeutic management of NAFLD[182]. Insulin resistance, as a major player in the pathogenesis of NAFLD, is an obvious target of therapeutic intervention by way of insulin sensitizing agents[177]. With that being said, several studies have looked at the effects of metformin on liver function test levels and histology in those with NASH. In initial work, use of metformin showed a reduction in insulin resistance and aminotransferase levels; however, no changes were noted in the participants liver histology[183,184]. A meta-analysis found that in combination with lifestyle changes, metformin did not improve liver function test profiles or liver histology compared with lifestyle modification alone[177]. Although some evidence exists of NASH's histological improvement by metformin intervention (study confounded by weight loss), the current AASLD practice guideline recommendation is not to use metformin for the specific treatment of liver disease in adults with NASH[177,185]. The thiazolidinediones (TZDs), specifically pioglitazone, was shown in meta-analysis to improve steatosis and inflammation but not fibrosis with the caveat that TZDs long term safety profile is still under investigation[177]. The current recommendation, according to the AASLD Practice Guideline for NAFLD, Pioglitazone can be used in the treatment of steatohepatitis in those who have biopsy confirmed NASH with the understanding that trials were conducted in NASH patients without diabetes[177]. Vitamin E, an anti-oxidant, has been investigated for use in the treatment of NASH as oxidative stress is considered to be a major player in hepatocyte injury and disease progression[186,187]. Several studies have produced data to suggest that the use of vitamin E leads to improved steatosis, reduced inflammation and ballooning, decreased liver function test values, resolution of steatohepatitis with no effect on hepatic fibrosis[177]. However, concerns over the use of vitamin E and associated increases in all-cause mortality and an increased risk of prostate cancer in men have been raised[188,189]. As it currently stands, vitamin E should be considered in the therapeutic regimen of patients with biopsy proven NASH who also are non-diabetics[177]. Other therapies such as Pentoxifylline (shown to improve hepatic steatosis with no effect on insulin resistance), obeticholic acid (improves insulin resistance, hepatic steatosis, hepatic inflammation, and hepatic fibrosis), Orlistat (improves insulin resistance), ursodeoxycholic acid (improves insulin resistance and hepatic steatosis), Statins (improves hepatic steatosis), and Omega-3 (improves hepatic steatosis), and glucagon-like peptide 1 receptor agonists (improves hepatic steatosis) have been investigated and have shown varying and often limited benefit[190]. Finally, up and coming agents to be aware of: PPARα/δ agonists, chemokine receptor (CCR)2/CCR5 antagonists and numerous fatty acid/bile acid conjugates and antifibrotic agents are being investigated for use in NASH and the results of these studies/trials will reveal what benefit if any they will have on the NALFD landscape[32].

According to the most recent American College of Gastroenterology and American Gastroenterological Association guidelines, the screening of adults in primary care clinics or high-risk groups (*i.e.,* those attending diabetes or obesity clinics) for NAFLD is not recommended and the

systematic screening of family members for NAFLD is also discouraged[191]. This due to the lack of evidence or current understanding regarding the long-term benefits and cost effectiveness of screening and the current uncertainties related to diagnostic tests and treatment options[191]. However, other screening guidelines suggest the implementation of a screening policy in those who are at high risk for NAFLD identified by the presence of metabolic risk factors and/or IR[191].

CONCLUSION

NAFLD is a growing epidemic, not only in the United States, but worldwide in part due to obesity and insulin resistance leading to liver accumulation of triglycerides and free fatty acids. Liver steatosis may be innocuous in most occasions but the progression and development of fibrosis is not and often heralds a poor prognosis. Numerous risk factors for the development of NAFLD have been espoused with most having some form of metabolic derangement or insulin resistance at the core of its pathophysiology. Additionally, access and decreasing cost for high quality and powered genetic scrutiny will no doubt provide future clinicians with a great deal of information and opportunity for enhanced targeted treatment. The same can be said for the development of advanced imaging and biochemical tests. As it currently stands several medications/supplements may be used in the treatment of NAFLD; however, none seem to be the "magic bullet" in curtailing this growing problem. Not enough can be said about the importance of lifestyle coupled with proper diet and appropriate exercise in the defense of developing NAFLD.

REFERENCES

1 **Sayiner M**, Koenig A, Henry L, Younossi ZM. Epidemiology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis in the United States and the Rest of the World. *Clin Liver Dis* 2016; **20**: 205-214 [PMID: 27063264 DOI: 10.1016/j.cld.2015.10.001]

2 **Kanwar P**, Kowdley KV. The Metabolic Syndrome and Its Influence on Nonalcoholic Steatohepatitis. *Clin Liver Dis* 2016; **20**: 225-243 [PMID: 27063266 DOI: 10.1016/j.cld.2015.10.002]

3 **Calzadilla Bertot L**, Adams LA. The Natural Course of Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 2016; **17**: pii: E774 [PMID: 27213358 DOI: 10.3390/ijms17050774]

4 **Powell EE**, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990; **11**: 74-80 [PMID: 2295475]

5 **Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669 [PMID: 10051466 DOI: 10.1002/hep.510290347]

6 **Poonawala A**, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. *Hepatology* 2000; **32**: 689-692 [PMID: 11003611 DOI: 10.1053/jhep.2000.17894]

7 **Teli MR**, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. *Hepatology* 1995; **22**: 1714-1719 [PMID: 7489979]

8 **Younossi ZM**, Stepanova M, Negro F, Hallaji S, Younossi Y, Lam B, Srishord M. Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine* (Baltimore) 2012; **91**: 319-327 [PMID: 23117851 DOI: 10.1097/MD.0b013e3182779d49]

9 **Patel V**, Sanyal AJ, Sterling R. Clinical Presentation and Patient Evaluation in Nonalcoholic Fatty Liver Disease. *Clin Liver Dis* 2016; **20**: 277-292 [PMID: 27063269 DOI: 10.1016/j.cld.2015.10.006]

10 **Federico A**, Dallio M, Masarone M, Persico M, Loguercio C. The epidemiology of non-alcoholic fatty liver disease and its connection with cardiovascular disease: role of endothelial dysfunction. *Eur Rev Med Pharmacol Sci* 2016; **20**: 4731-4741 [PMID: 27906428]

11 **Lonardo A**, Bellentani S, Argo CK, Ballestri S, Byrne CD, Caldwell SH, Cortez-Pinto H, Grieco A, Machado MV, Miele L, Targher G. Epidemiological modifiers of non-alcoholic fatty liver disease: Focus on high-risk groups. *Dig Liver Dis* 2015; **47**: 997-1006 [PMID: 26454786 DOI: 10.1016/j.dld.2015.08.004]

12 **Sung KC**, Wild SH, Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. *J Hepatol* 2014; **60**: 1040-1045 [PMID: 24445219 DOI: 10.1016/j.jhep.2014.01.009]

13 **Kalia HS**, Gaglio PJ. The Prevalence and Pathobiology of Nonalcoholic Fatty Liver Disease in Patients of Different Races or Ethnicities. *Clin Liver Dis* 2016; **20**: 215-224 [PMID: 27063265 DOI: 10.1016/j.cld.2015.10.005]

14 **Mohanty SR**, Troy TN, Huo D, O'Brien BL, Jensen DM, Hart J. Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. *J Hepatol* 2009; **50**: 797-804 [PMID: 19231016 DOI: 10.1016/j.jhep.2008.11.017]

15 **Satapathy SK**, Sanyal AJ. Epidemiology and Natural History of Nonalcoholic Fatty Liver Disease. *Semin Liver Dis* 2015; **35**: 221-235 [PMID: 26378640 DOI: 10.1055/s-0035-1562943]

16 **Brea A**, Puzo J. Non-alcoholic fatty liver disease and cardiovascular risk. *Int J Cardiol* 2013; **167**: 1109-1117 [PMID: 23141876 DOI: 10.1016/j.ijcard.2012.09.085]

17 **Goyal NP**, Schwimmer JB. The Progression and Natural History of Pediatric Nonalcoholic Fatty Liver Disease. *Clin Liver Dis* 2016; **20**: 325-338 [PMID: 27063272 DOI: 10.1016/j.cld.2015.10.003]

18 **Brumbaugh DE**, Tearse P, Cree-Green M, Fenton LZ, Brown M, Scherzinger A, Reynolds R, Alston M, Hoffman C, Pan Z, Friedman JE, Barbour LA. Intrahepatic fat is increased in the neonatal offspring of obese women with gestational diabetes. *J Pediatr* 2013; **162**: 930-936.e1 [PMID: 23260099 DOI: 10.1016/j.jpeds.2012.11.017]

19 **Patel KR**, White FV, Deutsch GH. Hepatic steatosis is prevalent in stillborns delivered to women with diabetes mellitus. *J Pediatr Gastroenterol Nutr* 2015; **60**: 152-158 [PMID: 25079479 DOI: 10.1097/mpg.0000000000000520]

20 **Nobili V**, Bedogni G, Alisi A, Pietrobattista A, Alterio A, Tiribelli C, Agostoni C. A protective effect of breastfeeding on the progression of non-alcoholic fatty liver disease. *Arch Dis Child* 2009; **94**: 801-805 [PMID: 19556219 DOI: 10.1136/adc.2009.159566]

21 **Schwimmer JB**. Clinical advances in pediatric nonalcoholic fatty liver disease. *Hepatology* 2016; **63**: 1718-1725 [PMID: 27100147 DOI: 10.1002/hep.28441]

22 **Schwimmer JB**, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006; **118**: 1388-1393 [PMID: 17015527 DOI: 10.1542/peds.2006-1212]

23 **Nobili V**, Alisi A, Grimaldi C, Liccardo D, Francalanci P, Monti L, Castellano A, de Ville de Goyet J. Non-alcoholic fatty liver disease and hepatocellular carcinoma in a 7-year-old obese boy: coincidence or comorbidity? *Pediatr Obes* 2014; **9**: e99-e102 [PMID: 24302697 DOI: 10.1111/j.2047-6310.2013.00209.x]

24 **Molleston JP**, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am J Gastroenterol* 2002; **97**: 2460-2462 [PMID: 12358273 DOI: 10.1111/j.1572-0241.2002.06003.x]

25 **Andersen CJ**, Fernandez ML. Dietary strategies to reduce metabolic syndrome. *Rev Endocr Metab Disord* 2013; **14**: 241-254 [PMID: 23943309 DOI: 10.1007/s11154-013-9251-y]

26 **Godos J**, Federico A, Dallio M, Scazzina F. Mediterranean diet and nonalcoholic fatty liver disease: molecular mechanisms of

protection. *Int J Food Sci Nutr* 2017; **68**: 18-27 [PMID: 27484357]
27 **Hamabe A**, Uto H, Imamura Y, Kusano K, Mawatari S, Kumagai K, Kure T, Tamai T, Moriuchi A, Sakiyama T, Oketani M, Ido A, Tsubouchi H. Impact of cigarette smoking on onset of nonalcoholic fatty liver disease over a 10-year period. *J Gastroenterol* 2011; **46**: 769-778 [PMID: 21302121 DOI: 10.1007/s00535-011-0376-z]
28 **Targher G**, Alberiche M, Zenere MB, Bonadonna RC, Muggeo M, Bonora E. Cigarette smoking and insulin resistance in patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1997; **82**: 3619-3624 [PMID: 9360516 DOI: 10.1210/jcem.82.11.4351]
29 **Rönnemaa T**, Rönnemaa EM, Puukka P, Pyörälä K, Laakso M. Smoking is independently associated with high plasma insulin levels in nondiabetic men. *Diabetes Care* 1996; **19**: 1229-1232 [PMID: 8908385]
30 **Carnethon MR**, Fortmann SP, Palaniappan L, Duncan BB, Schmidt MI, Chambless LE. Risk factors for progression to incident hyperinsulinemia: the Atherosclerosis Risk in Communities Study, 1987-1998. *Am J Epidemiol* 2003; **158**: 1058-1067 [PMID: 14630601]
31 **Weitzman M**, Cook S, Auinger P, Florin TA, Daniels S, Nguyen M, Winickoff JP. Tobacco smoke exposure is associated with the metabolic syndrome in adolescents. *Circulation* 2005; **112**: 862-869 [PMID: 16061737 DOI: 10.1161/circulationaha.104.520650]
32 **Marchesini G**, Day ChP, Dufour JF, Canbay A, Nobili V, Ratziu V, Tilg H, Roden M, Gastaldelli A, Yki-Järvinen H, Schick F, Vettor R, Frühbeck G, Mathus-Vliegen L. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]
33 **Zelber-Sagi S**, Godos J, Salomone F. Lifestyle changes for the treatment of nonalcoholic fatty liver disease: a review of observational studies and intervention trials. *Therap Adv Gastroenterol* 2016; **9**: 392-407 [PMID: 27134667 DOI: 10.1177/1756283x16638830]
34 **Keating SE**, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2012; **57**: 157-166 [PMID: 22414768 DOI: 10.1016/j.jhep.2012.02.023]
35 **Hashida R**, Kawaguchi T, Bekki M, Omoto M, Matsuse H, Nago T, Takano Y, Ueno T, Koga H, George J, Shiba N, Torimura T. Aerobic vs. resistance exercise in non-alcoholic fatty liver disease: A systematic review. *J Hepatol* 2017; **66**: 142-152 [PMID: 27639843 DOI: 10.1016/j.jhep.2016.08.023]
36 **Macut D**, Tziomalos K, Božić-Antić I, Bjekić-Macut J, Katsikis I, Papadakis E, Andrić Z, Panidis D. Non-alcoholic fatty liver disease is associated with insulin resistance and lipid accumulation product in women with polycystic ovary syndrome. *Hum Reprod* 2016; **31**: 1347-1353 [PMID: 27076501 DOI: 10.1093/humrep/dew076]
37 **Kahal H**, Abouda G, Rigby AS, Coady AM, Kilpatrick ES, Atkin SL. Glucagon-like peptide-1 analogue, liraglutide, improves liver fibrosis markers in obese women with polycystic ovary syndrome and nonalcoholic fatty liver disease. *Clin Endocrinol* (Oxf) 2014; **81**: 523-528 [PMID: 24256515 DOI: 10.1111/cen.12369]
38 **Vassilatou E**, Lafoyianni S, Vryonidou A, Ioannidis D, Kosma L, Katsoulis K, Papavassiliou E, Tzavara I. Increased androgen bioavailability is associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome. *Hum Reprod* 2010; **25**: 212-220 [PMID: 19887498 DOI: 10.1093/humrep/dep380]
39 **Markou A**, Androulakis II, Mourmouris C, Tsikkini A, Samara C, Sougioultzis S, Piaditis G, Kaltsas G. Hepatic steatosis in young lean insulin resistant women with polycystic ovary syndrome. *Fertil Steril* 2010; **93**: 1220-1226 [PMID: 19171337 DOI: 10.1016/j.fertnstert.2008.12.008]
40 **Kauffman RP**, Baker TE, Baker V, Kauffman MM, Castracane VD. Endocrine factors associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome: do androgens play a role? *Gynecol Endocrinol* 2010; **26**: 39-46 [PMID: 20001571 DOI: 10.3109/09513590903184084]
41 **Paschetta E**, Belci P, Alisi A, Liccardo D, Cutrera R, Musso G, Nobili V. OSAS-related inflammatory mechanisms of liver injury in nonalcoholic fatty liver disease. *Mediators Inflamm* 2015; **2015**: 815721 [PMID: 25873773 DOI: 10.1155/2015/815721]
42 **Tanné F**, Gagnadoux F, Chazouillères O, Fleury B, Wendum D, Lasnier E, Lebeau B, Poupon R, Serfaty L. Chronic liver injury during obstructive sleep apnea. *Hepatology* 2005; **41**: 1290-1296 [PMID: 15915459 DOI: 10.1002/hep.20725]
43 **Campos GM**, Bambha K, Vittinghoff E, Rabl C, Posselt AM, Ciovica R, Tiwari U, Ferrel L, Pabst M, Bass NM, Merriman RB. A clinical scoring system for predicting nonalcoholic steatohepatitis in morbidly obese patients. *Hepatology* 2008; **47**: 1916-1923 [PMID: 18433022 DOI: 10.1002/hep.22241]
44 **Aron-Wisnewsky J**, Minville C, Tordjman J, Lévy P, Bouillot JL, Basdevant A, Bedossa P, Clément K, Pépin JL. Chronic intermittent hypoxia is a major trigger for non-alcoholic fatty liver disease in morbid obese. *J Hepatol* 2012; **56**: 225-233 [PMID: 21703181 DOI: 10.1016/j.jhep.2011.04.022]
45 **Mishra P**, Nugent C, Afendy A, Bai C, Bhatia P, Afendy M, Fang Y, Elariny H, Goodman Z, Younossi ZM. Apnoeic-hypopnoeic episodes during obstructive sleep apnoea are associated with histological nonalcoholic steatohepatitis. *Liver Int* 2008; **28**: 1080-1086 [PMID: 18647236 DOI: 10.1111/j.1478-3231.2008.01822.x]
46 **Daltro C**, Cotrim HP, Alves E, de Freitas LA, Araújo L, Boente L, Leal R, Portugal T. Nonalcoholic fatty liver disease associated with obstructive sleep apnea: just a coincidence? *Obes Surg* 2010; **20**: 1536-1543 [PMID: 20556538 DOI: 10.1007/s11695-010-0212-1]
47 **Kallwitz ER**, Herdegen J, Madura J, Jakate S, Cotler SJ. Liver enzymes and histology in obese patients with obstructive sleep apnea. *J Clin Gastroenterol* 2007; **41**: 918-921 [PMID: 18090161 DOI: 10.1097/01.mcg.0000225692.62121.55]
48 **Polotsky VY**, Patil SP, Savransky V, Laffan A, Fonti S, Frame LA, Steele KE, Schweizter MA, Clark JM, Torbenson MS, Schwartz AR. Obstructive sleep apnea, insulin resistance, and steatohepatitis in severe obesity. *Am J Respir Crit Care Med* 2009; **179**: 228-234 [PMID: 18990675 DOI: 10.1164/rccm.200804-608OC]
49 **Anstee QM**, Day CP. The Genetics of Nonalcoholic Fatty Liver Disease: Spotlight on PNPLA3 and TM6SF2. *Semin Liver Dis* 2015; **35**: 270-290 [PMID: 26378644 DOI: 10.1055/s-0035-1562947]
50 **Willner IR**, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001; **96**: 2957-2961 [PMID: 11693332 DOI: 10.1111/j.1572-0241.2001.04667.x]
51 **Struben VM**, Hespenheide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. *Am J Med* 2000; **108**: 9-13 [PMID: 11059435]
52 **Schwimmer JB**, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, Shiehmorteza M, Yokoo T, Chavez A, Middleton MS, Sirlin CB. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 1585-1592 [PMID: 19208353 DOI: 10.1053/j.gastro.2009.01.050]
53 **Makkonen J**, Pietiläinen KH, Rissanen A, Kaprio J, Yki-Järvinen H. Genetic factors contribute to variation in serum alanine aminotransferase activity independent of obesity and alcohol: a study in monozygotic and dizygotic twins. *J Hepatol* 2009; **50**: 1035-1042 [PMID: 19303161 DOI: 10.1016/j.jhep.2008.12.025]
54 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]
55 **Browning JD**, Kumar KS, Saboorian MH, Thiele DL. Ethnic differences in the prevalence of cryptogenic cirrhosis. *Am J Gastroenterol* 2004; **99**: 292-298 [PMID: 15046220]
56 **Guerrero R**, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: an insulin resistance paradox? *Hepatology* 2009; **49**: 791-801 [PMID: 19105205 DOI: 10.1002/hep.22726]
57 **Bambha K**, Belt P, Abraham M, Wilson LA, Pabst M, Ferrell L, Unalp-Arida A, Bass N. Ethnicity and nonalcoholic fatty liver

disease. *Hepatology* 2012; **55**: 769-780 [PMID: 21987488 DOI: 10.1002/hep.24726]
58 **Ravi Kanth VV**, Sasikala M, Sharma M, Rao PN, Reddy DN. Genetics of non-alcoholic fatty liver disease: From susceptibility and nutrient interactions to management. *World J Hepatol* 2016; **8**: 827-837 [PMID: 27458502 DOI: 10.4254/wjh.v8.i20.827]
59 **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]
60 **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, Okanoue T. 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]
61 **Akuta N**, Kawamura Y, Arase Y, Suzuki F, Sezaki H, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Suzuki Y, Ikeda K, Kumada H. Relationships between Genetic Variations of PNPLA3, TM6SF2 and Histological Features of Nonalcoholic Fatty Liver Disease in Japan. *Gut Liver* 2016; **10**: 437-445 [PMID: 26610348 DOI: 10.5009/gnl15163]
62 **Kanth VV**, Sasikala M, Rao PN, Steffie Avanthi U, Rao KR, Nageshwar Reddy D. Pooled genetic analysis in ultrasound measured non-alcoholic fatty liver disease in Indian subjects: A pilot study. *World J Hepatol* 2014; **6**: 435-442 [PMID: 25018854 DOI: 10.4254/wjh.v6.i6.435]
63 **Bhatt SP**, Nigam P, Misra A, Guleria R, Pandey RM, Pasha MA. Genetic variation in the patatin-like phospholipase domain-containing protein-3 (PNPLA-3) gene in Asian Indians with nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2013; **11**: 329-335 [PMID: 23734760 DOI: 10.1089/met.2012.0064]
64 **Zhang Y**, Cai W, Song J, Miao L, Zhang B, Xu Q, Zhang L, Yao H. Association between the PNPLA3 I148M polymorphism and non-alcoholic fatty liver disease in the Uygur and Han ethnic groups of northwestern China. *PLoS One* 2014; **9**: e108381 [PMID: 25290313 DOI: 10.1371/journal.pone.0108381]
65 **Peng XE**, Wu YL, Lin SW, Lu QQ, Hu ZJ, Lin X. Genetic variants in PNPLA3 and risk of non-alcoholic fatty liver disease in a Han Chinese population. *PLoS One* 2012; **7**: e50256 [PMID: 23226254 DOI: 10.1371/journal.pone.0050256]
66 **Singal AG**, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, Waljee AK. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 325-334 [PMID: 24445574 DOI: 10.1038/ajg.2013.476]
67 **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]
68 **Xu R**, Tao A, Zhang S, Deng Y, Chen G. Association between patatin-like phospholipase domain containing 3 gene (PNPLA3) polymorphisms and nonalcoholic fatty liver disease: a HuGE review and meta-analysis. *Sci Rep* 2015; **5**: 9284 [PMID: 25791171 DOI: 10.1038/srep09284]
69 **Chalasani N**, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, Cui J, Taylor KD, Wilson L, Cummings OW, Chen YD, Rotter JI. Genome-wide association study identifies variants associated with histologic features of nonalcoholic Fatty liver disease. *Gastroenterology* 2010; **139**: 1567-1576, 1576.e1-6 [PMID: 20708005 DOI: 10.1053/j.gastro.2010.07.057]
70 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328 DOI: 10.1038/ng.2901]
71 **Mahdessian H**, Taxiarchis A, Popov S, Silveira A, Franco-Cereceda A, Hamsten A, Eriksson P, van't Hooft F. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *Proc Natl Acad Sci USA* 2014; **111**: 8913-8918 [PMID: 24927523 DOI: 10.1073/pnas.1323785111]
72 **Khatib MN**, Gaidhane S, Gaidhane AM, Simkhada P, Zahiruddin QS. Ghrelin O Acyl Transferase (GOAT) as a Novel Metabolic Regulatory Enzyme. *J Clin Diagn Res* 2015; **9**: LE01-LE05 [PMID: 25859472 DOI: 10.7860/JCDR/2015/9787.5514]
73 **Townsend SA**, Newsome PN. Non-alcoholic fatty liver disease in 2016. *Br Med Bull* 2016; **119**: 143-156 [PMID: 27543499 DOI: 10.1093/bmb/ldw031]
74 **Buzzetti E**, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016; **65**: 1038-1048 [PMID: 26823198 DOI: 10.1016/j.metabol.2015.12.012]
75 **Peverill W**, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *Int J Mol Sci* 2014; **15**: 8591-8638 [PMID: 24830559 DOI: 10.3390/ijms15058591]
76 **Petta S**, Valenti L, Bugianesi E, Targher G, Bellentani S, Bonino F. A "systems medicine" approach to the study of non-alcoholic fatty liver disease. *Dig Liver Dis* 2016; **48**: 333-342 [PMID: 26698409 DOI: 10.1016/j.dld.2015.10.027]
77 **Bugianesi E**, Moscatiello S, Ciaravella MF, Marchesini G. Insulin resistance in nonalcoholic fatty liver disease. *Curr Pharm Des* 2010; **16**: 1941-1951 [PMID: 20370677]
78 **Guilherme A**, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 2008; **9**: 367-377 [PMID: 18401346 DOI: 10.1038/nrm2391]
79 **Cusi K**. Role of insulin resistance and lipotoxicity in non-alcoholic steatohepatitis. *Clin Liver Dis* 2009; **13**: 545-563 [PMID: 19818304 DOI: 10.1016/j.cld.2009.07.009]
80 **Federico A**, Dallio M, Godos J, Loguercio C, Salomone F. Targeting gut-liver axis for the treatment of nonalcoholic steatohepatitis: translational and clinical evidence. *Transl Res* 2016; **167**: 116-124 [PMID: 26318867 DOI: 10.1016/j.trsl.2015.08.002]
81 **Watt KD**, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study. *Am J Transplant* 2010; **10**: 1420-1427 [PMID: 20486907 DOI: 10.1111/j.1600-6143.2010.03126.x]
82 **Bhagat V**, Mindikoglu AL, Nudo CG, Schiff ER, Tzakis A, Regev A. Outcomes of liver transplantation in patients with cirrhosis due to nonalcoholic steatohepatitis versus patients with cirrhosis due to alcoholic liver disease. *Liver Transpl* 2009; **15**: 1814-1820 [PMID: 19938128 DOI: 10.1002/lt.21927]
83 **Richards J**, Gunson B, Johnson J, Neuberger J. Weight gain and obesity after liver transplantation. *Transpl Int* 2005; **18**: 461-466 [PMID: 15773968 DOI: 10.1111/j.1432-2277.2004.00067.x]
84 **Lane JT**, Dagogo-Jack S. Approach to the patient with new-onset diabetes after transplant (NODAT). *J Clin Endocrinol Metab* 2011; **96**: 3289-3297 [PMID: 22058376 DOI: 10.1210/jc.2011-0657]
85 **Gitto S**, Villa E. Non-Alcoholic Fatty Liver Disease and Metabolic Syndrome after Liver Transplant. *Int J Mol Sci* 2016; **17**: 490 [PMID: 27049380 DOI: 10.3390/ijms17040490]
86 **Sprinzl MF**, Weinmann A, Lohse N, Tönissen H, Koch S, Schattenberg J, Hoppe-Lotichius M, Zimmermann T, Galle PR, Hansen T, Otto G, Schuchmann M. Metabolic syndrome and its association with fatty liver disease after orthotopic liver transplantation. *Transpl Int* 2013; **26**: 67-74 [PMID: 23126674 DOI: 10.1111/j.1432-2277.2012.01576.x]
87 **Mikolasevic I**, Orlic L, Hrstic I, Milic S. Metabolic syndrome and non-alcoholic fatty liver disease after liver or kidney transplantation. *Hepatol Res* 2016; **46**: 841-852 [PMID: 26713425 DOI: 10.1111/hepr.12642]
88 **Sanyal AJ**, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE, Ratziu V, McCullough A. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology* 2011; **54**:

344-353 [PMID: 21520200 DOI: 10.1002/hep.24376]
89 **Yeh MM**, Brunt EM. Pathological features of fatty liver disease. *Gastroenterology* 2014; **147**: 754-764 [PMID: 25109884 DOI: 10.1053/j.gastro.2014.07.056]
90 **Bedossa P**. Histological Assessment of NAFLD. *Dig Dis Sci* 2016; **61**: 1348-1355 [PMID: 26874689 DOI: 10.1007/s10620-016-4062-0]
91 **Burt AD**, Lackner C, Tiniakos DG. Diagnosis and Assessment of NAFLD: Definitions and Histopathological Classification. *Semin Liver Dis* 2015; **35**: 207-220 [PMID: 26378639 DOI: 10.1055/s-0035-1562942]
92 **Yip WW**, Burt AD. Alcoholic liver disease. *Semin Diagn Pathol* 2006; **23**: 149-160 [PMID: 17355088 DOI: 10.1053/j.semdp.2006.11.002]
93 **Sahini N**, Borlak J. Recent insights into the molecular pathophysiology of lipid droplet formation in hepatocytes. *Prog Lipid Res* 2014; **54**: 86-112 [PMID: 24607340 DOI: 10.1016/j.plipres.2014.02.002]
94 **Smagris E**, BasuRay S, Li J, Huang Y, Lai KM, Gromada J, Cohen JC, Hobbs HH. Pnpla3I148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. *Hepatology* 2015; **61**: 108-118 [PMID: 24917523 DOI: 10.1002/hep.27242]
95 **Singhi AD**, Jain D, Kakar S, Wu TT, Yeh MM, Torbenson M. Reticulin loss in benign fatty liver: an important diagnostic pitfall when considering a diagnosis of hepatocellular carcinoma. *Am J Surg Pathol* 2012; **36**: 710-715 [PMID: 22498821 DOI: 10.1097/PAS.0b013e3182495c73]
96 **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]
97 **Hall AR**, Dhillon AP, Green AC, Ferrell L, Crawford JM, Alves V, Balabaud C, Bhathal P, Bioulac-Sage P, Guido M, Hytiroglou P, Nakanuma Y, Paradis V, Quaglia A, Snover D, Theise N, Thung S, Tsui W, van Leeuwen DJ. Hepatic steatosis estimated microscopically versus digital image analysis. *Liver Int* 2013; **33**: 926-935 [PMID: 23560780 DOI: 10.1111/liv.12162]
98 **Karlas T**, Petroff D, Garnov N, Böhm S, Tenckhoff H, Wittekind C, Wiese M, Schiefke I, Linder N, Schaudinn A, Busse H, Kahn T, Mössner J, Berg T, Tröltzsch M, Keim V, Wiegand J. Non-invasive assessment of hepatic steatosis in patients with NAFLD using controlled attenuation parameter and 1H-MR spectroscopy. *PLoS One* 2014; **9**: e91987 [PMID: 24637477 DOI: 10.1371/journal.pone.0091987]
99 **Schwimmer JB**, Middleton MS, Behling C, Newton KP, Awai HI, Paiz MN, Lam J, Hooker JC, Hamilton G, Fontanesi J, Sirlin CB. Magnetic resonance imaging and liver histology as biomarkers of hepatic steatosis in children with nonalcoholic fatty liver disease. *Hepatology* 2015; **61**: 1887-1895 [PMID: 25529941 DOI: 10.1002/hep.27666]
100 **Harmon RC**, Tiniakos DG, Argo CK. Inflammation in nonalcoholic steatohepatitis. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 189-200 [PMID: 21476914 DOI: 10.1586/egh.11.21]
101 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219 [PMID: 12717402 DOI: 10.1053/jhep.2003.50193]
102 **Pais R**, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, Ratziu V. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. *J Hepatol* 2013; **59**: 550-556 [PMID: 23665288 DOI: 10.1016/j.jhep.2013.04.027]
103 **McPherson S**, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. *J Hepatol* 2015; **62**: 1148-1155 [PMID: 25477264 DOI: 10.1016/j.jhep.2014.11.034]
104 **Matsuda Y**, Takada A, Kanayama R, Takase S. Changes of hepatic microtubules and secretory proteins in human alcoholic liver disease. *Pharmacol Biochem Behav* 1983; **18** Suppl 1: 479-482 [PMID: 6634857]
105 **Schaff Z**, Lapis K. Fine structure of hepatocytes during the etiology of several common pathologies. *J Electron Microsc Tech* 1990; **14**: 179-207 [PMID: 2187062 DOI: 10.1002/jemt.1060140302]
106 **Gores GJ**, Herman B, Lemasters JJ. Plasma membrane bleb formation and rupture: a common feature of hepatocellular injury. *Hepatology* 1990; **11**: 690-698 [PMID: 2184116]
107 **Caldwell S**, Ikura Y, Dias D, Isomoto K, Yabu A, Moskaluk C, Pramoonjago P, Simmons W, Scruggs H, Rosenbaum N, Wilkinson T, Toms P, Argo CK, Al-Osaimi AM, Redick JA. Hepatocellular ballooning in NASH. *J Hepatol* 2010; **53**: 719-723 [PMID: 20624660 DOI: 10.1016/j.jhep.2010.04.031]
108 **Lackner C**, Gogg-Kamerer M, Zatloukal K, Stumptner C, Brunt EM, Denk H. Ballooned hepatocytes in steatohepatitis: the value of keratin immunohistochemistry for diagnosis. *J Hepatol* 2008; **48**: 821-828 [PMID: 18329127 DOI: 10.1016/j.jhep.2008.01.026]
109 **Kleiner DE**, Makhlouf HR. Histology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis in Adults and Children. *Clin Liver Dis* 2016; **20**: 293-312 [PMID: 27063270 DOI: 10.1016/j.cld.2015.10.011]
110 **Mann CD**, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. *Eur J Cancer* 2007; **43**: 979-992 [PMID: 17291746 DOI: 10.1016/j.ejca.2007.01.004]
111 **Gomaa AI**, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. *World J Gastroenterol* 2008; **14**: 4300-4308 [PMID: 18666317 DOI: 10.3748/wjg.14.4300]
112 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140 [PMID: 12105842 DOI: 10.1053/gast.2002.34168]
113 **Siegel AB**, Zhu AX. Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. *Cancer* 2009; **115**: 5651-5661 [PMID: 19834957 DOI: 10.1002/cncr.24687]
114 **Guzman G**, Brunt EM, Petrovic LM, Chejfec G, Layden TJ, Cotler SJ. Does nonalcoholic fatty liver disease predispose patients to hepatocellular carcinoma in the absence of cirrhosis? *Arch Pathol Lab Med* 2008; **132**: 1761-1766 [PMID: 18976012 DOI: 10.1043/1543-2165-132.11.1761]
115 **Takuma Y**, Nouso K. Nonalcoholic steatohepatitis-associated hepatocellular carcinoma: our case series and literature review. *World J Gastroenterol* 2010; **16**: 1436-1441 [PMID: 20333782 DOI: 10.3748/wjg.v16.i12.1436]
116 **Salomao M**, Remotti H, Vaughan R, Siegel AB, Lefkowitch JH, Moreira RK. The steatohepatitic variant of hepatocellular carcinoma and its association with underlying steatohepatitis. *Hum Pathol* 2012; **43**: 737-746 [PMID: 22018903 DOI: 10.1016/j.humpath.2011.07.005]
117 **Salomao M**, Yu WM, Brown RS, Emond JC, Lefkowitch JH. Steatohepatitic hepatocellular carcinoma (SH-HCC): a distinctive histological variant of HCC in hepatitis C virus-related cirrhosis with associated NAFLD/NASH. *Am J Surg Pathol* 2010; **34**: 1630-1636 [PMID: 20975341 DOI: 10.1097/PAS.0b013e3181f31caa]
118 **Jain D**, Nayak NC, Kumaran V, Saigal S. Steatohepatitic hepatocellular carcinoma, a morphologic indicator of associated metabolic risk factors: a study from India. *Arch Pathol Lab Med* 2013; **137**: 961-966 [PMID: 23808468 DOI: 10.5858/arpa.2012-0048-OA]
119 **Yeh MM**, Liu Y, Torbenson M. Steatohepatitic variant of hepatocellular carcinoma in the absence of metabolic syndrome or background steatosis: a clinical, pathological, and genetic study. *Hum Pathol* 2015; **46**: 1769-1775 [PMID: 26410018 DOI: 10.1016/j.humpath.2015.07.018]
120 **Schwimmer JB**, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, Lavine JE. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 641-649 [PMID: 16116629 DOI: 10.1002/hep.20842]
121 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ,

Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

122 **Bedossa P**. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. *Hepatology* 2014; **60**: 565-575 [PMID: 24753132 DOI: 10.1002/hep.27173]

123 **Younossi ZM**, Stepanova M, Rafiq N, Makhlouf H, Younoszai Z, Agrawal R, Goodman Z. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. *Hepatology* 2011; **53**: 1874-1882 [PMID: 21360720 DOI: 10.1002/hep.24268]

124 **Bedossa P**, Poitou C, Veyrie N, Bouillot JL, Basdevant A, Paradis V, Tordjman J, Clement K. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology* 2012; **56**: 1751-1759 [PMID: 22707395 DOI: 10.1002/hep.25889]

125 **Hjelkrem M**, Stauch C, Shaw J, Harrison SA. Validation of the non-alcoholic fatty liver disease activity score. *Aliment Pharmacol Ther* 2011; **34**: 214-218 [PMID: 21585409 DOI: 10.1111/j.1365-2036.2011.04695.x]

126 **Pawlik TM**, Olino K, Gleisner AL, Torbenson M, Schulick R, Choti MA. Preoperative chemotherapy for colorectal liver metastases: impact on hepatic histology and postoperative outcome. *J Gastrointest Surg* 2007; **11**: 860-868 [PMID: 17492335 DOI: 10.1007/s11605-007-0149-4]

127 **Zorzi D**, Laurent A, Pawlik TM, Lauwers GY, Vauthey JN, Abdalla EK. Chemotherapy-associated hepatotoxicity and surgery for colorectal liver metastases. *Br J Surg* 2007; **94**: 274-286 [PMID: 17315288 DOI: 10.1002/bjs.5719]

128 **Gentilucci UV**, Santini D, Vincenzi B, Fiori E, Picardi A, Tonini G. Chemotherapy-induced steatohepatitis in colorectal cancer patients. *J Clin Oncol* 2006; **24**: 5467; author reply 5467-5468 [PMID: 17135651 DOI: 10.1200/jco.2006.08.1828]

129 **Sakhuja P**. Pathology of alcoholic liver disease, can it be differentiated from nonalcoholic steatohepatitis? *World J Gastroenterol* 2014; **20**: 16474-16479 [PMID: 25469015 DOI: 10.3748/wjg.v20.i44.16474]

130 **O'Shea RS**, Dasarathy S, McCullough AJ. Alcoholic liver disease. *Am J Gastroenterol* 2010; **105**: 14-32; quiz 33 [PMID: 19904248 DOI: 10.1038/ajg.2009.593]

131 **Bahirwani R**, Reddy KR. Drug-induced liver injury due to cancer chemotherapeutic agents. *Semin Liver Dis* 2014; **34**: 162-171 [PMID: 24879981 DOI: 10.1055/s-0034-1375957]

132 **Lee WM**. Drug-induced hepatotoxicity. *N Engl J Med* 1995; **333**: 1118-1127 [PMID: 7565951 DOI: 10.1056/nejm199510263331706]

133 **Amacher DE**, Chalasani N. Drug-induced hepatic steatosis. *Semin Liver Dis* 2014; **34**: 205-214 [PMID: 24879984 DOI: 10.1055/s-0034-1375960]

134 **Schumacher JD**, Guo GL. Mechanistic review of drug-induced steatohepatitis. *Toxicol Appl Pharmacol* 2015; **289**: 40-47 [PMID: 26344000 DOI: 10.1016/j.taap.2015.08.022]

135 **Thomopoulos KC**, Arvaniti V, Tsamantas AC, Dimitropoulou D, Gogos CA, Siagris D, Theocharis GJ, Labropoulou-Karatza C. Prevalence of liver steatosis in patients with chronic hepatitis B: a study of associated factors and of relationship with fibrosis. *Eur J Gastroenterol Hepatol* 2006; **18**: 233-237 [PMID: 16462535]

136 **Machado MV**, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. *J Gastroenterol Hepatol* 2011; **26**: 1361-1367 [PMID: 21649726 DOI: 10.1111/j.1440-1746.2011.06801.x]

137 **Lonardo A**, Adinolfi LE, Restivo L, Ballestri S, Romagnoli D, Baldelli E, Nascimbeni F, Loria P. Pathogenesis and significance of hepatitis C virus steatosis: an update on survival strategy of a successful pathogen. *World J Gastroenterol* 2014; **20**: 7089-7103 [PMID: 24966582 DOI: 10.3748/wjg.v20.i23.7089]

138 **Vallet-Pichard A**, Mallet V, Pol S. Nonalcoholic fatty liver disease and HIV infection. *Semin Liver Dis* 2012; **32**: 158-166 [PMID: 22760655 DOI: 10.1055/s-0032-1316471]

139 **Adams LA**, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2004; **99**: 1316-1320 [PMID: 15233671 DOI: 10.1111/j.1572-0241.2004.30444.x]

140 **Yatsuji S**, Hashimoto E, Kaneda H, Taniai M, Tokushige K, Shiratori K. Diagnosing autoimmune hepatitis in nonalcoholic fatty liver disease: is the International Autoimmune Hepatitis Group scoring system useful? *J Gastroenterol* 2005; **40**: 1130-1138 [PMID: 16378177 DOI: 10.1007/s00535-005-1711-z]

141 **Híndi M**, Levy C, Couto CA, Bejarano P, Mendes F. Primary biliary cirrhosis is more severe in overweight patients. *J Clin Gastroenterol* 2013; **47**: e28-e32 [PMID: 23059407 DOI: 10.1097/MCG.0b013e318261e659]

142 **Haga Y**, Kanda T, Sasaki R, Nakamura M, Nakamoto S, Yokosuka O. Nonalcoholic fatty liver disease and hepatic cirrhosis: Comparison with viral hepatitis-associated steatosis. *World J Gastroenterol* 2015; **21**: 12989-12995 [PMID: 26675364 DOI: 10.3748/wjg.v21.i46.12989]

143 **Jackel-Cram C**, Babiuk LA, Liu Q. Up-regulation of fatty acid synthase promoter by hepatitis C virus core protein: genotype-3a core has a stronger effect than genotype-1b core. *J Hepatol* 2007; **46**: 999-1008 [PMID: 17188392 DOI: 10.1016/j.jhep.2006.10.019]

144 **Kim JY**, Song EH, Lee HJ, Oh YK, Choi KH, Yu DY, Park SI, Seong JK, Kim WH. HBx-induced hepatic steatosis and apoptosis are regulated by TNFR1- and NF-kappaB-dependent pathways. *J Mol Biol* 2010; **397**: 917-931 [PMID: 20156456 DOI: 10.1016/j.jmb.2010.02.016]

145 **Wong VW**, Wong GL, Choi PC, Chan AW, Li MK, Chan HY, Chim AM, Yu J, Sung JJ, Chan HL. Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. *Gut* 2010; **59**: 969-974 [PMID: 20581244 DOI: 10.1136/gut.2009.205088]

146 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

147 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]

148 **Evans CD**, Oien KA, MacSween RN, Mills PR. Non-alcoholic steatohepatitis: a common cause of progressive chronic liver injury? *J Clin Pathol* 2002; **55**: 689-692 [PMID: 12195000]

149 **Fassio E**, Alvarez E, Domínguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. *Hepatology* 2004; **40**: 820-826 [PMID: 15382171 DOI: 10.1002/hep.20410]

150 **Harrison SA**, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. *Am J Gastroenterol* 2003; **98**: 2042-2047 [PMID: 14499785 DOI: 10.1111/j.1572-0241.2003.07659.x]

151 **Hui AY**, Wong VW, Chan HL, Liew CT, Chan JL, Chan FK, Sung JJ. Histological progression of non-alcoholic fatty liver disease in Chinese patients. *Aliment Pharmacol Ther* 2005; **21**: 407-413 [PMID: 15709991 DOI: 10.1111/j.1365-2036.2005.02334.x]

152 **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]

153 **Singal AK**, Guturu P, Hmoud B, Kuo YF, Salameh H, Wiesner RH. Evolving frequency and outcomes of liver transplantation based on etiology of liver disease. *Transplantation* 2013; **95**: 755-760 [PMID: 23370710 DOI: 10.1097/TP.0b013e31827afb3a]

154 **Agopian VG**, Kaldas FM, Hong JC, Whittaker M, Holt C, Rana A, Zarrinpar A, Petrowsky H, Farmer D, Yersiz H, Xia V, Hiatt JR,

Busuttil RW. Liver transplantation for nonalcoholic steatohepatitis: the new epidemic. *Ann Surg* 2012; **256**: 624-633 [PMID: 22964732 DOI: 10.1097/SLA.0b013e31826b4b7e]

155 **Yang JD**, Abdelmalek MF, Pang H, Guy CD, Smith AD, Diehl AM, Suzuki A. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. *Hepatology* 2014; **59**: 1406-1414 [PMID: 24123276 DOI: 10.1002/hep.26761]

156 **Klair JS**, Yang JD, Abdelmalek MF, Guy CD, Gill RM, Yates K, Unalp-Arida A, Lavine JE, Clark JM, Diehl AM, Suzuki A. A longer duration of estrogen deficiency increases fibrosis risk among postmenopausal women with nonalcoholic fatty liver disease. *Hepatology* 2016; **64**: 85-91 [PMID: 26919573 DOI: 10.1002/hep.28514]

157 **Lomonaco R**, Ortiz-Lopez C, Orsak B, Finch J, Webb A, Bril F, Louden C, Tio F, Cusi K. Role of ethnicity in overweight and obese patients with nonalcoholic steatohepatitis. *Hepatology* 2011; **54**: 837-845 [PMID: 21674556 DOI: 10.1002/hep.24483]

158 **Liu YL**, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, Allison ME, Alexander GJ, Piguet AC, Anty R, Donaldson P, Aithal GP, Francque S, Van Gaal L, Clement K, Ratziu V, Dufour JF, Day CP, Daly AK, Anstee QM. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014; **5**: 4309 [PMID: 24978903 DOI: 10.1038/ncomms5309]

159 **Valenti L**, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, Nobili V, Mozzi E, Roviaro G, Vanni E, Bugianesi E, Maggioni M, Fracanzani AL, Fargion S, Day CP. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 1209-1217 [PMID: 20373368 DOI: 10.1002/hep.23622]

160 **Hossain N**, Afendy A, Stepanova M, Nader F, Srishord M, Rafiq N, Goodman Z, Younossi Z. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1224-1229, 1229.e1-2 [PMID: 19559819 DOI: 10.1016/j.cgh.2009.06.007]

161 **Singh S**, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015; **13**: 643-654.e1-9; quiz e39-40 [PMID: 24768810 DOI: 10.1016/j.cgh.2014.04.014]

162 **de Freitas AC**, Campos AC, Coelho JC. The impact of bariatric surgery on nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care* 2008; **11**: 267-274 [PMID: 18403923 DOI: 10.1097/MCO.0b013e3282fbd33f]

163 **Verna EC**, Berk PD. Role of fatty acids in the pathogenesis of obesity and fatty liver: impact of bariatric surgery. *Semin Liver Dis* 2008; **28**: 407-426 [PMID: 18956297 DOI: 10.1055/s-0028-1091985]

164 **Mathurin P**, Hollebecque A, Arnalsteen L, Buob D, Leteurtre E, Caiazzo R, Pigeyre M, Verkindt H, Dharancy S, Louvet A, Romon M, Pattou F. Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. *Gastroenterology* 2009; **137**: 532-540 [PMID: 19409898 DOI: 10.1053/j.gastro.2009.04.052]

165 **Grimm IS**, Schindler W, Haluszka O. Steatohepatitis and fatal hepatic failure after biliopancreatic diversion. *Am J Gastroenterol* 1992; **87**: 775-779 [PMID: 1590319]

166 **Rabl C**, Campos GM. The impact of bariatric surgery on nonalcoholic steatohepatitis. *Semin Liver Dis* 2012; **32**: 80-91 [PMID: 22418890 DOI: 10.1055/s-0032-1306428]

167 **Wieser V**, Adolph TE, Enrich B, Moser P, Moschen AR, Tilg H. Weight loss induced by bariatric surgery restores adipose tissue PNPLA3 expression. *Liver Int* 2017; **37**: 299-306 [PMID: 27514759 DOI: 10.1111/liv.13222]

168 **Vilar-Gomez E**, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, Diago M, Romero-Gomez M. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* 2015; **149**: 367-378.e5; quiz e14-5 [PMID: 25865049 DOI: 10.1053/j.gastro.2015.04.005]

169 **Cho JY**, Chung TH, Lim KM, Park HJ, Jang JM. The impact of weight changes on nonalcoholic Fatty liver disease in adult men with normal weight. *Korean J Fam Med* 2014; **35**: 243-250 [PMID: 25309705 DOI: 10.4082/kjfm.2014.35.5.243]

170 **You DM**, Volk CG, Philo L, Partridge BJ. Weight loss outcomes after liver biopsy in patients with nonalcoholic fatty liver disease. *Dig Liver Dis* 2014; **46**: 1136-1137 [PMID: 25241133 DOI: 10.1016/j.dld.2014.08.042]

171 **Dyson J**, Jaques B, Chattopadyhay D, Lochan R, Graham J, Das D, Aslam T, Patanwala I, Gaggar S, Cole M, Sumpter K, Stewart S, Rose J, Hudson M, Manas D, Reeves HL. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014; **60**: 110-117 [PMID: 23978719 DOI: 10.1016/j.jhep.2013.08.011]

172 **Park EJ**, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Osterreicher CH, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010; **140**: 197-208 [PMID: 20141834 DOI: 10.1016/j.cell.2009.12.052]

173 **Zámbó V**, Simon-Szabó L, Szelényi P, Kereszturi E, Bánhegyi G, Csala M. Lipotoxicity in the liver. *World J Hepatol* 2013; **5**: 550-557 [PMID: 24179614 DOI: 10.4254/wjh.v5.i10.550]

174 **Mittal S**, Sada YH, El-Serag HB, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA, Davila JA. Temporal trends of nonalcoholic fatty liver disease-related hepatocellular carcinoma in the veteran affairs population. *Clin Gastroenterol Hepatol* 2015; **13**: 594-601.e1 [PMID: 25148760 DOI: 10.1016/j.cgh.2014.08.013]

175 **Kawamura Y**, Arase Y, Ikeda K, Seko Y, Imai N, Hosaka T, Kobayashi M, Saitoh S, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Ohmoto Y, Amakawa K, Tsuji H, Kumada H. Large-scale long-term follow-up study of Japanese patients with non-alcoholic Fatty liver disease for the onset of hepatocellular carcinoma. *Am J Gastroenterol* 2012; **107**: 253-261 [PMID: 22008893 DOI: 10.1038/ajg.2011.327]

176 **Yatsuji S**, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J Gastroenterol Hepatol* 2009; **24**: 248-254 [PMID: 19032450 DOI: 10.1111/j.1440-1746.2008.05640.x]

177 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

178 **Wieckowska A**, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology* 2006; **44**: 27-33 [PMID: 16799979 DOI: 10.1002/hep.21223]

179 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

180 **Afonso MB**, Rodrigues PM, Simão AL, Castro RE. Circulating microRNAs as Potential Biomarkers in Non-Alcoholic Fatty Liver Disease and Hepatocellular Carcinoma. *J Clin Med* 2016; **5**: pii: E30 [PMID: 26950158 DOI: 10.3390/jcm5030030]

181 **Ban LA**, Shackel NA, McLennan SV. Extracellular Vesicles: A New Frontier in Biomarker Discovery for Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 2016; **17**: 376 [PMID: 26985892 DOI: 10.3390/ijms17030376]

182 **Rotman Y**, Sanyal AJ. Current and upcoming pharmacotherapy for non-alcoholic fatty liver disease. *Gut* 2017; **66**: 180-190 [PMID: 27646933 DOI: 10.1136/gutjnl-2016-312431]

183 **Uygun A**, Kadayifci A, Isik AT, Ozgurtas T, Deveci S, Tuzun A,

Yesilova Z, Gulsen M, Dagalp K. Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2004; **19**: 537-544 [PMID: 14987322]

184 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. *Lancet* 2001; **358**: 893-894 [PMID: 11567710]

185 **Loomba R**, Lutchman G, Kleiner DE, Ricks M, Feld JJ, Borg BB, Modi A, Nagabhyru P, Sumner AE, Liang TJ, Hoofnagle JH. Clinical trial: pilot study of metformin for the treatment of non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2009; **29**: 172-182 [PMID: 18945255 DOI: 10.1111/j.1365-2036.2008.03869.x]

186 **Harrison SA**, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2003; **98**: 2485-2490 [PMID: 14638353 DOI: 10.1111/j.1572-0241.2003.08699.x]

187 **Dufour JF**, Oneta CM, Gonvers JJ, Bihl F, Cerny A, Cereda JM, Zala JF, Helbling B, Steuerwald M, Zimmermann A. Randomized placebo-controlled trial of ursodeoxycholic acid with vitamin e in nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2006; **4**: 1537-1543 [PMID: 17162245 DOI: 10.1016/j.cgh.2006.09.025]

188 **Miller ER**, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* 2005; **142**: 37-46 [PMID: 15537682]

189 **Klein EA**, Thompson IM, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, Minasian LM, Ford LG, Parnes HL, Gaziano JM, Karp DD, Lieber MM, Walther PJ, Klotz L, Parsons JK, Chin JL, Darke AK, Lippman SM, Goodman GE, Meyskens FL, Baker LH. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2011; **306**: 1549-1556 [PMID: 21990298 DOI: 10.1001/jama.2011.1437]

190 **Mintziori G**, Polyzos SA. Emerging and future therapies for nonalcoholic steatohepatitis in adults. *Expert Opin Pharmacother* 2016; **17**: 1937-1946 [PMID: 27564402 DOI: 10.1080/14656566.2016.1225727]

191 **Nascimbeni F**, Pais R, Bellentani S, Day CP, Ratziu V, Loria P, Lonardo A. From NAFLD in clinical practice to answers from guidelines. *J Hepatol* 2013; **59**: 859-871 [PMID: 23751754 DOI: 10.1016/j.jhep.2013.05.044]

P- Reviewer: Borzio M, Fan XM, Marchesini GM, Sinakos E, Zheng SJ
S- Editor: Kong JX **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2017 June 8; 9(16): 733-745

DOI: 10.4254/wjh.v9.i16.733

ISSN 1948-5182 (online)

MINIREVIEWS

Abdominal cross-sectional imaging of the associating liver partition and portal vein ligation for staged hepatectomy procedure

Michele Zerial, Dario Lorenzin, Andrea Risaliti, Chiara Zuiani, Rossano Girometti

Michele Zerial, Chiara Zuiani, Rossano Girometti, Institute of Diagnostic Radiology, Department of Medicine, University of Udine, University Hospital "S. Maria della Misericordia", 33100 Udine, Italy

Dario Lorenzin, Andrea Risaliti, Clinical Surgery, Department of Medicine, University of Udine, University Hospital "S. Maria della Misericordia", 33100 Udine, Italy

Author contributions: Girometti R and Lorenzin D projected the paper; Zerial M, Girometti R and Zuiani C planned the review structure; Lorenzin D and Risaliti A performed surgeries and collected surgical images; Zerial M reviewed radiological literature; Lorenzin D and Risaliti A reviewed surgical literature; Zerial M, Zuiani C and Girometti R reviewed radiological images and selected them; Zerial M wrote the paper; Zuiani C and Girometti R prepared the tables; Zerial M and Lorenzin M prepared the figures; Girometti R, Lorenzin D, Zuiani C and Risaliti A reviewed the paper for structure and content; Girometti R supervised the work.

Conflict-of-interest statement: None of the authors has conflicts of interest to disclose. This paper has been presented as an electronic poster at the European Congress of Radiology (ECR) 2016 and European Society of Gastrointestinal and Abdominal Radiology (ESGAR) annual meeting 2016.

Manuscript source: Invited manuscript

Correspondence to: Rossano Girometti, MD, Institute of Diagnostic Radiology, Department of Medicine, University of Udine, University Hospital "S. Maria della Misericordia", p.le S. Maria della Misericordia, 15, 33100 Udine, Italy. rgirometti@sirm.org

Telephone: +39-0432-559266

Fax: +39-0432-559867

Received: January 27, 2017
Peer-review started: February 12, 2017
First decision: March 10, 2017
Revised: March 22, 2017
Accepted: April 6, 2017
Article in press: April 10, 2017
Published online: June 8, 2017

Abstract

Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) is a recently introduced technique aimed to perform two-stage hepatectomy in patients with a variety of primary or secondary neoplastic lesions. ALPSS is based on a preliminary liver resection associated with ligation of the portal branch directed to the diseased hemiliver (DH), followed by hepatectomy after an interval of time in which the future liver remnant (FLR) hypertrophied adequately (partly because of preserved arterialization of the DH). Multidetector computed tomography (MDCT) and magnetic resonance imaging (MRI) play a pivotal role in patients' selection and FLR assessment before and after the procedure, as well as in monitoring early and late complications, as we aim to review in this paper. Moreover, we illustrate main abdominal MDCT and MRI findings related to ALPPS.

Key words: Hepatectomy; Computed tomography; Magnetic resonance imaging; Associating liver partition and portal vein ligation for staged hepatectomy; Liver surgery

Core tip: Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) is a variant

of two-stage hepatectomy aimed to obtain rapid hypertrophy of the future liver remnant. Given its recent introduction, there are still controversies on indications and safety issues. Cross-sectional imaging by means of multidetector computed tomography (MDCT) and magnetic resonance imaging (MRI) play a key role in the multidisciplinary process of patients' selection and postoperative management. This review aims to emphasize such a role and illustrate main abdominal ALPPS-related findings on MDCT or MRI.

Zerial M, Lorenzin D, Risaliti A, Zuiani C, Girometti R. Abdominal cross-sectional imaging of the associating liver partition and portal vein ligation for staged hepatectomy procedure. *World J Hepatol* 2017; 9(16): 733-745 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i16/733.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i16.733

INTRODUCTION

Resection is the only treatment proven to achieve long-term survival in patients with primary hepatic malignancies or selected liver metastases[1,2]. Over the last years, advances in surgical techniques, systemic chemotherapy and intensive care improved the outcome of liver resection, leading to wider criteria for operability compared to the past[3]. However, adequate future liver remnant (FLR) (*i.e.*, the liver remnant planned to be left *in situ*) is still a critical factor in selecting patients when extended hepatectomy is required, given the need to minimize the risk of postoperative liver failure[4,5]. FLR should be at least 25%-30% of the liver volume in patients with normal preoperative liver function, 30% in chronic liver disease, and 40% in the setting of chemotherapy-related injury or cirrhosis[6,7]. Borderline FLR volumes pose the dilemma of whether attempting radical surgery *vs* performing palliative treatments[7].

In the 2000s, two-stage hepatectomy after preoperative percutaneous portal vein embolization (PVE) or portal vein ligation (PVL) has been proposed as a strategy to resect primarily inoperable tumors after having increased the FLR[8,9]. This approach combines the technical advantages of two-stage hepatectomy (*i.e.*, wedge resections of lesions in the FLR in the case of bilobar tumors) with the compensatory hypertrophy of the FLR induced by PVE or PVL performed at the time of first surgery[10]. The mechanism with which PVE and PVL lead to hypertrophic FLR is complex, involving both the diversion of portal blood flow and release of growth factors[7]. Since hypertrophy usually takes at least 4 wk to be completed, this technique shows high failure rate because of insufficient FLR growth and/or tumor progression during the interval of time between the two stages[11,12].

Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) is a two-stage hepatectomy procedure introduced in September 2007 by Schnitzbauer *et al*[13] to obtained more rapid and larger increase of the FLR volume compared to conventional staged hepatectomy (40%-80% within 6-9 d *vs* 8%-27% within up to 60 d, respectively)[4,6,7,13-15]. The key technical point in ALPPS is the preservation of hepatic artery blood flow to the diseased hemiliver (DH) at the time of first surgical stage. Preserved arterialization leads the DH to act as a vital auxiliary liver and assist the growth of FLR through metabolic and synthetic functions[16,17]. ALPPS achieves a high rate of tumor complete resection (83%)[18], given the successful rate of adequate FLR growth (78%-91%)[19]. Additionally, the reduced interval of time between surgical steps translates into lower tumor progression rate, less adhesions during second surgery, faster patients recovery and prompter starting of adjuvant chemotherapy[4,15,20,21].

ALPPS is becoming increasingly popular in patients candidate to extended hepatectomy. To our knowledge, though imaging plays a key-role in planning the procedure and monitoring the results of both surgical stages, radiological findings related to ALPSS have been poorly reported. In this review, we aimed to summarize the current role for multidetector computed tomography (MDCT) and magnetic resonance imaging (MRI) in the procedure, which enable detailed view of pre- and postoperative anatomy, as well as prompt and reliable identification of complications. We also illustrated main cross-sectional imaging findings related to ALPPS, with special emphasis on normal aspects.

ALPSS: INDICATIONS AND TECHNIQUE

Indications

There is controversy on which lesions should be treated with ALPPS[6], given initially reported high mortality rates (up to 22% in some series)[22]. It should be kept in mind that ALPPS is an "extrema ratio" procedure to be proposed after careful, multidisciplinary patient selection[6,23,24]. Morbidity and mortality amount up to 14% and 6.6% in experienced centers applying strict selection criteria[10,25-27]. Best results have been obtained in patients with bilobar metastases from colorectal cancer with predictable radical resection, absence of extrahepatic disease and partial or complete response to chemotherapy[2]. Other treatable lesions include hepatocellular carcinoma, cholangiocarcinoma (intrahepatic or hilar), gallbladder carcinoma, and metastases from breast cancer or neuroendocrine tumors[7,25,26]. However, higher postoperative mortality was reported for non-colorectal liver metastases[7]. ALPPS can be also offered as first-line treatment or salvage-therapy after failed PVE[20,25,28-32].

Contraindications to ALPPS include unresectable lesions in the FLR, unresectable extrahepatic metastases, infiltration of the retrohepatic avascular space, severe portal hypertension, high anesthesiology risk, medical contraindications to major hepatectomy, impossibility to achieve negative margins, and unresectable primary

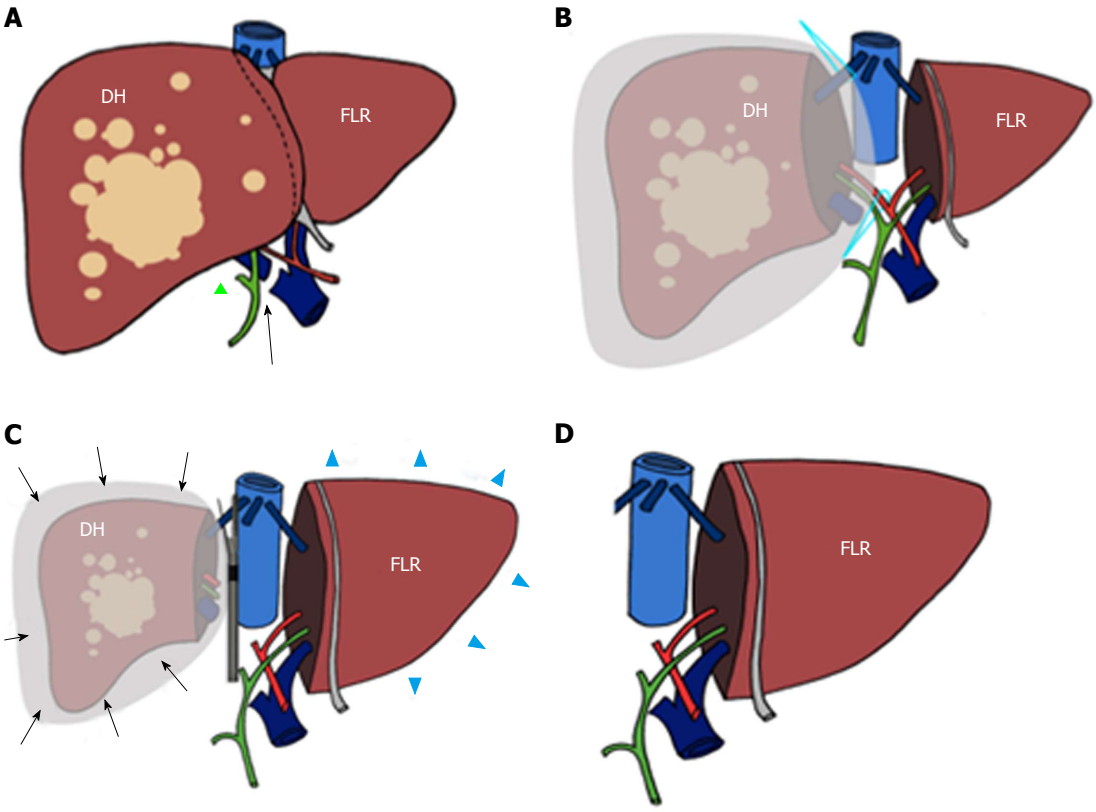

Figure 1 Scheme of trisectionectomy associating liver partition and portal vein ligation procedure. During surgical stage 1 the right portal vein is sectioned and sutured (arrow in A) after performing cholecystectomy (green triangle in A). Subsequently, the diseased hemiliver (DH) is sectioned from the future liver remnant (FLR) and wrapped with a bag (B). At the time of surgical stage 2 (C), hypertrophy of the (FLR) (blue arrowheads in D) and atrophy of the DH (arrows) have been obtained. Associating liver partition and portal vein ligation for staged hepatectomy procedure is then completed by removing the DH (D).

tumor in extrahepatic locations[26]. ALPPS is not recommended in patients with advanced liver cirrhosis, because liver regeneration in the context of chronic liver disease is less predictable[7,33]. On the other hand, some Authors attempted ALPPS in selected cirrhotic patients[34].

Technique

Elective indication to ALPPS is right trisectionectomy[7], in which FLR and DH consist of Couinaud segments 2-3 *vs* 4-8 (Figures 1 and 2), respectively. Other technical approaches include right hepatectomy (leaving a segments 2-4 FLR), left hepatectomy (leaving segments 5-8 FLR), central hepatectomy (segments 4, 5 and 8 FLR) or monosegmental ALPPS[35-37].

ALPSS includes two consecutive surgical stages (stage 1 and stage 2). During stage 1, the portal branch directed to the DH side is sectioned and sutured in order to divert the portal flow to the FLR. Hepatectomy is subsequently performed to separate the FLR from DH completely (complete ALPPS) or partially (partial ALPPS)[32,38]. If affected by metastases, the FLR is cleaned up by wedge resections and/or intraoperative radiofrequency ablation[17,26]. At the end of the procedure, DH is left in situ, often after having enveloped it into a hermetic bag made of plastic or a biodegradable type- I acellular collagen membrane[39]. The rationale for using the bag is to avoid adhesions and obtain an easier removal of DH on surgical stage 2, as well as better drainage or identification of collections (Figures 1 and 2)[7]. The purpose of stage 1 is to induce hypertrophy of the FLR (in which arterial and portal vascular supply is preserved) and atrophy of the DH (in which arterial supply alone is preserved). Cholecystectomy is also performed[40]. In the case of perihilar cholangiocarcinoma, biliary continuity is obtained by performing Roux-en-Y bilioenteric anastomosis[26]. After stage 1 completion, two drains are placed along the transection line and within the plastic bag, respectively.

Stage 2 is scheduled 7-14 d from stage 1[2]. Hepatectomy is completed by removing atrophic DH after transecting the serving hepatic artery, hepatic duct and hepatic veins (*e.g.*, right hepatic and middle hepatic veins in the case of right trisectionectomy, or right hepatic vein only in the case of right hepatectomy).

IMAGING TECHNIQUES

First-line imaging after both surgical stages 1 and 2 is represented by ultrasonography (US) with Color-Doppler examination. In our experience, US permits a "quick-and-dirty" evaluation at patient's bedside to screen for gross complications (*e.g.*, collections) and assess the patency of FLR portal vein, hepatic artery branches and hepatic vein. However, early postoperative US is limited by lack of patients' collaboration and reduced acoustic

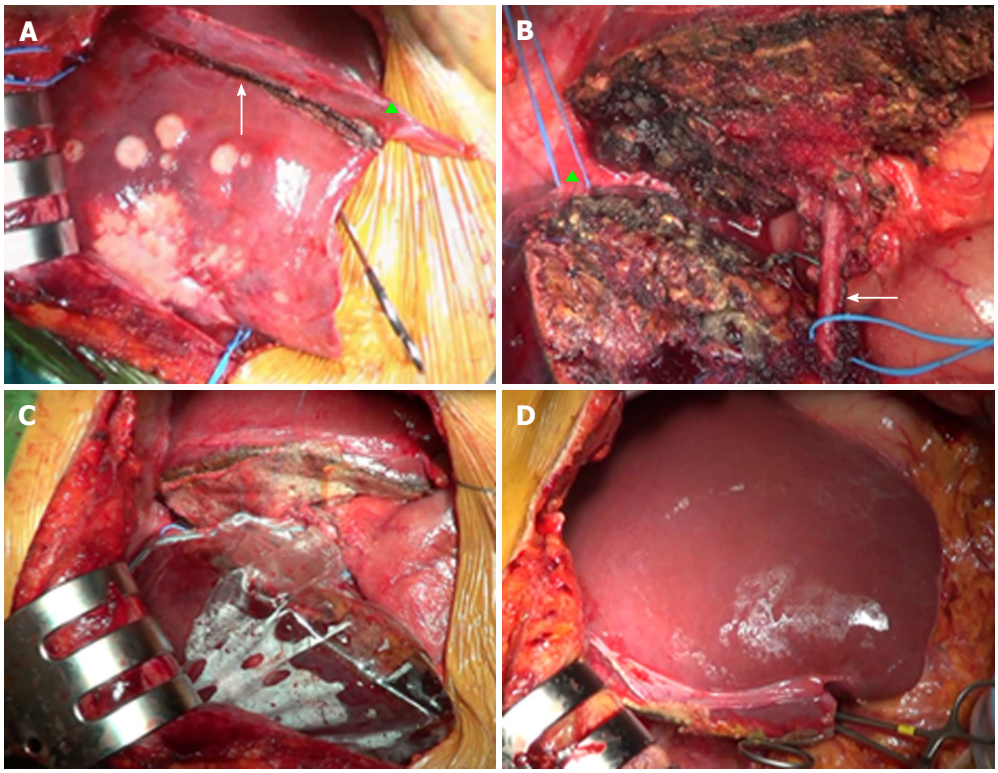

Figure 2 Surgical overview of the associating liver partition and portal vein ligation for staged hepatectomy procedure. A: Intraoperative findings during stage 1, with evidence of resection line (arrow) on the right side of ligamentum falciforme (green triangle); B: Resected liver with right hepatic vein (green triangle) and right hepatic artery (arrow) encircled by a vessel loop to simplify their identification during stage 2; C: After transection, diseased hemiliver is enveloped with a plastic bag; D: Pronounced hypertrophy of future liver remnant during intraoperative stage 2.

windows because of bowel gas and surgical dressing material[41]. Furthermore, US lacks panoramicity, *i.e.*, the capability to represent a section or a 3D reconstruction of the entire liver within a single image. Consequently, though this technique is useful in initial diagnosis of liver abnormalities, it has no direct role in selecting patients for ALPPS (*e.g.*, by assessing the number of lesions in the FLR or estimating its volume). Thus, cross-sectional imaging with MDCT and/or MRI is mandatory in the preoperative patients' selection, in evaluating postoperative increase in FLR volume and in assessing complications.

Because of wide panoramicity, fast acquisition time and lesser costs, MDCT should be regarded as the cross-sectional modality of choice to image patients before and after ALPPS. Our institutional protocol is summarized in Table 1. Fast acquisition makes MDCT feasible in less collaborating patients, with the possibility to extend the examination to the thorax and/or the lower abdomen if needed. Moreover, the multiphasic MDCT protocol has the advantage of providing all-in-one evaluation of liver neoplasms (in terms of both tumor burden and characterization), extrahepatic disease or complications, and the status of arterial, portal and venous structures for the purpose of preoperative planning and complications assessment. Multiplanar reformations and 3D reconstruction are of help in interpreting images and communicating imaging results to referring clinicians.

Given limited availability and longer acquisition times, MRI should be reserved to inconclusive MDCT cases, especially in the preoperative phase, *i.e.*, when there is less risk of image quality degradation because of reduced patients' collaboration. Similarly to other liver applications[41-44], MRI should be performed with 1.5 Tesla or 3.0 Tesla magnets, equipped with highly performing gradients and multi-element surface coils (preferably 8-16 elements) implementing parallel imaging. Our MRI protocol is illustrated in Table 2.

Hepatobiliary contrast agents such as gadoxetic acid and/or gadobenate dimeglumine improve the detection and characterization of focal liver lesions by representing the vascularity and the presence/absence of hepatocellular contrast uptake at one time[45,46]. When liver metastases are the cause for ALPPS, preoperative MRI with diffusion-weighted imaging and hepatobiliary contrast agents should be regarded as the method of choice for detailed identification of small lesions potentially affecting ALPPS feasibility or FLR cleaning up[47]. Furthermore, hepatobiliary contrast agents are of help in assessing tumor relapse after surgery.

Magnetic resonance cholangiopancreatography (MRCP) should be used preoperatively to evaluate whether biliary tree anatomic variants are at risk of increasing surgical difficulty, or to assign the Bismuth category of cholangiocarcinoma extension[48]. In the postoperative phases, this technique can be of help in assessing the content of fluid collections (fluid *vs* hemorrhagic) or early and late biliary complications. In

Table 1 Institutional multiphasic multidetector computed tomography protocol for evaluating associating liver partition and portal vein ligation for staged hepatectomy patients before and after surgery (LightSpeed HD, General Electrics, Milwaukee, United States)

Scan phase (timing from contrast injection)	Scal lenght	Scanning parameters	Rationale in the preoperative phase	Rationale in the postoperative phases
Unenhanced	Upper abdomen	KVp 120 mA modulated between 200-450 Tube rotation 0.6 s Pitch 0.984 Noise index 16.10 Collimation 1.25 mm (0.625 for the angiographic phase) Image reconstruction thickness 1.25 mm	Identifying potential confounders in image interpretation (*e.g.*, lesion's or vascular calcifications). Measuring baseline attenuation of target lesions (*e.g.*, fat-containing HCC) or in diffuse liver disease (*e.g.*, steatosis)	Identifying potential confounders in image interpretation (*e.g.*, surgical clips). Measuring the attenuation of intra-abdominal collections (biloma *vs* hematoma)
Angiographic phase (20)	Upper abdomen		This phase is not required if recent prior imaging is available. Assessing the patency and anatomic variants of the hepatic artery and its branches, both on source images and MIP reconstructions	This phase in not mandatory in repeated follow-up examinations Assessing the sources of suspicious active postoperative bleeding
Delayed arterial (35-40 s)	Upper abdomen		Assessing hypervascular focal liver lesions (malignant and benign ones)	Assessing the patency of the hepatic artery and its branches. Identifying the recurrence of hypervascular tumors in the delayed post-operative period
Venous (70 s)	Whole abdomen		Assessing lesions' enhancement pattern for the purpose of identification/characterization. Assessing the patency and anatomic variants of the portal trunk and intrahepatic branches, both on source images and MIP reconstructions. Identifying additional abdominal findings potentially contraindicating ALPSS. Assessing for signs of chronic liver disease (including splenomegaly, venous collaterals and ascites)	Assessing the portal status (absence of flow in the ligated portal branch and patency of the FLR branch). Assessing successful tumor cleaning up in the FLR before surgical stage 2. Ruling out thrombosis of the portal braches, hepatic veins and inferior vena cava. Identifying tumor relapse
Delayed (3-5 min)	Upper or whole abdomen, depending on findings on previous scans		Assessing lesions' enhancement pattern for the purpose of identification/characterization. Identifying additional findings potentially contraindicating ALPSS (*e.g.*, peritoneal carcinosis). This phase is not mandatory	Assessing venous bleeding. This phase in not mandatory

MIP: Maximum intensity projection; FLR: Future liver remnant; ALPPS: Associating liver partition and portal vein ligation for staged hepatectomy; HCC: Hepatocellular carcinoma.

particular, 3D T1-weighted MRCP acquired in the delayed phase after gadoxetic acid administration is useful in confirming clinical suspicion of biliary leakage (*e.g.*, persisting postoperative fluid collections associated with clinical sign of biliary sepsis) by showing active contrast extravasation[44]. The presence of endobag after surgical stage 1 can avoid gadoxetic acid-based MRCP, since bile leakage can be actively monitored through the internal surgical drainage.

ROLE FOR IMAGING

Preoperative imaging

Preoperative findings are essential to understand whether ALPPS is feasible or not based on tumor burden, liver status and presence of ancillary findings with potential surgical significance. There are five main goals of cross-sectional imaging in this setting.

The first task for imaging is accurate detection and characterization of liver lesions. Radiologists should carefully report the number, size, and location of individual lesions, as well as their relationship with surgically relevant anatomic structures, including the hepatic artery, main portal branches, hepatic veins, and first- to second-order biliary branches (Figure 3). This will help the surgeon to establish lesions resectability and the risk for intraoperative complications (*e.g.*, lesions close to the retrohepatic course of inferior vena cava, a region at higher risk of intraoperative bleeding). Second, imaging aims to evaluate the status of liver parenchyma, looking for signs of cirrhosis, cholestasis, steatosis or any other pathologic change attributable to the effects of lesions, diffuse liver disease or chemotherapy. Liver status may influence operability, regardless of the FLR volume (see below). Third, it is crucial to identify vascular and biliary anatomy variants of potential surgical significance (*e.g.*, aberrant and/or accessory branches)[49]. Fourth, any extrahepatic finding potentially affecting the feasibility of ALPPS should be

Table 2 Institutional magnetic resonance imaging protocol with *i.v.* administration gadoxetic acid (0.025 mmol/kg at an injection rate of 1 mL/s) for evaluating associating liver partition and portal vein ligation for staged hepatectomy patients before and after surgery

Sequence	Weightening	Acquisition plane	Technical clues	Rationale in the preoperative phase	Rationale in the postoperative phase
Half fourier acquisition single-shot turbo spin echo/ single shot fast spin echo	T2	Coronal, transverse	-	Ruling out signs of chronic liver disease, including splenomegaly and/or ascites. Detection of parenchymal low signal intensity in iron accumulation	Detection of perihepatic/ abdominal collection and/or ascites
GE in-phase/out of-phase	T1	Transverse	Dual echo, breath hold sequence with slice thickness 6 mm	Characterization of fat-containing lesions. Detection of signal intensity patterns of liver steatosis or hemochromatosis	Evaluation of the postoperative status of liver parenchyma. Characterization of tumor recurrence
MRCP	T2	Radial coronal acquisition (2D) or oblique coronal (3D)	2D and/or 3D technique	Evaluation of anatomic variants complicating or contraindicating surgery. Assessing the Bismuth category of hilar cholangiocarcinoma	Assessment of biliary strictures (site, extent) and biliary dilation upstream
Dynamic study with fat saturated 3D GE	T1	Transverse	Thin slice thickness (3 mm). Baseline acquisition followed by early arterial, late arterial, venous and delayed phases	Detection and characterization of liver lesions	Detection and characterization of parenchymal abnormalities, including tumor recurrence
Single-shot echoplanar imaging	Diffusion	Transverse	*b* values 50 and 400 and 800 s/mm^2 (1.5T) or 50 and 800 and 1200 s/mm^2 (3.0T). Nominal acquisition time about 3 min (1.5T) and 4 min (3T)	Detection and characterization of smaller lesions (< 1 cm in size)	Detection of parenchymal/ periportal edema. Detection and characterization of smaller lesions (< 1 cm in size)
Fat saturated Turbo spin echo	T2	Transverse	Respiratory triggered, with slice thickness 6 mm. Nominal acquisition time 1.50 min	Detection and characterization of liver lesions.	Detection of parenchymal/ periportal edema. Detection and characterization of liver lesions. Assessment of collections
GE in-phase/out of-phase	T1	Transverse	Same sequence as (2), acquired in the hepatobiliary phase (15-20 min after contrast injection)	Detection and characterization of liver lesions	Detection and characterization of liver abnormalities
Fat saturated 3D GE	T1	Transverse	Same sequence as (4), with modified flip angle (35°) to increase lesion-to-parenchyma conspicuity. Acquired in the hepatobiliary phase		
Contrast-enhanced MRCP	T1	Oblique coronal	Thin-slice (1 mm) fat saturated 3D fast low angle shot (FLASH) sequence acquired	Functional evaluation of biliary obstruction (if present)	Detection of active bile leakage. Functional assessment of bile duct strictures and patency of bilioenteric anastomosis

GE: Gradient echo; MRCP: Magnetic resonance cholangiopancreatography.

evaluated, including large, inoperable primary cancer on other sites, as well as portal hypertension (including splenomegaly and venous collaterals).

The final key step in preoperative imaging is liver volumetry (LV) of the FLR and the whole liver. FLR volume should be calculated by excluding major vessels and FLLs, in order to obtain a reasonable estimate of final viable liver tissue supporting liver function. FLR should be no lower than 25%-30% of preoperative liver volume in patients with normal liver function, and no lower than 40% in patients with underlying chronic liver disease or liver dysfunction (including the effects of chemotherapy)[7,23,50-52]. Many dedicated liver volumetry software are currently available, most times implemented in the picture archive and communication systems used for routine image analysis. In our Institution, abdominal radiologists perform LV together with liver surgeons, with the objective of reliable volumes definition according to

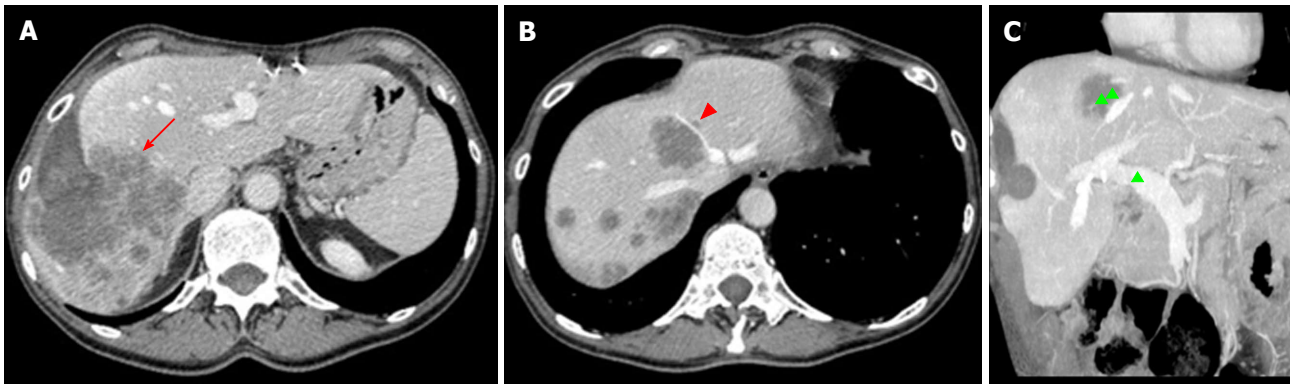

Figure 3 Radiologists should carefully report the number, size, and location of individual lesions, as well as their relationship with surgically relevant anatomic structures, including the hepatic artery, main portal branches, hepatic veins, and first- to second-order biliary branches. A: Preoperative assessment with computed tomography in a 64 male years old patient showing colorectal metastases on the right hepatic lobe (red arrow). Right trisectionectomy associating liver partition and portal vein ligation for staged hepatectomy was planned; B: One small satellite lesion was found on the left side of the middle hepatic vein (arrowhead), indicating the need for future liver remnant clean-up during stage 1; C: No vascular involvement was shown, as exemplified by patent main portal trunk and intrahepatic branches (green triangle), except for infiltration of the middle hepatic vein (double green triangle). Based on this finding, a wide free margin between the line of resection and the middle hepatic vein was obtained.

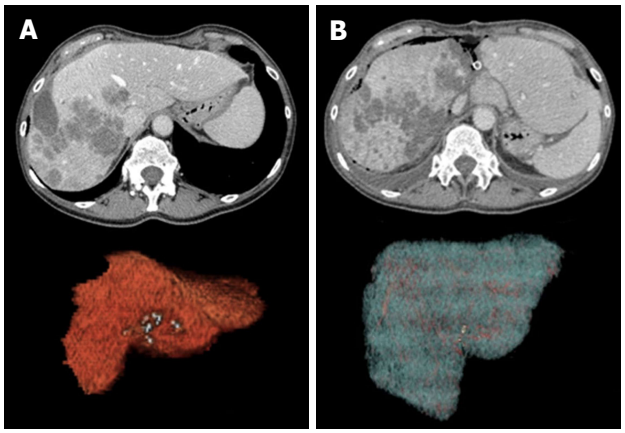

Figure 4 Evolution of the future liver remnant (liver segments 2 + 3) before (A) and after stage 1 surgery (B). Future liver remnant remnant almost doubled in volume (from 280 cm^3 to 468 cm^3), showing clear enlargement on 2D images and volume rendering reconstructions.

the intended lines of resection.

Imaging after surgical stage 1

Goals: In uncomplicated patients, post-stage 1 imaging is performed at the time adequate FLR hypertrophy is expectedly achieved (about 6-9 d from surgery)[7]. Cross-sectional imaging is mandatory to calculate the increase in volume of the FLR using LV (Figure 4), to confirm tumor-free status of the FLR, and to verify the expected changes in the DH (atrophy and persistent portal devascularization).

In the case of US and/or clinical suspicion, MDCT or MRI must be anticipated to guarantee early assessment and intervention. Cross-sectional imaging is also of help in ruling-out surgical complications or insufficient FLR volume as a cause for postoperative liver failure.

Normal findings: Normal hypertrophic FLR is represented in Figure 4. Enlargement can be easily appreciated on transverse and reformatted 2D images, though precise estimation should be always performed on 3D reconstructions obtained with LV. The magnitude of expected FLR increase ranges between 61% and 93% compared to the baseline volume[50]. In our center, a minimum increase of 40% is needed for completing the procedure. It is of paramount importance to distinguish between true parenchymal hypertrophy and liver enlargement from postoperative liver edema or congestion. Measurement of Hounsfield units (HU) on MDCT can be of help in the distinction, since edematous parenchyma shows significantly lower attenuation compared to unaffected liver[20]. In rare cases in which doubts persist, MRI can be of help in differential diagnosis by showing parenchymal and/or prominent periportal edema.

FLR and DH are often surrounded by a thin rim of free fluid, which is usually more prominent around the DH when the endobag is on site. Of note, thin walls make the endobag usually not directly visible on images. Small air bubbles are frequently mixed within the perihepatic fluid, sometimes at a larger extent along the line of hepatectomy (Figure 5). It is crucial not to misdiagnose this normal finding with an infected collection, which is usually larger, lenticular or round in shape and sometimes well-encapsulated on contrast-enhanced images. Mild periportal edema is commonly present as a thin hypodense (on MDCT) or hyperintense (on T2-weighted MRI images) halo surrounding the intrahepatic portal branches.

Except for the portal branches of the DH, the vascular supply to the liver is preserved, with the hepatic artery for the DH appearing slightly hypertrophic compared to the baseline examination to compensate for portal occlusion. No biliary dilation should be observed, in both the DH and FLR.

Main complications: Postoperative complications of ALPPS include bleeding, bile leakage, fluid or bile collections,

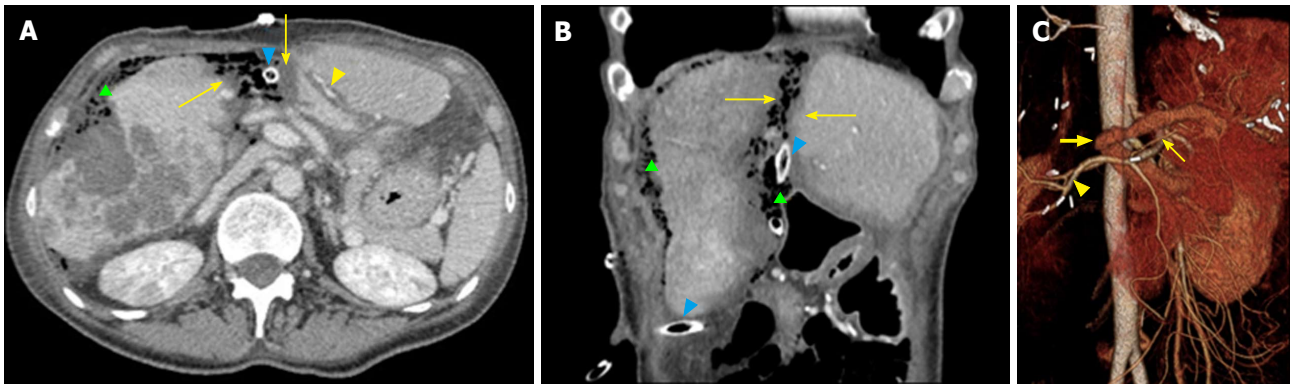

Figure 5 Normal findings on computed tomography after stage 1 surgery on transverse (A) and coronally-reformatted images (B), as well as volume rendering 3D reconstruction (C) (right trisectionectomy associating liver partition and portal vein ligation for staged hepatectomy). A thin rim of free fluid with air bubbles is visible along the surface of diseased hemiliver (right liver lobe), suggesting its accumulation within the plastic bag (green triangle on A and B). A similar finding can be appreciated along the line of transection. Mild periportal edema (yellow arrowhead in A), thin hypodense bands along the edges of surgical resection (arrows on both A and B) and drains (blue arrowheads) are visible. Main right portal branch was ligated and transected (thick arrow in C). Hepatic artery branches are patent (C), including right hepatic artery, which shows mild hypertophy (arrowhead), and left hepatic artery (thin arrow).

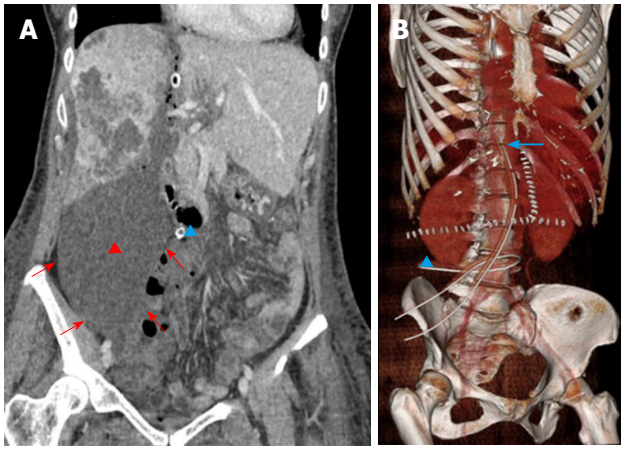

Figure 6 Biloma in a 49-year-old female patient who underwent associating liver partition and portal vein ligation for staged hepatectomy because of peripheral cholangiocarcinoma of the right liver lobe. A: Computed tomography was performed because of bile flowing from the right drainage (blue triangle). The examination confirmed a large fluid collection beneath the DH (red triangle), which distended the plastic bag (red arrows). Biloma was removed with the DH during stage 2 surgery, resolving the biliary leakage originating from right transection surface; B: Normal position of the two drains on volume rendering reconstruction. Left drain has a vertical course along the line of transection up to the inferior margin of the diaphragm (arrow). Right drain has an horizontal course beneath DH (triangle), with its his placed within the plastic bag, in order to drain collections. DH: Diseased hemiliver.

biliary fistula, cholangitis, portal vein thrombosis (PVT), hepatic vein and hepatic arterial thrombosis, hepatic dysfunction, liver failure, persistent postoperative ascites, pleural effusion, prolonged ileus, coagulation disorders, cardiovascular, respiratory, and renal system dysfunction, encephalopathy and infection[5,53]. Clinical presentation is often challenging, since patient's signs and symptoms tend to be non-specific. They include fever, abdominal pain, jaundice, ascites, pleural effusion, abnormal liver tests and bleeding or bile within the drains[54]. Post-hepatectomy liver failure has been specifically defined according to so called 50-50 criteria (prothrombin time < 50% and total serum bilirubin > 50 mmol/L on postoperative day 5 or after)[55]. Imaging is recommended in symptomatic patients to rule-out vascular, biliary or parenchymal causes. The most common ALPPS complications encountered on abdominal cross-sectional imaging are collections, hemorrhage and vascular thrombosis.

Collections are represented by hematoma (up to 50% of cases), biloma (25%) and infected collections (25%)[56]. Collections tend to origin from the resection surfaces, *i.e.*, (assuming right trisectionectomy) in the subphrenic space if originating from the FLR, and within the endobag if originating from DH. Small bilomas and/or transient hematomas are common during the first post-operative days, being rapidly reabsorbed or showing no tendency to increase. On the contrary, collections with large size or increasing in volume over a few days should be regarded as pathological (Figure 6). Bilomas are virtually indistinguishable from serous collections on MDCT, since they present homogeneous fluid content (< 30 HU) without contrast-enhancement. Active biliary leakage can be shown on gadoxetic acid-enhanced MRCP because of contrast extravasation from bile ducts or liver surface into the collection[57,58]. Early diagnosis of biliary leakage is important to prevent biliary sepsis. In this case, stage 2 might be anticipated before the FLR is sufficiently hypertrophied, even if at risk of subsequent insufficient liver function. Hematomas usually show more heterogeneous content than bilomas, with mixed internal areas of low and high attenuation (> 30 HU) on MDCT reflecting the presence of fibrin septa and clots. On MRI, bilomas appears as fluid collections with hypointensity on T1-weighted images and hyperintensity on T2-weightd images, whereas hematomas show typical hyperintensity on T1-weighted fat suppressed images. Treatment options for collections include drainage under sonographic or MDCT guidance, as well as surgical toilette in more extensive cases[3,59]. Infected collections typically show small air bubbles from anaerobic bacteria, and may be surrounded by thickened contrast-enhancing walls of peripheral inflammatory tissue.

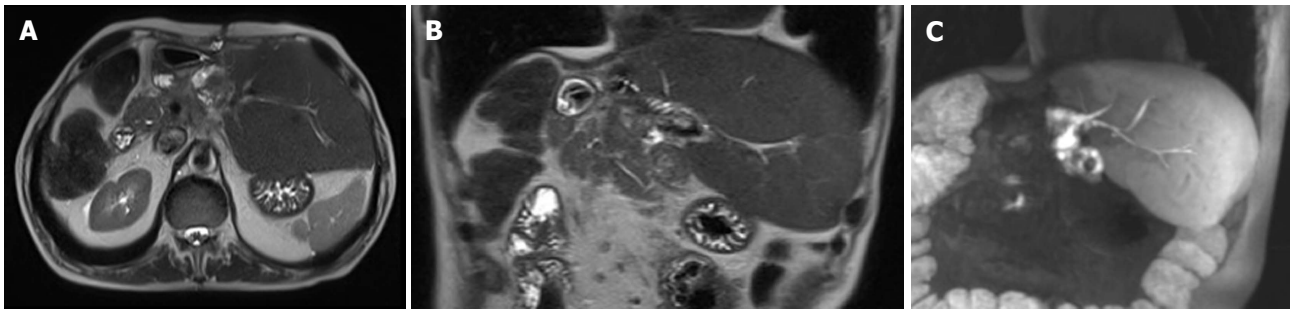

Figure 7 Bilioenteric anastomosis between the jejunum and biliary branches for hepatic segments 2-3 after right trisectionectomy associating liver partition and portal vein ligation for staged hepatectomy performed for hilar colangiocarcinoma showing. A, B: Magnetic resonance imaging single-shot turbo spin echo T2 weighted images acquired on transverse (A) and coronal planes (B) show absence of biliary dilatation; C: This finding was confirmed on thick maximum intensity projection coronally-reformatted image acquired on the hepatobiliary phase after gadoxetic acid administration, demonstrating regular flow of hyperintense bile.

Postoperative hemorrhage generally arises within 48 h from intervention, commonly originating from the resection margins (*e.g.*, because of an arterial branch truncation or congestion of the hepatic vein due to stenosis or ligation), incomplete intraoperative hemostasis or dehiscence of vascular sutures[3]. MDCT with angiographic phase should be promptly performed to identify the site of bleeding and guide embolization or surgery.

The most threatening vascular complication after stage 1 is portal thrombosis. This rare condition may affect the portal trunk and/or the FLR branch, thus affecting the hypertrophy process. Not surprisingly, patients showing extensive PVT are at high risk of liver failure and death[7,26,28]. Color Doppler US has a primary role in detecting thrombosis. Similarly to other postoperative scenarios[41], thrombosis manifests with absent flow, with our without direct demonstration of an intraluminal echogenic thrombus on B-mode. Although no specific data on ALPPS have been reported, to our knowledge, contrast-enhanced US is supposedly of help in confirming absent contrast arrival in thrombotic vessels[41]. Post-contrast MDCT and/or MRI acquired on venous and delayed phases are useful to confirm color Doppler findings, as well as to map the extent of thrombosis (portal trunk and/or FLR main branch and/or intrahepatic branches) and the degree of occlusion (partial or complete filling defects). Contrast enhancement of vascular walls is an additional findings of thrombosis, likely representing contrast engorgement within dilated vasa vasorum[60,61]. Partial thrombosis may benefit from medical therapy, whereas complete thrombosis requires thrombolysis.

Imaging after surgical stage 2

Goals: Early cross-sectional imaging is usually not required in the case of an uncomplicated clinical course. Chest X-ray and abdominal US with color Doppler interrogation of major vessels are usually sufficient to monitor the patient in the first weeks after the intervention. MDCT and/or MRI should be ordered in the case of suspicious complications and/or inconclusive findings on US. On the contrary, cross-sectional imaging has a major role in the delayed postoperative period, mainly in assessing tumor recurrence and/or late complications with or without prior US.

Recommended imaging follow-up includes US and MDCT or MRI scan after 3 and 6-12 mo from surgery, respectively[54]. However, there is no definite schedule for imaging controls, which should be tailored to patients according to the type and extent of the operated tumor, concomitant chemotherapy and history of major complications after surgical stage 1 and/or 2. MRI is reserved to cases of suspicious biliary complications or for characterizing ambiguous CT findings.

Normal findings: Asymptomatic, small amounts of intra-abdominal air or small fluid collections are common findings in the postoperative phase. Air is usually reabsorbed early, whereas collections can persist up to two months after surgery[56]. Another frequent finding is represented by a hypoattenuating linear band adjacent to liver raw surface (about 30%-50% of cases), which has been related to the effects of parenchymal devascularization or bile/blood accumulation[56]. No vascular or biliary abnormalities should be found (Figure 7).

Of note, transitory splenic enlargement is commonly encountered within 6 mo from hepatectomy. The degree of splenomegaly is generally proportional to the volume of liver resection, with average increase in splenic volume of about 40% compared to the preoperative period[62-64].

Main complications: Complications after stage 2 may be classified into early and late, depending on the onset from surgery. Early complications occur within a few weeks from stage 2, and manifest with a clinical and radiological spectrum similar to that following stage 1 surgery. Thus, hematomas/bilomas (Figure 8), bleeding, vascular thrombosis and pleural effusion represent main expected findings, presenting as described above. Late complications are stage 2 specific, and tend to occur from 3 to about 6 mo after this surgical step. The most frequent and relevant ones are tumor recurrence and biliary complications. The treatment of late complications may be challenging, especially if further surgery is

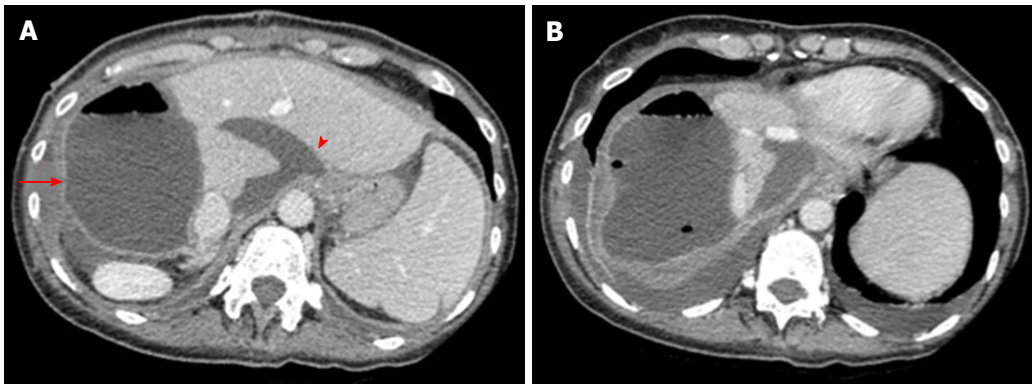

Figure 8 Intrabdominal collection 9 d after stage 2 surgery in a 49-year-old female patient with fever and altered liver function tests. A: Large, encapsulated collection with fluid-air level was shown after diseased hemiliver removal, with mild parietal enhancement (arrow). Part of the collection surrounded liver segment I (arrow head); B: Bilateral pleural effusion coexisted.

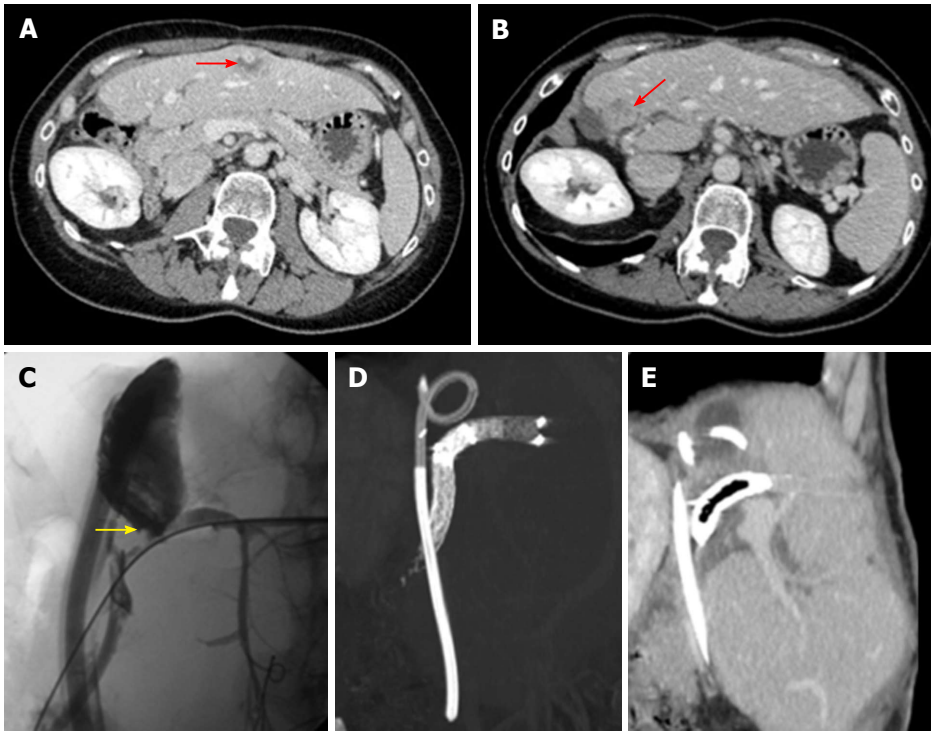

Figure 9 Recurrence appears with multidetector computed tomography and/or magnetic resonance imaging signs of the original tumor, though recurrence can manifest with pleomorphic, nonspecific appearance in our experience. A, B: Multifocal recurrent cholangiocarcinoma presenting 16 mo after right trisectionectomy associating liver partition and portal vein ligation for staged hepatectomy. Lesions showed atypical persistently hypovascular appearance on dynamic contrast-enhanced multidetector computed tomography; C-E: Wedge resection of recurrences was complicated by biliary leakage, as shown on percutaneous transhepatic cholangiography (arrow in C), treated by positioning a drainage within the biloma and a biliary stent graft, as shown on maximum intensity projection reconstruction in D and oblique sagittal reformation in E.

needed. Indeed, additional interventions may in turn increase the risk of morbidity (Figure 9).

The International ALPSS registry[27] reported disease-free survival of 73% and 59% at 1 and 2 years after ALPSS, respectively, with median survival of 14 mo. Recurrence appears with MDCT and/or MRI signs of the original tumor, though recurrence can manifest with pleomorphic, nonspecific appearance in our experience (Figure 9). Suspicious solid lesions should be regarded as tumor recurrence, regardless of the fact they mimic preoperative lesions or not.

Late biliary complications include stricture and fistula. Because of the recent introduction of ALPSS, it is difficult to quantify the prevalence of these complications, which are generally rare in experienced centers. Strictures are multifactorial in origin, having been related to mechanical stress from FLR enlargement and rotation, as well as to iatrogenic causes (inaccurately placed clips, injury, periductal bile leakage and ischemia due to injured FLR hepatic artery)[65,66].

MRCP is the elective tool to assess the site of obstruction, which appears as a focal zone of absent signal on fluid-

Table 3 Overview of normal and abnormal findings after surgical stages 1 and 2

Postoperative phase	Normal findings		Abnormal findings
	Goals of ALPPS	Findings not to be confused with pathological aspects	prompting intervention
After surgical stage 1	Hypertrophic FLR (≥ 40% of baseline preoperative volume)	Thin rim of free fluid around both FLR and DH	Large, persisting collections (hematoma, bilomas, infected collections)
		Air bubbles within the perihepatic fluid, especially on the hepatectomy line	Bleeding
		Mild periportal edema	Biliary dilation
		Hypertrophy of hepatic artery for the DH	Bile leakage/fistula
			Portal vein thrombosis
After surgical stage 2	Uncomplicated appearance of the FLR (*e.g.*, no relapsing focal liver lesions)	Thin rim of free fluid around FLR	Early complications
		Air bubbles	see surgical stage 1
		Hypoattenuating linear band adjacent to liver raw surface	Late complications (3-6 mo)
			Biliary stricture
		Rotation of hypertrophic FLR	Biliary fistula
		Transitory splenomegaly	Tumor recurrence

ALPPS: Associated liver partition and portal vein ligation for staged hepatectomy; FLR: Future liver remnant; DH: Diseased hemiliver.

sensitive images, as well as the degree of proximal biliary dilation[42]. Strictures of the bilioenteric anastomosis should be evaluated with gadoxetic acid-based MRCP, which shows lack of contrast flow from the biliary tree to the anastomotic bowel loop[65,67]. This technique is of help also in identifying the site of bile extravasation when chronic biliary fistula is suspected. Similarly to other clinical scenarios[42], MRCP is electively ordered in patients with low pre-test probability of biliary complications, since a negative result is reliable enough to avoid invasive procedures of direct cholangiography. On the other hand, MRCP is effective also in patients with high pre-test probability of disease, since it provides a panoramic and detailed representation of pathological findings, *i.e.*, an accurate road-map for planning the most appropriate interventional approach. Most bilomas and strictures are treated with endoscopic sphincterotomy and balloon dilation followed by endoprothesis placement.

An overall view of normal postoperative findings and complications after both surgical stages 1 and 2 is provided in Table 3.

CONCLUSION

ALPSS is an increasingly popular two-stage hepatectomy technique associated with portal ligation aimed to obtain rapid and adequate FLR hypertrophy, thus extending operability in patients with massive primary or secondary neoplastic liver involvement.

Cross-sectional imaging, especially MDCT, plays a key role in planning ALPPS procedure and monitoring different surgical stages. In particular, MDCT is the main instrument to provide liver volumetry, which is of special importance in assessing technique feasibility and assessing variation in volume of the FLR between surgical stages. MDCT also confirm a clinical or sonographic suspicion of complications, including collections, bilomas, hematomas, post-surgical bleeding, PVT, and tumor recurrence. MRI should be used as a problem-solving tool in both preoperative and postoperative phases, whereas MRCP has an elective role in assessing biliary complications.

ACKNOWLEDGMENTS

The Authors thank Dr. Iliana Bednarova', MD (Institute of Radiology, University of Udine, Italy) for having revised English language.

REFERENCES

1 **Agrawal S**, Belghiti J. Oncologic resection for malignant tumors of the liver. *Ann Surg* 2011; **253**: 656-665 [PMID: 21475004 DOI: 10.1097/SLA.0b013e3181fc08ca]
2 **Torres OJ**, Fernandes ES, Herman P. ALPPS: past, present and future. *Arq Bras Cir Dig* 2015; **28**: 155-156 [PMID: 26537135 DOI: 10.1590/S0102-67202015000300001]
3 **Jin S**, Fu Q, Wuyun G, Wuyun T. Management of post-hepatectomy complications. *World J Gastroenterol* 2013; **19**: 7983-7991 [PMID: 24307791 DOI: 10.3748/wjg.v19.i44.7983]
4 **Schnitzbauer A,** Lang S A, Fichtner-Feigl S, Loss M, Kroemer A, Goessmann H, Farkas SA, Kirchner G, Jung EM, Scherer MN, Piso P, Lang H, Schlitt HJ. In situ split with portal vein ligation induces rapid left lateral lobe hypertrophy enabling two-staged extended right hepatic resection. Berlin: Oral Presentation, 2010: 35
5 **Herman P**, Krüger JA, Perini MV, Coelho FF, Cecconello I. High Mortality Rates After ALPPS: the Devil Is the Indication. *J Gastrointest Cancer* 2015; **46**: 190-194 [PMID: 25682120 DOI: 10.1007/s12029-015-9691-6]
6 **Bertens KA**, Hawel J, Lung K, Buac S, Pineda-Solis K, Hernandez-Alejandro R. ALPPS: challenging the concept of unresectability--a systematic review. *Int J Surg* 2015; **13**: 280-287 [PMID: 25496851 DOI: 10.1016/j.ijsu.2014.12.008]
7 **Zhang GQ**, Zhang ZW, Lau WY, Chen XP. Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS): a new strategy to increase resectability in liver surgery. *Int J Surg* 2014; **12**: 437-441 [PMID: 24704086 DOI: 10.1016/j.ijsu.2014.03.009]
8 **Adam R**, Miller R, Pitombo M, Wicherts DA, de Haas RJ, Bitsakou G, Aloia T. Two-stage hepatectomy approach for initially unresectable colorectal hepatic metastases. *Surg Oncol Clin N Am* 2007; **16**: 525-536, viii [PMID: 17606192 DOI: 10.1016/j.soc.2007.04.016]
9 **Adam R**, Laurent A, Azoulay D, Castaing D, Bismuth H. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. *Ann Surg* 2000; **232**: 777-785 [PMID: 11088072]
10 **Hernandez-Alejandro R**, Bertens KA, Pineda-Solis K, Croome KP. Can we improve the morbidity and mortality associated with the associating liver partition with portal vein ligation for staged

hepatectomy (ALPPS) procedure in the management of colorectal liver metastases? *Surgery* 2015; **157**: 194-201 [PMID: 25282528 DOI: 10.1016/j.surg.2014.08.041]

11 **Kianmanesh R**, Farges O, Abdalla EK, Sauvanet A, Ruszniewski P, Belghiti J. Right portal vein ligation: a new planned two-step all-surgical approach for complete resection of primary gastrointestinal tumors with multiple bilateral liver metastases. *J Am Coll Surg* 2003; **197**: 164-170 [PMID: 12831938 DOI: 10.1016/S1072-7515(03)00334-X]

12 **Jaeck D**, Oussoultzoglou E, Rosso E, Greget M, Weber JC, Bachellier P. A two-stage hepatectomy procedure combined with portal vein embolization to achieve curative resection for initially unresectable multiple and bilobar colorectal liver metastases. *Ann Surg* 2004; **240**: 1037-1049; discussion 1049-1051 [PMID: 15570209 DOI: 10.1097/01.sla.0000145965.86383.89]

13 **Schnitzbauer AA**, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, Fichtner-Feigl S, Lorf T, Goralcyk A, Hörbelt R, Kroemer A, Loss M, Rümmele P, Scherer MN, Padberg W, Königsrainer A, Lang H, Obed A, Schlitt HJ. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 2012; **255**: 405-414 [PMID: 22330038 DOI: 10.1097/SLA.0b013e31824856f5]

14 **Wiederkehr JC**, Avilla SG, Mattos E, Coelho IM, Ledesma JA, Conceição AF, Wiederkehr HA, Wiederkehr BA. Associating liver partition with portal vein ligation and staged hepatectomy (ALPPS) for the treatment of liver tumors in children. *J Pediatr Surg* 2015; **50**: 1227-1231 [PMID: 25783345 DOI: 10.1016/j.jpedsurg.2014.10.019]

15 **de Santibañes E**, Clavien PA. Playing Play-Doh to prevent post-operative liver failure: the "ALPPS" approach. *Ann Surg* 2012; **255**: 415-417 [PMID: 22330039 DOI: 10.1097/SLA.0b013e 318248577d]

16 **Sala S**, Ardiles V, Ulla M, Alvarez F, Pekolj J, de Santibañes E. Our initial experience with ALPPS technique: encouraging results. *Updates Surg* 2012; **64**: 167-172 [PMID: 22903531 DOI: 10.1007/s13304-012-0175-y]

17 **Torres OJ**, Moraes-Junior JM, Lima e Lima NC, Moraes AM. Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS): a new approach in liver resections. *Arq Bras Cir Dig* 2012; **25**: 290-292 [PMID: 23411931 DOI: 10.1590/S0102-67202012000400015]

18 **Schadde E**, Ardiles V, Slankamenac K, Tschuor C, Sergeant G, Amacker N, Baumgart J, Croome K, Hernandez-Alejandro R, Lang H, de Santibañes E, Clavien PA. ALPPS offers a better chance of complete resection in patients with primarily unresectable liver tumors compared with conventional-staged hepatectomies: results of a multicenter analysis. *World J Surg* 2014; **38**: 1510-1519 [PMID: 24748319 DOI: 10.1007/s00268-014-2513-3]

19 **Schadde E**, Schnitzbauer AA, Tschuor C, Raptis DA, Bechstein WO, Clavien PA. Systematic review and meta-analysis of feasibility, safety, and efficacy of a novel procedure: associating liver partition and portal vein ligation for staged hepatectomy. *Ann Surg Oncol* 2015; **22**: 3109-3120 [PMID: 25448799 DOI: 10.1245/s10434-014-4213-5]

20 **Knoefel WT**, Gabor I, Rehders A, Alexander A, Krausch M, Schulte am Esch J, Fürst G, Topp SA. In situ liver transection with portal vein ligation for rapid growth of the future liver remnant in two-stage liver resection. *Br J Surg* 2013; **100**: 388-394 [PMID: 23124776 DOI: 10.1002/bjs.8955]

21 **Li L,** Ewald F, Gulati A, Nashan B. Associating liver partition and portal vein ligation for staged hepatectomy: From technical evolution to oncological benefit. *World J Gastrointest Surg* 2016; **8:** 124-133 [PMID: 26981186 DOI: 10.4240/wjgs.v8.i2.124]

22 **Li J**, Girotti P, Königsrainer I, Ladurner R, Königsrainer A, Nadalin S. ALPPS in right trisectionectomy: a safe procedure to avoid postoperative liver failure? *J Gastrointest Surg* 2013; **17**: 956-961 [PMID: 23288719 DOI: 10.1007/s11605-012-2132-y]

23 **Torres OJ**, Fernandes Ede S, Oliveira CV, Lima CX, Waechter FL, Moraes-Junior JM, Linhares MM, Pinto RD, Herman P, Machado MA. Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS): the Brazilian experience. *Arq Bras Cir Dig* 2013; **26**: 40-43 [PMID: 23702869 DOI: 10.1590/S0102-67202013000100009]

24 **Nadalin S**, Capobianco I, Li J, Girotti P, Königsrainer I, Königsrainer A. Indications and limits for associating liver partition and portal vein ligation for staged hepatectomy (ALPPS). Lessons Learned from 15 cases at a single centre. *Z Gastroenterol* 2014; **52**: 35-42 [PMID: 24420797 DOI: 10.1055/s-0033-1356364]

25 **Peteja M,** Pelikan A, Vavra P, Lerch M, Ihnat P, Zonca P and Janout V. Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy: A Contemporary Surgical Oncology Conundrum. *J Gastrointest Dig Syst* 2015; **5:** 3 [DOI: 10.4172/2161-069X.1000296]

26 **Alvarez FA**, Ardiles V, Sanchez Claria R, Pekolj J, de Santibañes E. Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS): tips and tricks. *J Gastrointest Surg* 2013; **17**: 814-821 [PMID: 23188224 DOI: 10.1007/s11605-012-2092-2]

27 **Schadde E**, Ardiles V, Robles-Campos R, Malago M, Machado M, Hernandez-Alejandro R, Soubrane O, Schnitzbauer AA, Raptis D, Tschuor C, Petrowsky H, De Santibanes E, Clavien PA. Early survival and safety of ALPPS: first report of the International ALPPS Registry. *Ann Surg* 2014; **260**: 829-836; discussion 836-838 [PMID: 25379854 DOI: 10.1097/SLA.0000000000000947]

28 **Tschuor Ch**, Croome KP, Sergeant G, Cano V, Schadde E, Ardiles V, Slankamenac K, Clariá RS, de Santibañes E, Hernandez-Alejandro R, Clavien PA. Salvage parenchymal liver transection for patients with insufficient volume increase after portal vein occlusion -- an extension of the ALPPS approach. *Eur J Surg Oncol* 2013; **39**: 1230-1235 [PMID: 23994139 DOI: 10.1016/j.ejso.2013.08.009]

29 **Björnsson B**, Gasslander T, Sandström P. In situ split of the liver when portal venous embolization fails to induce hypertrophy: a report of two cases. *Case Rep Surg* 2013; **2013**: 238675 [PMID: 24383035 DOI: 10.1155/2013/238675]

30 **Jackson T**, Siegel KA, Siegel CT. Rescue ALPPS: Intraoperative Conversion to ALPPS during Synchronous Resection of Rectal Cancer and Liver Metastasis. *Case Rep Surg* 2014; **2014**: 487852 [PMID: 25506458 DOI: 10.1155/2014/487852]

31 **Vyas SJ**, Davies N, Grant L, Imber CJ, Sharma D, Davidson BR, Malago M, Fusai G. Failure of portal venous embolization. ALPPS as salvage enabling successful resection of bilobar liver metastases. *J Gastrointest Cancer* 2014; **45** Suppl 1: 233-236 [PMID: 25081490 DOI: 10.1007/s12029-014-9643-6]

32 **Edmondson MJ**, Sodergren MH, Pucher PH, Darzi A, Li J, Petrowsky H, Campos RR, Serrablo A, Jiao LR. Variations and adaptations of associated liver partition and portal vein ligation for staged hepatectomy (ALPPS): Many routes to the summit. *Surgery* 2016; **159**: 1058-1072 [PMID: 26747229 DOI: 10.1016/j.surg.2015.11.013]

33 **van Lienden KP**, van den Esschert JW, de Graaf W, Bipat S, Lameris JS, van Gulik TM, van Delden OM. Portal vein embolization before liver resection: a systematic review. *Cardiovasc Intervent Radiol* 2013; **36**: 25-34 [PMID: 22806245 DOI: 10.1007/s00270-012-0440-y]

34 **Vennarecci G**, Laurenzi A, Santoro R, Colasanti M, Lepiane P, Ettorre GM. The ALPPS procedure: a surgical option for hepatocellular carcinoma with major vascular invasion. *World J Surg* 2014; **38**: 1498-1503 [PMID: 24146197 DOI: 10.1007/s00268-013-2296-y]

35 **Gauzolino R**, Castagnet M, Blanleuil ML, Richer JP. The ALPPS technique for bilateral colorectal metastases: three "variations on a theme". *Updates Surg* 2013; **65**: 141-148 [PMID: 23690242 DOI: 10.1007/s13304-013-0214-3]

36 **Schadde E**, Malagó M, Hernandez-Alejandro R, Li J, Abdalla E, Ardiles V, Lurje G, Vyas S, Machado MA, de Santibañes E. Monosegment ALPPS hepatectomy: extending resectability by rapid hypertrophy. *Surgery* 2015; **157**: 676-689 [PMID: 25712199 DOI: 10.1016/j.surg.2014.11.015]

37 **de Santibañes M**, Alvarez FA, Santos FR, Ardiles V, de Santibañes E. The associating liver partition and portal vein ligation for staged hepatectomy approach using only segments I and IV as future liver remnant. *J Am Coll Surg* 2014; **219**: e5-e9 [PMID: 24974268 DOI:

10.1016/j.jamcollsurg.2014.01.070]
38 **Petrowsky H**, Györi G, de Oliveira M, Lesurtel M, Clavien PA. Is partial-ALPPS safer than ALPPS? A single-center experience. *Ann Surg* 2015; **261**: e90-e92 [PMID: 25706390 DOI: 10.1097/SLA.0000000000001087]
39 **Brustia R**, Scatton O, Soubrane O. Variation on a Theme: Alternative to Plastic Bag in ALPPS Procedures: Feasibility and Clinical Safety of COVA+™ Membrane in ALPPS Procedures. *World J Surg* 2015; **39**: 3023-3027 [PMID: 26319257 DOI: 10.1007/s00268-015-3209-z]
40 **Dokmak S**, Belghiti J. Which limits to the "ALPPS" approach? *Ann Surg* 2012; **256**: e6; author reply e16-e17 [PMID: 22895355 DOI: 10.1097/SLA.0b013e318265fd64]
41 **Girometti R**, Como G, Bazzocchi M, Zuiani C. Post-operative imaging in liver transplantation: state-of-the-art and future perspectives. *World J Gastroenterol* 2014; **20**: 6180-6200 [PMID: 24876739 DOI: 10.3748/wjg.v20.i20.6180]
42 **Fulcher AS**, Turner MA. Orthotopic liver transplantation: evaluation with MR cholangiography. *Radiology* 1999; **211**: 715-722 [PMID: 10352596 DOI: 10.1148/radiology.211.3.r99jn17715]
43 **Ito K**, Siegelman ES, Stolpen AH, Mitchell DG. MR imaging of complications after liver transplantation. *AJR Am J Roentgenol* 2000; **175**: 1145-1149 [PMID: 11000180 DOI: 10.2214/ ajr.175.4.1751145]
44 **Girometti R**, Cereser L, Bazzocchi M, Zuiani C. Magnetic resonance cholangiography in the assessment and management of biliary complications after OLT. *World J Radiol* 2014; **6**: 424-436 [PMID: 25071883 DOI: 10.4329/wjr.v6.i7.424]
45 **Palmucci S**. Focal liver lesions detection and characterization: The advantages of gadoxetic acid-enhanced liver MRI. *World J Hepatol* 2014; **6**: 477-485 [PMID: 25067999 DOI: 10.4254/wjh.v6.i7.477]
46 **Seale MK**, Catalano OA, Saini S, Hahn PF, Sahani DV. Hepatobiliary-specific MR contrast agents: role in imaging the liver and biliary tree. *Radiographics* 2009; **29**: 1725-1748 [PMID: 19959518 DOI: 10.1148/rg.296095515]
47 **Matos AP**, Velloni F, Ramalho M, AlObaidy M, Rajapaksha A, Semelka RC. Focal liver lesions: Practical magnetic resonance imaging approach. *World J Hepatol* 2015; **7**: 1987-2008 [PMID: 26261689 DOI: 10.4254/wjh.v7.i16.1987]
48 **Valls C**, Ruiz S, Martinez L, Leiva D. Radiological diagnosis and staging of hilar cholangiocarcinoma. *World J Gastrointest Oncol* 2013; **5**: 115-126 [PMID: 23919105 DOI: 10.4251/wjgo.v5.i7.115]
49 **Catalano OA**, Singh AH, Uppot RN, Hahn PF, Ferrone CR, Sahani DV. Vascular and biliary variants in the liver: implications for liver surgery. *Radiographics* 2008; **28**: 359-378 [PMID: 18349445 DOI: 10.1148/rg.282075099]
50 **Adams RB**, Aloia TA, Loyer E, Pawlik TM, Taouli B, Vauthey JN; Americas Hepato-Pancreato-Biliary Association; Society of Surgical Oncology; Society for Surgery of the Alimentary Tract. Selection for hepatic resection of colorectal liver metastases: expert consensus statement. *HPB* (Oxford) 2013; **15**: 91-103 [PMID: 23297719 DOI: 10.1111/j.1477-2574.2012.00557.x]
51 **Ferrero A**, Viganò L, Polastri R, Muratore A, Eminefendic H, Regge D, Capussotti L. Postoperative liver dysfunction and future remnant liver: where is the limit? Results of a prospective study. *World J Surg* 2007; **31**: 1643-1651 [PMID: 17551779 DOI: 10.1007/s00268- 007-9123-2]
52 **Shoup M**, Gonen M, D'Angelica M, Jarnagin WR, DeMatteo RP, Schwartz LH, Tuorto S, Blumgart LH, Fong Y. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. *J Gastrointest Surg* 2003; **7**: 325-330 [PMID: 12654556 DOI: 10.1016/S1091-255X(02)00370-0]
53 **Schindl MJ**, Redhead DN, Fearon KC, Garden OJ, Wigmore SJ; Edinburgh Liver Surgery and Transplantation Experimental Research Group (eLISTER). The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. *Gut* 2005; **54**: 289-296 [PMID: 15647196 DOI: 10.1136/gut.2004.046524]
54 **Vivarelli M**, Vincenzi P, Montalti R, Fava G, Tavio M, Coletta M, Vecchi A, Nicolini D, Agostini A, Ahmed EA, Giovagnoni A, Mocchegiani F. ALPPS Procedure for Extended Liver Resections: A Single Centre Experience and a Systematic Review. *PLoS One* 2015; **10**: e0144019 [PMID: 26700646 DOI: 10.1371/journal.pone.0144019]
55 **Balzan S**, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828, discussion 828-829 [PMID: 16327492 DOI: 10.1097/01.sla.0000189131.90876.9e]
56 **Mulé S**, Colosio A, Cazejust J, Kianmanesh R, Soyer P, Hoeffel C. Imaging of the postoperative liver: review of normal appearances and common complications. *Abdom Imaging* 2015; **40**: 2761-2776 [PMID: 26023007 DOI: 10.1007/s00261-015-0459-z]
57 **Melamud K**, LeBedis CA, Anderson SW, Soto JA. Biliary imaging: multimodality approach to imaging of biliary injuries and their complications. *Radiographics* 2014; **34**: 613-623 [PMID: 24819784 DOI: 10.1148/rg.343130011]
58 **Hoeffel C**, Azizi L, Lewin M, Laurent V, Aubé C, Arrivé L, Tubiana JM. Normal and pathologic features of the postoperative biliary tract at 3D MR cholangiopancreatography and MR imaging. *Radiographics* 2006; **26**: 1603-1620 [PMID: 17102039 DOI: 10.1148/rg.266055730]
59 **Yamashita Y,** Hamatsu T, Rikimaru T, Tanaka S, Shirabe K, Shimada M, Sugimachi K. Bile Leakage After Hepatic Resection. *Annals of Surgery* 2001; **233:** 45-50
60 **Tirumani SH**, Shanbhogue AK, Vikram R, Prasad SR, Menias CO. Imaging of the porta hepatis: spectrum of disease. *Radiographics* 2014; **34**: 73-92 [PMID: 24428283 DOI: 10.1148/rg.341125190]
61 **Parvey HR**, Raval B, Sandler CM. Portal vein thrombosis: imaging findings. *AJR Am J Roentgenol* 1994; **162**: 77-81 [PMID: 8273695 DOI: 10.2214/ajr.162.1.8273695]
62 **Petrovai G**, Truant S, Langlois C, Bouras AF, Lemaire S, Buob D, Leteurtre E, Boleslawski E, Pruvot FR. Mechanisms of splenic hypertrophy following hepatic resection. *HPB* (Oxford) 2013; **15**: 919-927 [PMID: 23458075 DOI: 10.1111/hpb.12056]
63 **Jacobs KE**, Visser BC, Gayer G. Changes in spleen volume after resection of hepatic colorectal metastases. *Clin Radiol* 2012; **67**: 982-987 [PMID: 22608244 DOI: 10.1016/j.crad.2012.03.013]
64 **Yin S**, Wang H, Park O, Wei W, Shen J, Gao B. Enhanced liver regeneration in IL-10-deficient mice after partial hepatectomy via stimulating inflammatory response and activating hepatocyte STAT3. *Am J Pathol* 2011; **178**: 1614-1621 [PMID: 21435447 DOI: 10.1016/j.ajpath.2011.01.001]
65 **Ward J**, Sheridan MB, Guthrie JA, Davies MH, Millson CE, Lodge JP, Pollard SG, Prasad KR, Toogood GJ, Robinson PJ. Bile duct strictures after hepatobiliary surgery: assessment with MR cholangiography. *Radiology* 2004; **231**: 101-108 [PMID: 14990819 DOI: 10.1148/radiol.2311030017]
66 **Sharma S**, Gurakar A, Jabbour N. Biliary strictures following liver transplantation: past, present and preventive strategies. *Liver Transpl* 2008; **14**: 759-769 [PMID: 18508368 DOI: 10.1002/lt.21509]
67 **Boraschi P**, Donati F. Biliary-enteric anastomoses: spectrum of findings on Gd-EOB-DTPA-enhanced MR cholangiography. *Abdom Imaging* 2013; **38**: 1351-1359 [PMID: 23820693 DOI: 10.1007/s00261-013-0007-7]

P- Reviewer: Shan YF, Richter B **S- Editor**: Song XX **L- Editor**: A
E- Editor: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2017 June 8; 9(16): 746-751

DOI: 10.4254/wjh.v9.i16.746

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Prospective Study

Low bone mineral density and the severity of cholestasis in biliary atresia

Krittapak Homchan, Tawatchai Chaiwatanarat, Wanvisa Udomsinprasert, Voranush Chongsrisawat, Yong Poovorawan, Sittisak Honsawek

Krittapak Homchan, Voranush Chongsrisawat, Yong Poovorawan, Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok 10330, Thailand

Tawatchai Chaiwatanarat, Department of Radiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Wanvisa Udomsinprasert, Sittisak Honsawek, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Author contributions: Homchan K carried out laboratory work, collected the data, and analyzed the data; Udomsinprasert W collected blood samples and assisted in analysis of data; Chaiwatanarat T, Chongsrisawat V and Poovorawan Y examined all the patients and collected clinical data; Honsawek S designed the study, carried out laboratory work, analyzed the data, wrote the manuscript, and revised the manuscript for final submission.

Supported by The Thailand Research Fund (RSA5880019); the Research Chair Grant from the National Science and Technology Development Agency; and National Research University Project, through the Ageing Cluster (NRU59-056-AS), Chulalongkorn University.

Institutional review board statement: This study was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University.

Informed consent statement: All study participants provided written informed consent prior to study enrollment.

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

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at sittisak.h@chula.ac.th. Participants gave informed consent for data sharing.

Manuscript source: Invited manuscript

Correspondence to: Sittisak Honsawek, MD, PhD, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, 1873 Rama IV Road, Patumwan, Bangkok 10330, Thailand. sittisak.h@chula.ac.th
Telephone: +66-2-2564482
Fax: +66-2-2564482

Received: January 30, 2017
Peer-review started: February 12, 2017
First decision: March 28, 2017
Revised: April 19, 2017
Accepted: May 12, 2017
Article in press: May 15, 2017
Published online: June 8, 2017

Abstract

AIM

To investigate the prevalence of osteopenia and osteoporosis in postoperative biliary atresia (BA) children and the association of bone mineral density (BMD) and biochemical parameters in postKasai BA subjects.

METHODS

A total of 70 patients with postKasai BA were enrolled in this prospective study. The patients were classified into two groups according to their jaundice status. BMD of the lumbar spine was analyzed using dual energy

X-ray absorptiometry.

RESULTS

The prevalence of low bone mass (osteopenia and osteoporosis) in BA patients were 51.4% (36 out of 70). Ten patients (35.7%) in the jaundice group and 8 patients (19.0%) in the non-jaundice group had osteopenia. Sixteen patients (57.1%) in the jaundice group and 2 patients (4.8%) in the no jaundice group had osteoporosis. In addition, lumbar spine BMD Z-score was substantially lower in the jaundice BA patients compared with non-jaundice patients. BA subjects with persistent jaundice had significantly lower serum 25-hydroxyvitamin D than those without jaundice. Further analysis revealed that lumbar spine BMD was correlated with age (r = 0.774, P < 0.001), serum albumin (r = 0.333, P = 0.005), total bilirubin (r = -0.476, P < 0.001), aspartate aminotransferase (r = -0.583, P < 0.001), alanine aminotransferase (r = -0.428, P < 0.001), and alkaline phosphatase(r = -0.456, P < 0.001).

CONCLUSION

Low BMD was associated with biochemical parameters reflecting the severity of cholestasis in postKasai BA patients.

Key words: Bone mineral density; Jaundice; Biliary atresia; Cholestasis; Severity

Core tip: Recent evidences have highlighted the importance of bone mineral density (BMD) in chronic liver disease including biliary atresia (BA). This study revealed that BA patients with persistent jaundice had significantly lower BMD and 25-hydroxyvitamin D than those without jaundice. Furthermore, lumbar spine BMD was correlated with hepatic dysfunction suggesting that low BMD was associated with outcome parameters reflecting the severity of cholestasis in postoperative BA patients.

Homchan K, Chaiwatanarat T, Udomsinprasert W, Chongsrisawat V, Poovorawan Y, Honsawek S. Low bone mineral density and the severity of cholestasis in biliary atresia. *World J Hepatol* 2017; 9(16): 746-751 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i16/746.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i16.746

INTRODUCTION

Biliary atresia (BA) is a progressive, idiopathic, necro-inflammatory process resulting in obliteration of the extrahepatic biliary tree resulting in intrahepatic cholestasis, hepatic fibrosis, biliary cirrhosis, and advanced chronic liver failure[1]. It is a rare disease, with the reported prevalence ranging from 1 in 5000 to 1 in 19000 live births[2]. It is the most common cause of neonatal jaundice for which surgery is indicated and also the most common indication for liver transplantation in children. The pathogenesis of BA has remained a mystery. Most of the causal theories include defects resulting from a viral infection or toxin exposure, defects in morphogenesis, genetic predisposition, defects in prenatal circulation and immune dysregulation[3-5].

Low bone mass is frequent in patients with chronic liver disorder including BA. Metabolic bone disease is a common disorder that can be found in patients with hepatic osteodystrophy, particularly those affected by chronic cholestasis[6,7]. Its etiology is complex and multifactorial and presents as osteopenia and osteoporosis which should be investigated and diagnosed early in patients with chronic liver disease in order to minimize the risk of fractures and improve their quality of life[8,9]. The purpose of this study was to determine bone mineral density (BMD) from postKasai BA children and to investigate the association of BMD and outcome parameters in postoperative BA patients.

MATERIALS AND METHODS

Patients

This investigation was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University and was conducted in compliance with the Declaration of Helsinki. All parents of BA children were informed of the study's objectives, and written informed consent was derived from the parents prior to the participants entering the study.

A total of 70 postKasai BA subjects (30 males and 40 females; mean age 7.6 ± 0.5 years) who attended the follow-up visit in Pediatric Liver Clinic at King Chulalongkorn Memorial Hospital were recruited in the present study. Among the 70 BA children in this study, none of them had any evidence of residual infection or ascending cholangitis or clotting abnormalities during venipuncture. None had experienced liver transplantation. To compare the clinical outcomes among BA subjects, they were allocated into two groups corresponding to their levels of serum total bilirubin (TB): Non-jaundiced group (TB < 2.0 mg/dL, n = 42) and persistently jaundiced group (TB ⩾ 2.0 mg/dL, n = 28).

Laboratory tests

Venous blood specimens were procured from each subject, centrifuged, and then kept at -80 ℃ until measurement. Liver function tests including TB, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assessed using Hitachi 912 automated chemical analyzer at the central laboratory of our hospital. Serum 25-hydroxyvitamin D [25(OH)D] levels were analyzed using automated chemiluminescent immunoassay (Diasorin, Saluggia, Italy).

BMD assessments

Dual-energy X-ray absorptiometry scans (Hologic QDR

Table 1 Demographic data and laboratory parameters of biliary atresia patients based on status of jaundice

BA patients	Total	Jaundice	No jaundice	*P*-value
n	70	28	42	
Gender (male/female)	30:40	12:16	18:24	0.5
Age (yr)	7.6 ± 0.5	6.3 ± 0.8	8.6 ± 0.6	0.01
Albumin (g/dL)	3.9 ± 0.1	3.2 ± 0.3	4.3 ± 0.1	< 0.001
Total bilirubin (mg/dL)	3.8 ± 0.7	8.2 ± 1.5	0.9 ± 0.1	< 0.001
Direct bilirubin (mg/dL)	2.5 ± 0.6	5.8 ± 1.1	0.2 ± 0.1	< 0.001
AST (IU/L)	148.8 ± 13.7	235.9 ± 20.9	90.8 ± 11.3	< 0.001
ALT (IU/L)	133.3 ± 12.8	183.4 ± 18.4	99.8 ± 15.7	0.001
ALP (IU/L)	501.7 ± 36.3	681.6 ± 46.3	381.8 ± 43.3	< 0.001
25(OH)D (ng/mL)	25.3 ± 1.1	16.0 ± 1.8	30.1 ± 0.7	< 0.001
Lumbar BMD (g/cm^2)	0.5 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	< 0.001
Lumbar BMD Z-score	-1.2 ± 0.2	-2.3 ± 0.2	-0.4 ± 0.1	< 0.001

Data are expressed as mean and SEM. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BA: Biliary atresia; BMD: Bone mineral density; 25(OH)D: 25-hydroxyvitamin D.

2000, Hologic Inc., Waltham, MA, United States) were performed on the lumbar spine (anteroposterior lumbar vertebrae L1-L4) of every subject for BMD assessments. BMD was reported as grams of mineral per square centimeter (g/cm^2) and Z-scores. Z-scores of BMD were expressed as numbers of standard deviations from the mean BMD of age matched norms. Children were categorized into normal, osteopenia, and osteoporosis based on World Health Organization (WHO) criteria. Osteoporosis was designated as a lumbar spine BMD equal to or exceeding 2.5 standard deviations (SD) below the average values (Z score $\leqslant$ -2.5). Osteopenia was designated as a lumbar spine BMD below 2.5 SD but above 1 SD under the average values (-2.5 < Z score < -1.0). Normal BMD was designated as a lumbar spine BMD equal to or below 1 SD under the average values (Z score $\geqslant$ -1.0).

Statistical analysis

Statistical analysis was performed using the statistical package for social sciences software, version 22.0 for Windows. All values are expressed as a mean ± standard error. Demographic and clinical data between groups were compared by χ^2 tests and unpaired Student's *t* tests, where appropriate. Comparisons of clinical data and biochemical markers among patients with normal, osteopenia, and osteoporosis were analyzed using one-way analysis of variance (ANOVA) with Tukey post hoc test if ANOVA showed significance. Correlations between numerical data were acquired using the Pearson correlation coefficient (*r*). A *P*-value < 0.05 indicated statistically significant.

RESULTS

Comparisons between BA subjects with and without persistent jaundice

Seventy postKasai BA patients were enrolled in this prospective study. The characteristics and laboratory parameters of BA children with persistent jaundice compared to BA children without jaundice are described in Table 1. Jaundice BA subjects had markedly lower serum albumin levels than non-jaundice BA children. On the other hand, serum bilirubin, AST, ALT, ALP were considerably higher in BA cases with jaundice than those without jaundice. Subsequent analysis demonstrated that lumbar spine BMD and serum 25-hydroxyvitamin D values of jaundice BA subjects were significantly lower than those of non-jaundice BA subjects ($P < 0.001$).

Correlation of lumbar spine BMD and outcome parameters in BA subjects

The prevalence of low bone mass (osteopenia and osteoporosis) in BA subjects were 51.4% (36 out of 70). Ten patients (35.7%) in the jaundice group and 8 patients (19.0%) in the non-jaundice group had osteopenia. Sixteen patients (57.1%) in the jaundice group and 2 patients (4.8%) in the no jaundice group had osteoporosis. Subsequently, BA patients were divided into tertiles based on the WHO criteria. The first tertile included 34 patients with BMD Z-scores from 0 to -1 (considered as normal), the second tertile included 18 patients with Z-scores from -1.0 to -2.5 (considered as osteopenia), and the third tertile included 18 patients with Z-score lower than -2.5 (considered as osteoporosis). There was no statistically significant difference in gender and age distribution among the three tertiles (Table 2). However, serum albumin, serum bilirubin, AST, ALT, serum 25(OH)D and lumbar spine BMD were significantly different between the three tertiles. Further analysis revealed that lumbar spine BMD was correlated with age ($r = 0.774$, $P < 0.001$), serum albumin ($r = 0.333$, $P = 0.005$), TB ($r = -0.476$, $P < 0.001$), AST ($r = -0.583$, $P < 0.001$), ALT ($r = -0.428$, $P < 0.001$), and ALP ($r = -0.456$, $P < 0.001$). The correlations between lumbar spine BMD, age, serum albumin, serum TB, AST, ALT, ALP are illustrated in Figure 1.

DISCUSSION

BA is a serious cholestatic liver disease in neonates. The obstruction of bile flow in BA results in worsening

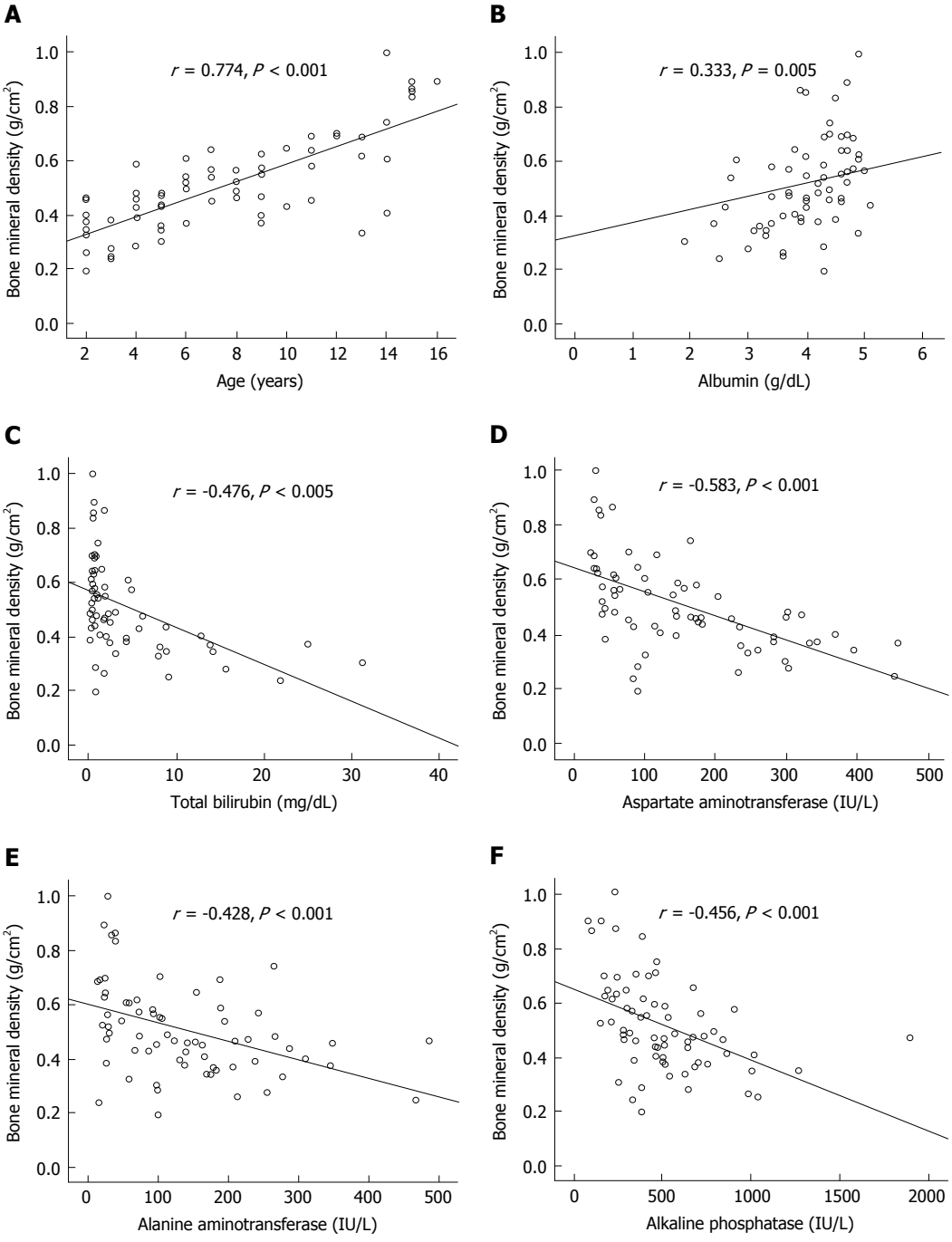

Figure 1 Scatter diagram and correlation analysis in biliary atresia patients. Lumbar spine bone mineral density are correlated with age (A), serum albumin (B), total bilirubin (C), aspartate aminotransferase (D), alanine aminotransferase (E), alkaline phosphatase (F).

cholestasis, liver fibrosis and cirrhosis, which lead to portal hypertension and eventually end-stage liver failure in children. Early diagnosis and timely Kasai porto-enterostomy to restore bile flow can help avoid the need of liver transplantation during childhood in a number of patients[10]. Despite a number of extensive clinical research studies on BA, the etiology and pathogenesis of BA are largely unknown.

In the recent years, serum 25-hydroxyvitamin D level was decreased in BA patients with low BMD[11]. Additionally, circulating leptin and osteoprotegerin levels has been shown to be correlated with BMD and the presence of jaundice in BA, suggesting that leptin and osteoprotegerin could play a pontential role in maintaining bone mass of BA patients[12,13].

The current study showed that postoperative BA patients with jaundice had significantly lower lumbar spine BMD than those without jaundice. Moreover, we have illustrated that the prevalence rates of osteopenia and osteoporosis in jaundiced BA subjects were higher in comparison with those in non-jaundiced children. Further analysis revealed an inverse association between lumbar spine BMD and serum TB and liver synthetic function. The explanation for these findings may be attributable to

Table 2 Comparison of clinical characteristics and laboratory parameters among biliary atresia patients with normal, osteopenic, and osteoporotic bone mineral density Z-scores at the lumbar spine

Characteristics	Normal	Osteopenia	Osteoporosis	*P*-value
n	34	18	18	
Gender (male/female)	15/19	7/11	8/10	0.3
Age (yr)	8.2 ± 0.7	7.7 ± 1.1	6.5 ± 1.0	0.4
Albumin (g/dL)	4.1 ± 0.2	4.0 ± 0.1	3.3 ± 0.2	< 0.05
Total bilirubin (mg/dL)	1.0 ± 0.2	2.8 ± 0.7	10.0 ± 2.1	< 0.001
Direct bilirubin (mg/dL)	0.4 ± 0.1	1.6 ± 0.5	7.3 ± 1.7	< 0.001
AST (IU/L)	95.6 ± 13.7	177.1 ± 24.8	221.2 ± 31.2	< 0.001
ALT (IU/L)	104.2 ± 18.2	164.6 ± 23.7	156.8 ± 25.1	< 0.001
ALP (IU/L)	429.1 ± 55.7	538.4 ± 55.2	602.3 ± 71.3	0.08
25(OH)D (ng/mL)	33.2 ± 0.7	26.3 ± 0.5	14.3 ± 1.5	< 0.01
Lumbar BMD (g/cm^2)	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	< 0.001

Data are expressed as mean and SEM. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BA: Biliary atresia; BMD: Bone mineral density; 25(OH)D: 25-hydroxyvitamin D.

decreased osteoblastic function or increased osteoclastic resorption in BA patients. It has been documented that osteoblast proliferation was inhibited by unconjugated bilirubin *in vitro* and by the serum of jaundiced patients, indicating that bilirubin might have a direct effect on bone metabolism[14,15]. A number of BA cases eventually become advanced stage of liver disease and pediatric liver transplantation is the treatment strategy of choice for improving quality of life in BA children. Recent study has reported that successful liver transplantation could improve biochemical markers of bone formation and resorption suggesting acceleration of growth process in BA children[16]. However, the connection between cholestasis and low bone mass in BA patients merits further investigations.

Some caveats need to be acknowledged regarding the current study. First, the number of patients and controls enrolled in the present study was relative small. This could reduce the statistical power of these results. Accordingly, prospective longitudinal study with a larger population is warranted to elucidate the exact relationship between BMD, outcome parameters, and the severity in BA subjects. Secondly, inadequate measurement of plausible confounding factors including comorbidities needed to be taken under advisement. Moreover, another limitation of our study is the lack of Child-Pugh and Model for End-Stage Liver Disease (MELD) scores. Future study is also required to evaluate the Child-Pugh and MELD values for predicting of chronic liver disease severity. Ultimately, the paucity of quantitative bone histomorphometry analysis which may render evidence as to whether bone was correlated with BMD data. Therefore, more research will be needed in order to better comprehend the precise role of bone mass in the severity of postKasai BA.

To summarize, the current study demonstrated that BA subjects with persistent jaundice had significantly lower BMD than those without jaundice. Additionally, lumbar spine BMD was correlated with hepatic dysfunction suggesting that low BMD was associated with outcome parameters reflecting the severity of cholestasis in postKasai BA patients.

COMMENTS

Background

Biliary atresia (BA) is a severe congenital cholestatic liver disease with an unknown etiology. Metabolic bone disorder (osteopenia and osteoporosis) can be complicated by existing chronic liver diseases including BA. There is evidence that serum markers of bone metabolism correlated with the degree of jaundice in BA.

Research frontiers

In recent years, much research has revealed that vitamin D deficiency is associated with the severity of hepatic fibrosis or reduced bone mineral density (BMD) in patients with chronic liver disease. This study showed that lumbar spine BMD and 25-hydroxyvitamin D level in BA patients with jaundice were lower than those without jaundice. Moreover, low BMD was associated with serum bilirubin and liver function.

Innovations and breakthroughs

Jaundiced BA patients showed significantly lower lumbar spine BMD and 25-hydroxyvitamin D than in non-jaundiced BA patients. Additionally, lumbar spine BMD correlated with hepatic function markers, which reflect the severity of cholestasis in postKasai BA patients.

Applications

BMD could be used to assist clinicians in assessing the progression of cholestasis. This study highlights the need of vitamin D supplementation and its potential in maintaining bone mass in persistently jaundiced BA children.

Terminology

BMD is the amount of bone mineral per unit volume of the bone tissue and is used as an indirect parameter of bone health. BMD measurements of the patients are generally compared to those from age-matched population and are expressed as Z-score. Osteopenia is defined as Z-score between -1 and -2.5, and osteoporosis as Z-score < -2.5.

Peer-review

A very interesting study to explore the prevalence of osteopenia and osteoporosis in post-Kasai BA children and the association of bone mineral density and biochemical parameters in postoperative BA patients.

REFERENCES

1 **Kobayashi H**, Stringer MD. Biliary atresia. *Semin Neonatol* 2003; **8**: 383-391 [PMID: 15001126 DOI: 10.1016/S1084-2756(03)00065-4]
2 **Balistreri WF**, Grand R, Hoofnagle JH, Suchy FJ, Ryckman FC, Perlmutter DH, Sokol RJ. Biliary atresia: current concepts and

research directions. Summary of a symposium. *Hepatology* 1996; **23**: 1682-1692 [PMID: 8675193 DOI: 10.1002/hep.510230652]
3 **Bassett MD**, Murray KF. Biliary atresia: recent progress. *J Clin Gastroenterol* 2008; **42**: 720-729 [PMID: 18496390 DOI: 10.1097/MCG.0b013e3181646730]
4 **Erlichman J**, Hohlweg K, Haber BA. Biliary atresia: how medical complications and therapies impact outcome. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 425-434 [PMID: 19673629 DOI: 10.1586/egh.09.30]
5 **A-Kader HH**, Abdel-Hameed A, Al-Shabrawi M, Mohsen N, El-Karaksy H, Hassanein B, Elsayed B, Abdel-Khalik MK, Karjoo M. Is biliary atresia an autoimmune disease? *Eur J Gastroenterol Hepatol* 2003; **15**: 447 [PMID: 12655270]
6 **Rouillard S**, Lane NE. Hepatic osteodystrophy. *Hepatology* 2001; **33**: 301-307 [PMID: 11124849 DOI: 10.1053/jhep.2001.20533]
7 **Pusl T**, Beuers U. Extrahepatic manifestations of cholestatic liver diseases: pathogenesis and therapy. *Clin Rev Allergy Immunol* 2005; **28**: 147-157 [PMID: 15879620 DOI: 10.1385/CRIAI: 28: 2: 147]
8 **Sanchez AJ**, Aranda-Michel J. Liver disease and osteoporosis. *Nutr Clin Pract* 2006; **21**: 273-278 [PMID: 16772544 DOI: 10.1177/0115426506021003273]
9 **Okada T**, Honda S, Miyagi H, Minato M, Taketomi A. Hepatic osteodystrophy complicated with bone fracture in early infants with biliary atresia. *World J Hepatol* 2012; **4**: 284-287 [PMID: 23293713 DOI: 10.4254/wjh.v4.i10.284]
10 **Hartley JL**, Davenport M, Kelly DA. Biliary atresia. *Lancet* 2009; **374**: 1704-1713 [PMID: 19914515 DOI: 10.1016/S0140-6736(09)60946-6]
11 **Chongsrisawat V**, Ruttanamongkol P, Chaiwatanarat T, Chandrakamol B, Poovorawan Y. Bone density and 25-hydroxyvitamin D level in extrahepatic biliary atresia. *Pediatr Surg Int* 2001; **17**: 604-608 [PMID: 11727049 DOI: 10.1007/s003830100003]
12 **Honsawek S**, Chaiwatanarat T, Chongsrisawat V, Thawornsuk N, Vejchapipat P, Poovorawan Y. Circulating leptin levels and bone mineral density in children with biliary atresia. *Acta Paediatr* 2008; **97**: 206-211 [PMID: 18177445 DOI: 10.1111/j.1651-2227.2007.00596.x]
13 **Honsawek S**, Chaiwatanarat T, Vejchapipat P, Chongsrisawat V, Thawornsuk N, Poovorawan Y. Relationships between OPG, RANKL, bone metabolism, and bone mineral density in biliary atresia. *Pediatr Surg Int* 2009; **25**: 261-267 [PMID: 19184056 DOI: 10.1007/s00383-009-2325-y]
14 **Ruiz-Gaspà S**, Dubreuil M, Guañabens N, Combalia A, Peris P, Monegal A, Parés A. Ursodeoxycholic acid decreases bilirubin-induced osteoblast apoptosis. *Eur J Clin Invest* 2014; **44**: 1206-1214 [PMID: 25331234 DOI: 10.1111/eci.12355]
15 **Janes CH**, Dickson ER, Okazaki R, Bonde S, McDonagh AF, Riggs BL. Role of hyperbilirubinemia in the impairment of osteoblast proliferation associated with cholestatic jaundice. *J Clin Invest* 1995; **95**: 2581-2586 [PMID: 7769100 DOI: 10.1172/JCI117959]
16 **Teisseyre M**, Pawłowska J, Kryśkiewicz E, Karczmarewicz E, Czubkowski P, Dadalski M, Jankowska I, Teisseyre J, Ismail H, Lorenc R. Bone mineral metabolism in children with biliary atresia after living related liver transplantation. Evaluation of selected parameters. *Ann Transplant* 2007; **12**: 19-25 [PMID: 18173062]

P- Reviewer: Balaban YH, Chiu KW, Kaya M **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.f6publishing.com

World J Hepatol 2017 June 8; 9(16): 752-756

DOI: 10.4254/wjh.v9.i16.752

ISSN 1948-5182 (online)

CASE REPORT

Successful surgical resection of ruptured cholangiolocellular carcinoma: A rare case of a primary hepatic tumor

Shota Akabane, Takushiro Ban, Shunsaku Kouriki, Hiroyuki Tanemura, Haruhiro Nakazaki, Masayuki Nakano, Nobuaki Shinozaki

Shota Akabane, Takushiro Ban, Shunsaku Kouriki, Hiroyuki Tanemura, Haruhiro Nakazaki, Nobuaki Shinozaki, Department of Surgery, Shonan Fujisawa Tokushukai Hospital, Fujisawa City, Kanagawa 251-0041, Japan

Masayuki Nakano, Department of Diagnostic Pathology, Shonan Fujisawa Tokushukai Hospital, Fujisawa City, Kanagawa 251-0041, Japan

Author contributions: All authors contributed to the acquisition of the data and to the writing and revision of this manuscript.

Institutional review board statement: This case report was reviewed and approved by Shonan Fujisawa Tokushukai Hospital Institutional Review Board.

Informed consent statement: The patient has provided informed consent for the publication of this case report and accompanying images.

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

Manuscript source: Unsolicited manuscript

Correspondence to: Shota Akabane, MD, Department of Surgery, Shonan Fujisawa Tokushukai Hospital, 1-5-1 Kandai Tsujido, Fujisawa City, Kanagawa 251-0041, Japan. akap.sh.3381@gmail.com
Telephone: +81-80-34641295
Fax: +81-426-745266

Received: January 17, 2017
Peer-review started: January 19, 2017
First decision: March 8, 2017
Revised: April 3, 2017
Accepted: April 23, 2017
Article in press: April 24, 2017
Published online: June 8, 2017

Abstract

Spontaneous rupture is one of the most fatal complications of hepatic tumors such as hepatocellular carcinoma. In fact, many studies have shown that the in-hospital and 30-d mortality rates are as high as 25%-100%. Cholangiolocellular carcinoma (CoCC) is a rare primary hepatic tumor, usually small in size, that is thought to originate from the ductules and/or canals of Hering. Here, we present a case of spontaneous rupture of a CoCC that was successfully resected by radical surgery. Although CoCC is a rare primary hepatic tumor, it demonstrates certain specific clinical features, including a better prognosis than for other primary liver cancers, and thus should be distinguished from those other cancers. Moreover, CoCC can appear as a ruptured huge tumor, and when it does, radical hepatectomy can be an effective measure to achieve both absolute hemostasis and curability of tumor.

Key words: Hepatic tumor; Rupture; Cholangiolocellular carcinoma; Resection; Pathology

Core tip: Spontaneous rupture is one of the most fatal complications of hepatic tumors such as hepatocellular carcinoma. Here, we present a case of spontaneous rupture of a cholangiolocellular carcinoma (CoCC) that was successfully resected by radical surgery. Although CoCC is a rare primary hepatic tumor, it demonstrates certain specific clinical features, including a better prognosis

than for other primary liver cancers, and thus should be distinguished from those other cancers. Moreover, CoCC can appear as a ruptured huge tumor, and when it does, radical hepatectomy can be an effective measure to achieve both absolute hemostasis and curability of tumors.

Akabane S, Ban T, Kouriki S, Tanemura H, Nakazaki H, Nakano M, Shinozaki N. Successful surgical resection of ruptured cholangiolocellular carcinoma: A rare case of a primary hepatic tumor. *World J Hepatol* 2017; 9(16): 752-756 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i16/752.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i16.752

INTRODUCTION

Spontaneous tumor rupture is one of the most fatal complications of hepatic tumors such as hepatocellular carcinoma (HCC). In fact, many studies have shown that the in-hospital and 30-d mortality rates are as high as 25%-100%[1]. Cholangiolocellular carcinoma (CoCC) is a rare primary hepatic tumor first described by Steiner and Higginson[2]. Subsequent reports characterized it based on small cords resembling cholangioles (canals of Hering). Although CoCC was previously classified as a special type of intrahepatic cholangiocarcinoma (ICC), as a result of recent advancements in the field, it is now considered to originate from hepatic stem/progenitor cells.

Here, we present a case of spontaneous rupture of a CoCC that was successfully resected by radical surgery.

CASE REPORT

An 80-year-old Japanese woman presented with right upper abdominal pain that had developed within 2-3 h along with hypotension. She had not experienced vomiting or diarrhea. Her medical history included hypertension and dyslipidemia. On physical examination, there was tenderness in the right upper abdomen, and her body temperature was 36.5 ℃. Laboratory tests were negative for anemia and thrombocytopenia and revealed bilirubin, transaminase and albumin levels in the normal range. A contrast-enhanced computed tomography (CT) scan showed a huge tumor (12 cm × 7 cm × 9 cm) located in the right anterior segment of the liver along with extrahepatic hematoma (Figure 1).

The tumor was hyperattenuating relative to the noncancerous liver parenchyma in the arterial phase and was hypo- or isoattenuating in the delayed phase, with central necrosis appearing as a low-density area. The axial T1-weighted gradient-echo image showed a hypointense mass in the right anterior segment of the liver, and the axial T2-weighted spin-echo image with fat suppression showed an isointense mass with a large central hyperintense area (Figure 2). In addition, the penetrating portal tract showed hyperintensity.

The patient was subsequently diagnosed with a ruptured hepatic tumor. Although emergency transcatheter arterial embolization (TAE) was considered, she was hemodynamically stable due to fluid resuscitation and blood transfusion. As a result, primary right hemihepatectomy was performed. Following surgery, the patient was admitted to the intensive care unit and transferred to the general ward 5 d after surgery. Although it took time to improve her nutritional status and to rehabilitate her, she was discharged 30 d later without any complications.

Histologically, the tumor was mainly composed of small, monotonous glands formed into antler-like anastomosing patterns, embedded in the fibrous stroma to various degrees of fibrous stroma and lacking mucin production (Figure 3). The presence of CoCC cells was confirmed by positive staining for cytokeratin 19 (CK19) and membranous positive staining for epithelial membrane antigen (EMA), but no positive staining for hepatocyte paraffin 1 (HepPar1) was present (Figure 4).

So far, the patient has attended the outpatient clinic for follow-up for 1 year after surgery, with no signs of recurrence detected on CT scans.

DISCUSSION

This case highlights two important considerations. First, although CoCC is a rare primary hepatic tumor, it demonstrates certain specific clinical features and hence should be distinguished from other primary liver cancers, such as HCC or ICC. Second, to the best of our knowledge, this is the first report describing the presentation of a CoCC with spontaneous tumor rupture that was successfully resected.

CoCC is derived from the cholangioles or canals of Hering and is characterized by small cords resembling cholangioles and ductular reaction-like anastomosing glands in abundant fibrous stroma. The canals of Hering are found in portal tracts of all sizes, where the canals connect with the bile duct. The ductules contain hepatic progenitor cells that can differentiate into both hepatocytes and cholangiocytes. Therefore, in the case of tumors derived from hepatic progenitor cells, characteristics of hepatocytic and cholangiocytic differentiation can be alternately displayed within the same tumor.

The clinical characteristics and imaging features of CoCC are similar to those of HCC and ICC. Many CoCC patients are infected with hepatitis C virus or hepatitis B virus, and angiographical hypervascularity is one of the characteristics of CoCC[3]; therefore, it is not surprising that CoCC has often been mistaken for HCC in the clinic[4].

Histologically, the presence of CoCC cells is further confirmed by either positive staining for CK19 or membranous positive staining for mucin core protein 1 and/or membranous positive staining for EMA but negative staining for HepPar1.

Patients with CoCC demonstrate favorable long-term survival after curative surgery. CoCC has been shown to be less invasive in the portal vein, as the number of patients with remaining portal tracts within their tumors was significantly higher in a CoCC group than in an

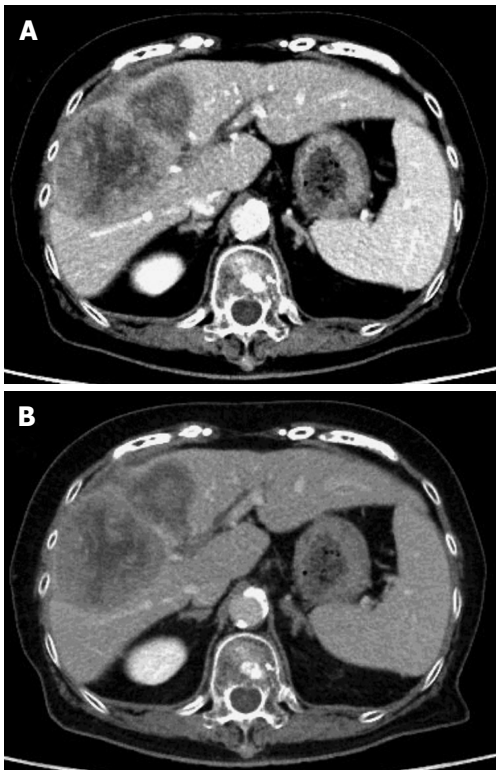

Figure 1 Contrast enhanced computed tomography scan showed a tumor located in the right anterior compartment of the liver with extrahepatic hematoma. The tumors were hyperattenuating relative to the noncancerous liver parenchyma on arterial-phase (A) and hypo- or isoattenuating on delayed phase (B).

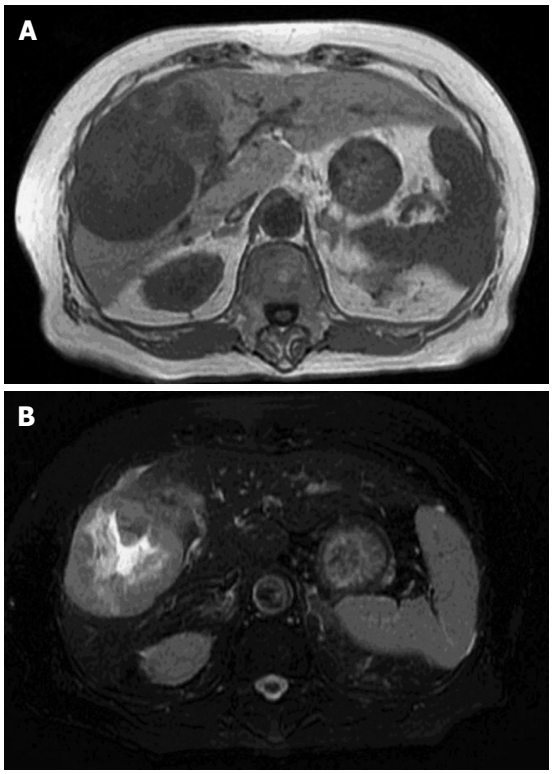

Figure 2 The axial T1-weighted gradient-echo image showed a hypointense mass in the right anterior segment of the liver (A), and the axial T2-weighted spin-echo image with fat suppression showed an isointense mass with a large central hyperintense area (B).

ICC group[5]. Moreover, the number of patients with intrahepatic metastasis was significantly lower in a CoCC group than in an ICC group[6]. Furthermore, the 5-year overall survival rate and recurrence-free survival rate were significantly higher in a CoCC group than in an ICC group[7].

Spontaneous tumor rupture is one of the most fatal complications of hepatic tumors and is mainly determined by the growth characteristics of the tumor. The mechanism of tumor rupture has not been fully characterized; however, the literature[8] suggests that the pathogenesis may be associated with expansive growth and intratumoral pressure, which may cause tumor vein compression and congestion. The rapid growth of tumors also results in an insufficient blood supply to the tumors *in vivo*, causing tumor hypoxia-ischemia to occur, in turn resulting in significant necrosis.

As mentioned previously, CoCC grows relatively slowly and is less invasive, which results in a smaller tumor size (mean: 3.5 cm) than for other hepatic tumors[9]. To the best of our knowledge, this is the first report describing CoCC presenting as a huge tumor that had ruptured.

Emergency fluid resuscitation is a key therapeutic step for patients with hepatic tumor rupture. In particular, patients admitted to hospitals are treated with fast rehydration, anti-shock treatment, blood transfusion, and other supporting treatments to stabilize their circulation so that transhepatic artery angiography and embolization (that is, TAE) can be performed for hemostasis. For patients with continued bleeding, primary surgeries are performed to stop the bleeding. Hepatectomy, however, not only can stop bleeding immediately but also can make the radical resection of hepatic lesions possible as well as allowing for better long-term outcomes. There is still a possibility of rebleeding, even with temporarily controlled bleeding, in the case of spontaneous hepatic tumor ruptures[10,11]; hence, radical hepatectomy is an effective measure to address such emergencies. However, liver resection would be risky for patients with relatively poor liver function and/or severe liver cirrhosis[12]. Thus, TAE is more advantageous during initial hemostasis in emergency procedures for patients with hepatic tumor ruptures, especially in the case of high-surgical-risk patients. Once initial hemostasis is achieved using active supportive therapy, the patients may undergo staged hepatectomy.

In the case described here, the tumor was so huge that TAE was assumed to be anatomically difficult. In addition, the patient was hemodynamically stable due to fluid resuscitation, and her hepatic function was competent; thus, primary right hemi-hepatectomy was performed as a radical treatment.

CoCC is a rare primary hepatic tumor that demonstrates a better prognosis than for other primary liver cancers, such as HCC or ICC, and thus should be dis-

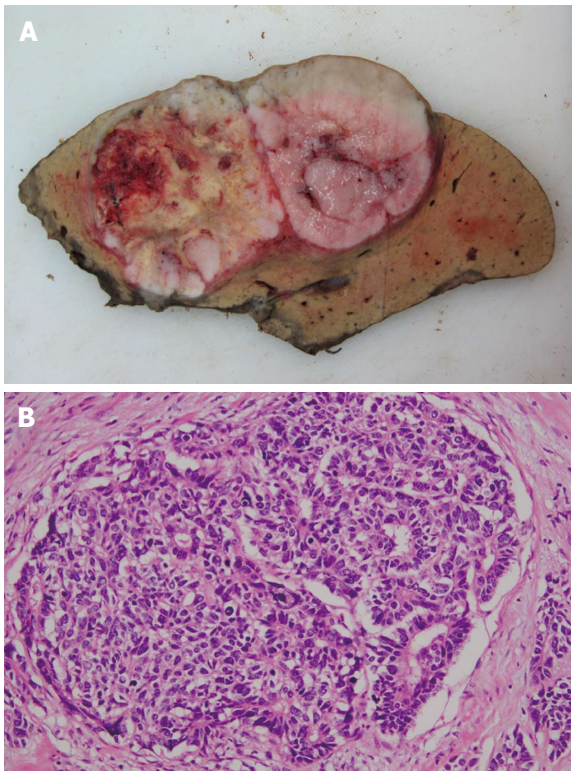

Figure 3 Histologically, the tumor was mainly composed of small, monotonous glands formed into antler-like anastomosing patterns, embedded in the fibrous stroma to various degrees of fibrous stroma and lacking mucin production. A: Macroscopically, an expanding tumor located in the right anterior compartment of the liver with extrahepatic hematoma with central necrosis; B: HE staining demonstrated the tumors mainly composed of small, monotonous glands and embedded in various degrees of fibrous stroma without mucin production.

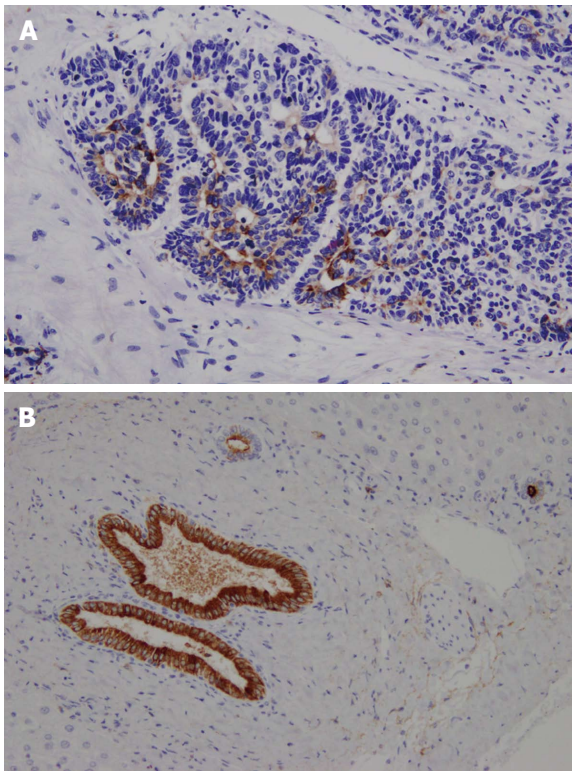

Figure 4 Cholangiolocellular carcinoma cells were confirmed by positive staining for cytokeratin 19 (A) and membranous positive staining for epithelial membrane antigen (B).

tinguished from those other cancers.

Moreover, CoCC can appear as a ruptured huge tumor, and when it does, radical hepatectomy can be an effective measure to achieve both absolute hemostasis and tumor cure.

COMMENTS

Case characteristics

An 80-year-old Japanese woman presented with right upper abdominal pain that had developed within 2-3 h along with hypotension.

Clinical diagnosis

There was tenderness in the right upper abdomen, and her body temperature was 36.5 ℃.

Differential diagnosis

Hepatocellular carcinoma, intrahepatic cholangiocarcinoma, metastatic hepatic tumor or hepatic abscess.

Laboratory diagnosis

Laboratory test results were within the normal range. In particular, the patient was negative for anemia and thrombocytopenia, and her bilirubin, transaminase and albumin levels were in the normal range.

Imaging diagnosis

A contrast-enhanced computed tomography scan showed a huge tumor located in the right anterior segment of the liver along with extrahepatic hematoma.

Pathological diagnosis

The tumor was mainly composed of small, monotonous glands formed into antler-like anastomosing patterns, embedded in the fibrous stroma to various degrees and lacking mucin production.

Treatment

Fluid resuscitation, blood transfusion and primary right hemi-hepatectomy.

Related reports

Spontaneous tumor rupture is one of the most fatal complications of hepatic tumors, and it is reported that the in-hospital and 30-d mortality rates are as high as 25%-100%.

Term explanation

Cholangiolocellular carcinoma (CoCC) was previously classified as a special type of intrahepatic cholangiocarcinoma. However, as a result of recent advancements in the field, CoCC is now considered to originate from hepatic stem/progenitor cells.

Experiences and lessons

CoCC can appear as a ruptured huge tumor, and when it does, radical hepatectomy can be an effective measure to achieve both absolute hemostasis and tumor cure.

Peer-review

Nice case report of successful surgical treatment of rare primary hepatic tumor. Excellent illustrations.

REFERENCES

1 **Liu CL**, Fan ST, Lo CM, Tso WK, Poon RT, Lam CM, Wong J. Management of spontaneous rupture of hepatocellular carcinoma: single-center experience. *J Clin Oncol* 2001; **19**: 3725-3732 [PMID: 11533094 DOI: 10.1200/jco.2001.19.17.3725]

2 **Steiner PE**, Higginson J. Cholangiolocellular carcinoma of the liver. *Cancer* 1959; **12**: 753-759 [PMID: 13663020 DOI: 10.1002/1097-0142 (195907/08)12:4<753::AID-CNCR2820120420>3.0.CO;2-L]
3 **Kanamoto M**, Yoshizumi T, Ikegami T, Imura S, Morine Y, Ikemoto T, Sano N, Shimada M. Cholangiolocellular carcinoma containing hepatocellular carcinoma and cholangiocellular carcinoma, extremely rare tumor of the liver: a case report. *J Med Invest* 2008; **55**: 161-165 [PMID: 18319561]
4 **Fukukura Y**, Hamanoue M, Fujiyoshi F, Sasaki M, Haruta K, Inoue H, Aiko T, Nakajo M. Cholangiolocellular carcinoma of the liver: CT and MR findings. *J Comput Assist Tomogr* 2000; **24**: 809-812 [PMID: 11045707]
5 **Uenishi T**, Yamazaki O, Tanaka H, Takemura S, Yamamoto T, Tanaka S, Nishiguchi S, Kubo S. Serum cytokeratin 19 fragment (CYFRA21-1) as a prognostic factor in intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 2008; **15**: 583-589 [PMID: 17955299 DOI: 10.1245/s10434-007-9650-y]
6 **Guglielmi A**, Ruzzenente A, Campagnaro T, Pachera S, Valdegamberi A, Nicoli P, Cappellani A, Malfermoni G, Iacono C. Intrahepatic cholangiocarcinoma: prognostic factors after surgical resection. *World J Surg* 2009; **33**: 1247-1254 [PMID: 19294467 DOI: 10.1007/s00268-009-9970-0]
7 **Ariizumi S**, Kotera Y, Katagiri S, Nakano M, Nakanuma Y, Saito A, Yamamoto M. Long-term survival of patients with cholangiolocellular carcinoma after curative hepatectomy. *Ann Surg Oncol* 2014; **21** Suppl 3: S451-S458 [PMID: 24633664 DOI: 10.1245/s10434-014-3582-0]
8 **Yoshida H**, Mamada Y, Taniai N, Uchida E. Spontaneous ruptured hepatocellular carcinoma. *Hepatol Res* 2016; **46**: 13-21 [PMID: 25631290 DOI: 10.1111/hepr.12498]
9 **Maeno S**, Kondo F, Sano K, Takada T, Asano T. Morphometric and immunohistochemical study of cholangiolocellular carcinoma: comparison with non-neoplastic cholangiole, interlobular duct and septal duct. *J Hepatobiliary Pancreat Sci* 2012; **19**: 289-296 [PMID: 22179577 DOI: 10.1007/s00534-011-0483-5]
10 **Barosa R**, Figueiredo P, Fonseca C. Acute Anemia in a Patient With Hepatocellular Carcinoma. HCC Rupture With Intraperitoneal Hemorrhage. *Gastroenterology* 2015; **149**: e3-e4 [PMID: 26231603 DOI: 10.1053/j.gastro.2014.12.055]
11 **Recordare A**, Bonariol L, Caratozzolo E, Callegari F, Bruno G, Di Paola F, Bassi N. Management of spontaneous bleeding due to hepatocellular carcinoma. *Minerva Chir* 2002; **57**: 347-356 [PMID: 12029230]
12 **Han XJ**, Su HY, Shao HB, Xu K. Prognostic factors of spontaneously ruptured hepatocellular carcinoma. *World J Gastroenterol* 2015; **21**: 7488-7494 [PMID: 26139994 DOI: 10.3748/wjg.v21.i24.7488]

P- Reviewer: Galvao FHF, Kang KJ, Kamiyama T, Qin JM, Surani S, Tarazov PG **S- Editor**: Song XX **L- Editor**: A **E- Editor**: Li D

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com